



Hope or Hype: Stem Cells as Therapeutics in Retinal Degenerative Diseases

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“Netram Pradhanam Servendriyanam”; “Eyes are the precious gift given to humans by the Almighty” – verse from Bhagavad Gita emphasizes the importance of vision for the mankind. The most recent statistics from the World Health Organization (WHO) reveals that 39 million are blind worldwide, signifying the problem and a need to initiate formidable approaches to address the issue. Almost all the ocular diseases involving the retina, the innermost layer of the eye composed of light sensitive tissue, is characterized by degeneration of retinal cells. The treatment for retinal degenerative diseases is impeded for the want of suitable cells to replace those that are getting degenerated in response to the disease. Stem cell therapy offers a unique opportunity to replace the damaged cells with new ones. Stem cell-based therapeutic approaches can be broadly classified as endogenous and exogenous. The former approach utilizes activation of endogenous stem cells present in the retina for replacing the degenerating cells. The latter utilizes exogenous stem cells, such as mesenchymal stem cells, neural progenitors, embryonic stem cells, and induced pluripotent stem cell-derived retinal progenitors that are transplanted into the degenerating retina. In this book chapter, the key concepts involving both the endogenous and exogenous stem cells for retinal degenerative diseases and their potential advantages and the limitations will be discussed. The outcome of the recent clinical trials along with the future directions and the challenges of stem cell-based therapies will be briefly covered.

Vision is considered the most important sensory modalities that we possess. Blindness or loss of vision is a debilitating condition that affects the quality of life. The most recent statistics from the World Health Organization (WHO) reveals that nearly 285 million people are visually impaired in the world, with 39 million totally blind [117]. Retinal degenerative diseases are the leading causes of untreatable

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blindness in the world [35]. Unlike cataract or corneal disorder, where replacement of either a synthetic implant or live tissue is possible, the restoration of visual function due to retinal degeneration is precarious, owing to the complex structure of the retinal tissue and its neural nature.

8.1 Retina: The Delicate Screen in the Eye

Until studies by Cajal, the retina was considered as an inextricable membrane consisting of reticular and granular layers of uncertain significance. His studies revealed that the retina is a complex structure composed of different classes of neural cells that convey visual message toward the encephalic centers along well-defined paths [121].

Today, retina is defined as a light-sensitive membrane at the back of the eyeball which triggers nerve impulses that pass via the optic nerve to the brain, where a visual image is formed. The retina is organized into three layers of cell bodies and two layers of neuropil.

The three layers of cell bodies are the outer nuclear layer (ONL) which comprise the primary sensory neurons/photoreceptors (rods and cones), the inner nuclear layer (INL) which homes the interneurons (horizontal, amacrine, and bipolar cells), and the ganglion cell layer which contains the output neurons (retinal ganglion cells (RGCs)). In addition to the interneurons, the INL contain sole glia of the retina, the Muller glia [77]. Figure 8.1 shows a schematic representation of different layers of the retina and the diseases associated with each layer.

There are several subtypes of each type of neuron with at least 30 known types of RGCs and 33 types of amacrine cells. These subtypes show variations in their morphological characters, functions, and also in their spatial distributions and frequencies. The fundamental schema of the retina is conserved across vertebrates, and in all species, the light traverse the whole thickness of the retina before reaching the photoreceptors. Retinal pigment epithelium (RPE), which interfaces the retina and choroid, by definition, is not a part of the retinal tissue. However, it is primarily involved in the health of the retinal tissue by providing trophic support and hence for the purpose of the book chapter will be considered as part of the retina. Unlike cornea which possesses resident stem cells in the limbal region, the adult retina does not harbor authentic resident stem cells hence do not have the potential to replenish the retinal cells that are lost during degeneration.

8.2 Retinal Degenerative Diseases: Causes and Mechanisms

Retinal degeneration is defined as the deterioration of the retina caused by the progressive loss of the retinal cells. Retinal degenerative diseases are broadly classified as inherited monogenic or multifactorial depending on the cause of the disease. Classical examples of monogenic diseases include Leber's congenital amaurosis and retinitis pigmentosa, while the most common multifactorial retinal degenerative diseases are age-related macular degeneration, diabetic retinopathy, and glaucoma.

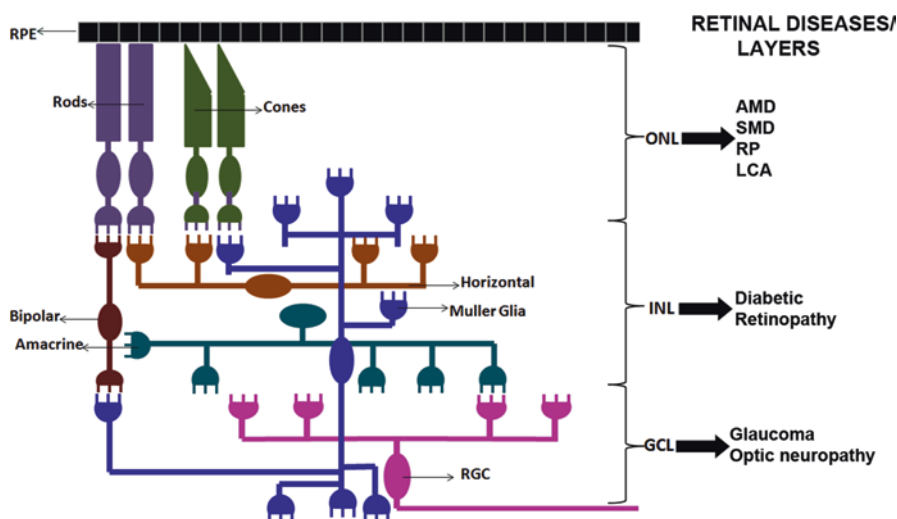


Fig. 8.1 Schematic representation of different layers of the retina with the most common retinal diseases associated with each layer. Abbreviations: *RPE* Retinal pigment epithelium, *RGC* Retinal ganglion cells, *ONL* Outer nuclear layer, *INL* Inner nuclear layer, *GCL* Ganglion cell layer, *AMD* Age-related macular degeneration, *SMD* Stargardt macular degeneration, *RP* Retinitis pigmentosa, *LCA* Leber's congenital amaurosis

Table 8.1 provides a list of common retinal degenerative diseases, the cells they affect and known causative factors. It can be realized from the table that the most vulnerable cell populations in the retina are the photoreceptors, retinal ganglion cells, and the retinal pigment epithelial cells.

The vulnerability of the photoreceptors to cell death is attributed to its unique physiology and biochemical properties. There seems to be a delicate balance maintained between the survival and death of the photoreceptors, and any minute defect seems to tip them toward cell death [171]. The well-known factors that make the photoreceptors vulnerable to cell death are the presence of photosensitizing molecules [1] and its lipid-rich outer segments [153]. These molecules on exposure to light, in the presence of oxygen, lead to the generation of reactive oxygen and nitrogen species that ultimately lead to cell damage. This is also one of the primary reasons for macular damage since the light has to focus on the central macula to ensure high visual acuity. The mechanism of the photooxidative damage suggests that blue light in the range of 450–495 nm in wavelength leads to activation of rhodopsin with the release of all-trans-retinal. Any defect in the visual cycle which inhibits the conversion of all-trans-retinal to 11-cis retinal lead to a buildup of toxic bisretinoids which upon photooxidation lead to the generation of reactive oxygen and nitrogen species [146, 173]. The presence of polyunsaturated fatty acid-rich outer segment of the photoreceptors leads to accumulation of lipid peroxidation products such as 4-hydroxy-2-nonenal and malondialdehyde. These form protein adducts which compromise the lysosomal proteolysis thereby inhibiting the exocytosis of

Table 8.1 List of the common retinal degenerative diseases with the cells affected and causative factors

Diseases	Cell types affected	Causative factors
Age-related macular degeneration	Rods, cones, retinal pigment epithelium	Complex, multiple genetic/ environmental factors
Stargardt disease	Rods, cones, retinal pigment epithelium	Genetic mutations in ABCA4; ELOVL4
Retinitis pigmentosa	Rods more than cones retinal pigment epithelium (progressive)	Genetic mutations in more than 150 genes
Leber's congenital amaurosis	Rods, cones retinal pigment epithelium (progressive)	Genetic mutations in RPE65; CRX; AIPL1; TULP1; CABP4; CEP290; RDH12
Diabetic retinopathy	Retinal blood vessel	Diabetes greater than 20 years
Glaucoma	Retinal ganglion cells, optic nerve	Complex, multiple genetic/ environmental factors
Optic neuropathy	Retinal ganglion cells, optic nerve	Ischemia, trauma, mutations in mitochondrial genes

undegraded protein adducts. The accumulation of these adducts ultimately leads to degeneration of RPE [79]. In addition, the protein adducts are also shown to activate the complement pathway thereby initiating the pathophysiology in disease such as age-related macular degeneration [61].

Unlike the photoreceptors and the RPE, photooxidative stress does not seem to be the primary cause of RGC cell death. RGCs have been shown to be susceptible to various modes of injury, particularly intraocular pressure elevation and ischemia. [174] suggested that the mechanism and extent of injury in the RGCs differ based on their subcellular components. In this study, the RGCs were divided into subcellular components based on their structure, composition of their extracellular environment, and energy consumption, all of which play an important role in their response to the insults [174]. The four subcellular components of RGCs are as follows: (1) RGC dendrites and synapse in the inner plexiform layer, (2) RGC cell bodies in the ganglion cell layer, (3) nonmyelinated axons of the RGCs in the retina and the optic nerve head, and (4) myelinated axons in the orbit and the cranial region [174]. Based on several studies, it is now accepted that the optic nerve head is the primary site of RGC damage in glaucomatous optic neuropathy [19, 105, 164]. It is suggested that increase in IOP and ischemia interfere with axonal transport and likely damage the mitochondria in the axons of the optic nerve head [24, 69, 78]. This leads to deprivation of neurotrophic factors from the cell body and synapse. Chronic deprivation of these factors initiates apoptosis in RGC ultimately leading to cell death [3].

Although the causative factors and mechanisms of retinal degeneration are profoundly different, they converge toward a single aspect, i.e., loss of retinal cells. Hence, therapeutic approaches which can replace the lost retinal cells can aid in

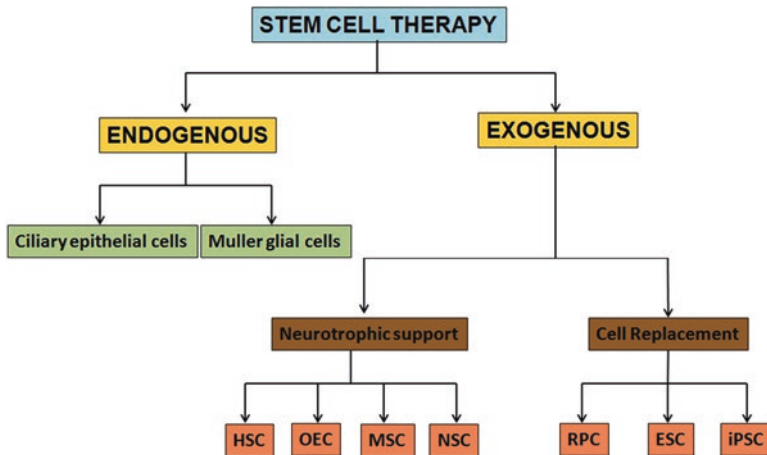


Fig. 8.2 Schematic representation of stem cell-based therapeutic strategies for retinal degenerative diseases. (1) Endogenous strategy involves activation of retinal stem cells/progenitors present in ciliary epithelium and Muller glial cells by growth factors or small molecules, and (2) exogenous strategy involves replacement of degenerated cells by transplantation of stem cells. The cells that are transplanted either provide neurotrophic support and prevent further degeneration (HSC; OEC; MSC; NSC) or transdifferentiate and form functional retinal cells (RPC; ESC; iPSC). Abbreviations: *HSC* hematopoietic stem cells (adult), *OEC* olfactory ensheathing cells (adult), *MSC* mesenchymal stem cells (adult), *NSC* neural stem cells (fetal/adult), *RPC* retinal progenitor cells (fetal), *ESC* embryonic stem cells (Embryonic), *iPSC* induced pluripotent stem cells (reprogrammed cells)

treating retinal degenerations. Figure 8.2 shows a broad representation of the two therapeutic strategies: (1) endogenous, activation of retinal stem cells/progenitors, and (2) exogenous, cell replacement therapies.

8.3 Regeneration of the Retina: Repairing the Retina from Within

Regeneration of either entire or a part of the retina has been observed in several organisms. The extent of regeneration seems to vary between different organisms and also on the developmental stage. Upon injury, retinal regeneration can follow either of the two strategies: (1) transdifferentiation, wherein differentiated cells dedifferentiate to a primitive progenitor type and redifferentiate into a new retinal cell, and (2) direct differentiation of quiescent population of stem cells.

While retinal regeneration is highly hampered during the adulthood in the mammals, it is widely demonstrated in adult fish, amphibians, and birds. In fact, fishes and amphibians regenerate the retina throughout their lives. This property is an absolute requirement in these species since their eyes grow throughout their life, and in order to meet the ever-increasing size of the eye, the retina also grows. This is accomplished by the presence of retinal stem and progenitor cells in the periphery

of the neural retina called the ciliary marginal zone (CMZ) [120]. It is also suggested that these stem/progenitor cells also contribute toward regeneration during retinal damage in the adulthood of lower vertebrates. However, the studies have indicated that different mechanisms are involved in the retinal development from CMZ during embryonic stages and that during the retinal regeneration during adulthood [40, 104, 151]. It is also noticed that in the teleost fish, the truly regenerated cells post-injury in the retina shows a “clumped” pattern, while the normal cells that are made from the CMZ as part of the normal retinal development (in uninjured sites) were more “random” suggesting difference in spatiotemporal patterns of regeneration [149–151]. In addition, several studies suggested that a complete destruction of the entire retina or at least greater than 30% of the retina was a requirement to initiate the regenerative response in the teleost fishes [13, 133]. Although the stem cells in the CMZ have the capacity to contribute to normal retinal development throughout adulthood and also post-injury, the regeneration was observed to be restricted to more peripheral regions of the retina. Several studies indicated that the regeneration of more central portions of the retina was not contributed by CMZ but by another set of putative stem cells [39, 41, 70, 163].

Initial studies in the direction of finding the other putative stem cells’ contribution to the retinal regeneration in injured retina suggested that these stem cells were predominantly harbored in the inner nuclear layer (INL) of the retina. These cells were initially identified as cells of rod lineage and named as proliferative inner nuclear cells (PINC) [38, 70]. However, further studies failed to demonstrate the association of these cells to retinal regeneration. In 2000, the study by Vihtelic and Hyde using light-induced photoreceptor damage in zebrafish suggested that the proliferating cells within the INL post-injury could be the Muller glial cells [163].

Fischer and Reh [41], using chick eye, convincingly proved that these proliferating cells in the INL are Muller glial cells, which upon injury dedifferentiate and reenter the cell cycle to give rise to retinal progenitor-like cells capable of differentiating into new retinal neurons [41]. The mechanism of Muller glia-directed retinal regeneration is very well established in the zebrafish model. It is now widely accepted that in response to the injury, growth factors and cytokines such as heparin-binding epidermal growth factor and tumor necrosis factor alpha are released by Muller glia in the site of injury which leads to an intrinsic genetic reprogramming of these cells [10, 74, 108, 131, 148, 165]. The reprogramming of these cells leads to interkinetic nuclear migration wherein the nucleus of these reprogrammed cells which usually lies in the INL migrates to the ONL. The interkinetic nuclear migration is then followed by an asymmetric cell division which leads to the generation of retinal progenitors (sometimes referred to as neurogenic clusters) [107]. These retinal progenitors are multipotential and migrate to different retinal layers giving rise to almost all the retinal neuron types. The signaling cascades involved in different steps of the retinal regeneration via activated Muller glial cells are varied and difficult to comprehend. The variation seen in different studies arises from the fact that the nature and the intensity of the injury vary immensely between these studies. The signaling cascades that are involved in light-induced damage are different from that involved in the chemical and mechanical damage. Knowledge from various

studies point toward activation of four main signaling pathways: the MAPK-ERK, Wnt – β catenin, Notch, and JAK-STAT pathways [109, 131, 165]. In addition to the activation of these pathways, inhibition of the TGF β and GSK3 β using pharmacological inhibitors also increased the efficiency of regeneration in the injured retina [83, 103, 131]. Although these signaling pathways contribute to the overall reprogramming of the Muller glial cells and its differentiation to different retinal neurons, it is not very clear as to the stages at which each signaling pathways specifically play a role. There is paucity in the studies demonstrating the role of different signaling pathways for directed differentiation of Muller glial cells to specific retinal neurons. Nevertheless, the factors involved in specific retinal neuron generation are emerging. For instance, *Insm1a*, a transcriptional repressor, is indicated in overall differentiation of Muller glial-derived retinal progenitors; signal transducers such as FGFR1 and Notch are implicated in rod photoreceptor differentiation [60, 126, 132, 165]; and *Mps1*, a regulator of mitotic checkpoint, and N-cadherin, cell adhesion molecules, have been attributed to cone photoreceptor and inner retinal neurons [107, 125], respectively.

These studies clearly demonstrate that both ciliary marginal zone cells and the Muller glia regenerate retina in lower vertebrates and chicks, and the intensity of this regeneration can be modulated by regulating specific signaling pathways. However, although this capacity is clearly evident in these nonmammalian animals, most studies have suggested that this capacity is very much reduced and hidden in the mammals. In the case of mammals, the regenerative ability of ciliary epithelial cells is controversial [2, 25, 160]. In addition, the mammalian Muller glial cells post-injury tends to undergo a different phenomenon known as reactive gliosis.

8.4 Muller Glia: A Ray of Hope for Endogenous Retinal Repair in Mammals

In the mammals, the Muller glial cells post-injury undergoes either a proliferative or a nonproliferative gliosis. The gliosis aids in neuroprotection by releasing neurotrophic factors which enable the retinal neurons to survive the injury [16]. However, chronic gliosis leads to neurodegeneration and interferes with the proper functioning of the retina [16, 50]. As stated earlier, the Muller glia in mammals is in the threshold of retinal regeneration and reactive gliosis. Any stimuli which tip off the balance from the gliotic response to retinal regeneration would authenticate Muller glial cells as an endogenous source for retinal repair.

In the studies involving rodents, it is shown that the Muller glial cells which are cultured *in vitro* under the influence of growth factors such as EGF, bFGF, and/or Shh dedifferentiate into retinal progenitor-like cells [26, 48, 72, 161, 166]. These cells have the potential to proliferate and differentiate into photoreceptors and retinal ganglion cells when specific cues are provided. In addition, these retinal progenitor-like cells, when transplanted into damaged rodent retina, were able to orchestrate retinal repair, albeit at a lower efficiency. These observations persuaded studies toward establishing external stimuli that are required to coax the Muller glial cells to reenter cell cycle and differentiate into other retinal cells that are lost during the injury.

In vivo stimulation of Muller glial cells in the rodents has been attempted by several groups with limited success. Different methods such as pharmacological damage and genetic engineering have been attempted to achieve this. These studies reveal that Notch-, Shh-, EGF-, Wnt-, and Ascl-1-dependent signaling could indeed stimulate proliferation of the Muller glial cells in vivo and also lead to neural regeneration in the injured rodent retina; however, these events were very rare [26, 29, 30–32, 72, 161, 166].

The well-studied model of retinal regeneration in mammals involves cytotoxicity induced by N-methyl-D-aspartate (NMDA), an amino acid derivative and an agonist of NMDA receptor with functions similar to glutamate [72]. In this model of retinal degeneration, the introduction of EGF growth factor led to the proliferation of Muller glia by activation of MAPK, PI3K, and BMP signaling. The activation of EGFR was observed upstream of these signaling pathways [161]. Low doses of glutamate also led to proliferation and neural regeneration in the retina, suggesting that retinal injury in the mammals might lead to increased glutamate secretion at the site of injury thereby stimulating the Muller glia to break quiescence and reenter cell cycle [157]. Compared to the signaling pathways involved in zebrafish or chick retinal regeneration post-injury, in mammals, the expression of Ascl-1, one of the immediate features in proliferating Muller glial cells, was not observed. Gain-of-function experiments with Ascl-1 in mouse retina post-injury increased the proliferation and differentiation toward bipolar neurons [123].

Taken together, these studies postulate Muller cells as a cellular source for healing the retina from within mammals. However, the findings in the mammalian studies have also left some pressing questions that are yet to be answered. (1) Why does the retina of lower vertebrates regenerate more readily than mammals'? (2) Although the Muller glial cells in the injured retina in mammals readily proliferate and dedifferentiate, what are the mechanisms that prevent their differentiation into other retinal neurons? Future studies involving high-throughput sequencing will enable identification of genetic and epigenetic differences between the lower vertebrate and mammalian retina in terms of their regenerative capacity. Those that are identified can then be tested in the mammalian models of retinal regeneration to improve the efficiency of endogenous repair.

The advantages of the endogenous repair are the following: (1) the approach does not entail transplant of genetically or chemically modified cells; (2) no risk of the immune response, chromosomal aberrations, or tumorigenicity; and (3) does not require cell infiltration or grafting to specific layer for functionality. The major drawbacks of the approach are (1) inefficient delivery of growth factor or small molecules to activate endogenous stem cells, (2) lower stability and bioavailability of the growth factor or small molecules that necessitate repeated injections, (3) increased possibility of unexpected physiological responses depending on the nature of the injury which cannot be predicted by any in vitro or in vivo models, and (4) the most regenerative potential of the Muller glial cells that is limited to outer retinal layers, i.e., photoreceptors. In view of the abovementioned limitations, cell replacement strategies involving exogenous stem cells are currently being explored.

8.5 Exogenous Stem Cell Therapy: Tackling Retinal Degeneration Using Non-native Cells

The rationale for stem cell therapy stems from the following facts of the inner retina: (i) the organ is supposedly immune-privileged (with some reservation); (2) earlier observations suggesting long-term survival of neural grafts; (3) possibility of intraocular route of administration of the exogenous cells, and (4) demonstration of migration and integration of transplanted cells into specific layers. Hence, the only constraint is to find the most reliable source of cells to replace the ones that are lost during the retinal degeneration.

The ideal characteristics of the source of the stem cells for cell-based therapies are (i) renewable, available in sufficient amounts; (ii) versatile, a single source that can generate different kinds of retinal cells; (iii) ethical and legal, both in accordance with accepted principles and beliefs; (iv) autologous, devoid of immune rejection; (v) cost-effective; and (vi) technically less challenging to isolate, culture, or transplant.

Several types of stem cells have been studied for their ability to replace or rejuvenate the degenerating retinal cells which have one or more of the ideal characteristics mentioned above. These cells can be broadly classified as adult stem cells, embryonic or fetal stem cells, and reprogrammed stem cells.

8.6 Adult Stem Cells

8.6.1 Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are the first stem cells that were discovered in 1963 by Ernest McCulloch and James Till [159]. These cells were predominantly found in the adult bone marrow and had the ability to give rise to the complete spectrum of blood and immune cells. HSCs are usually obtained from bone marrow aspirates collected from the hip bones (bone marrow-derived stem cells; BMSC) or by mobilization of these in peripheral blood using cytokines such as granulocyte colony-stimulating factors (G-CSF) [23, 80, 98]. Recently, HSCs have also been isolated from the umbilical cord blood and placenta [20].

With respect to retinitis pigmentosa mouse models rd1 and rd10, intravitreal injection of HSCs was shown to improve the visual parameters [112]. The improvement in the visual parameter was also attributed to the neurotrophic factors that are secreted by the lineage negative HSCs. In addition, this study also showed that the HSCs which are intravitreally injected in these models rescued mostly the cone photoreceptors which would have otherwise died due to the bystander effect of degenerating rod photoreceptors. Hence, this study provided the proof-of-principle that autologous BMSCs can slow down the progression of retinitis pigmentosa by secreting neurotrophic factors which orchestrate its function by upregulating anti-apoptotic genes and factors [112]. These preclinical studies eventually led to clinical trials involving the intravitreal injection of BMSCs in three individuals with

retinitis pigmentosa (discussed under the subheading stem cell-based clinical trials in retinal degenerative diseases).

8.6.2 Olfactory Ensheathing Cells

Olfactory ensheathing cells (OECs) are specialized glial cells that ensheath the axons of the olfactory nerve which connects the nose to the brain. They are predominantly present in the olfactory bulb and nasal mucosa. Studies have revealed that transplantation of OECs has potential in RGC regeneration in rat optic nerve transection models. Analysis of the transplanted animals 6 months posttransplantation revealed that the transplanted cells had the ability to survive, migrate, and integrate into the optic nerve [84, 86]. They also increased the survival of the RGCs post-axotomy and promoted their axonal growth. It was assumed that the effect of OECs on RGC survival and regeneration was due to the neurotrophic factors that they secreted.

The factors responsible for the effect of OECs on RGC survival was analyzed using an in vitro model of RGCs. In this study, scratch-insulted RGCs were exposed to adult OEC conditioned medium with and without neutralizing antibodies to brain-derived neurotrophic factor (BDNF). The study revealed that neutralization of BDNF in the conditioned medium attenuated the survival and neuroprotective effect of the OEC conditioned medium [168, 172]. Currently, the clinical trials of OECs are limited to spinal cord injury and ischemic strokes and have not been initiated with respect to retinal and/or optic nerve injury.

8.6.3 Adult Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are non-hematopoietic multipotent progenitors originally isolated and identified in the bone marrow by [42]. Although MSCs were initially isolated from the bone marrow, currently, several tissue sources of MSCs have been identified [62, 64, 95, 152]. Sources of mesenchymal stem cells other than the bone marrow are adipose tissue and peripheral blood [106]. MSCs from the lung and heart have also been isolated [63, 135]. In addition to the authentic adult MSCs, several birth-associated tissues such as umbilical cord, cord blood, placenta and amnion, and Wharton's jelly have been used as sources for isolating and enriching MSCs [12, 58, 75, 90, 93, 124, 147, 158]. Currently, MSCs have been identified from almost all the tissues in the body. MSCs have also been reported in the limbal and corneal tissues [14, 15, 122]. Since there is no concurrence with either morphology or functionality of MSCs identified in various tissues, a minimal set of criteria have been laid by the International Society for Cellular Therapy to define a set of cells as MSCs: (1) selective adherence of the cells to plastic surface of culture dishes; (2) minimum 95% positive for cell surface markers CD105, CD90, and CD73 and negative for CD34, CD45, CD14 or CD11b, CD79 or CD19, and

HLA-DR; and (3) capacity to differentiate into osteocytes, adipocytes, and chondrocytes [34].

MSCs are favored in the field of regenerative medicine because of the ease of logistics involved in isolation and expansion from the bone marrow and adipose tissues of adult patients to facilitate autologous transplantation. Thus, these cells do not entail ethical and immunological issues associated with many other stem cell sources. With respect to retinal degeneration, although few studies have indicated transdifferentiation potential of MSCs into photoreceptors or RPE cells, the results were not reproducible, and hence the potential of MSCs in generating retinal cells is controversial [7, 8, 66, 177]. However, the neuroprotective aspect of MSCs in various retinal degeneration model is well accepted. Unlike the OECs, the neuroprotective effect of MSCs are not restricted to inner retinal layer such as GCL but also reported in outer retinal layers such as photoreceptors and RPE. Both subretinal and intravitreal transplantations of bone marrow-derived MSCs have been attempted in RCS rats and mouse models of retinitis pigmentosa [7, 66]. The studies showed significant improvement in the retinal functionality attributed to the increased survival of photoreceptor and RPE cells compared to the untreated or sham-treated controls. In addition to the subretinal and intravitreal routes, intravenous administration of these MSCs has also been shown to improve the visual function, which makes the procedure less complex. It is assumed that the MSCs that are injected intravenously can home into the sites of injury, as observed in other central nervous system-related injuries [167]. More recently, intravenous injection of MSCs genetically engineered to secrete pigment epithelium-derived factor (PEDF) has been shown to be effective in treating laser-induced choroidal neovascularization in mice. The authors of the study suggest that the injected MSCs can be a source of long-term anti-angiogenic factor that otherwise is provided to patients as part of intermittent intravitreal injections [8].

With respect to inner retinal degenerations, MSC-based therapies have been evaluated in animal models of glaucoma, ischemic retinopathy, and diabetic retinopathy [37, 68, 130, 136, 179]. A preclinical rat model of ocular hypertension has been extensively used as a model to assess the effect of MSC-based therapy in glaucoma. Intravitreal transplantation of MSCs into the ocular hypertension models irrespective of the method employed for inducing hypertension (episcleral vein cauterization or laser photocoagulation of trabecular meshwork) showed significant RGC protection. [59] showed that the vitreous humor is impermeable to differentiation of BMSCs to neurons [59]. However, the study by [100] substantiated that the effect of intravitreal MSCs was due to the neurotrophic factors such as BDNF and NT-3 that diffused through the vitreous thereby conferring neuroprotection to RGCs in the optic nerve injury model [100]. It was confirmed that the dental pulp MSCs were more effective than the bone marrow in the RGC protection, and this was attributed to the increased level of neurotrophic factors secreted by dental pulp MSCs. In addition to BDNF, CNTF and bFGF have been found to be secreted by intravenously injected MSCs in ischemic retina model. The cells were shown to secrete these neurotrophic factors for at least 4 weeks [100].

Both local and systemic routes of administration have been shown to be effective in different studies. The most tested routes are intravitreal, subretinal, and intravenous. Although systemic administration proved to be effective in choroidal neovascularization model, it did not confer neuroprotection in glaucoma model [67, 68]. Several pros and cons of the routes of administration have been postulated. Intravitreal injection is localized, easy to perform, and less complex; however, it does not ensure close contact of the transplanted cells to the retina. In addition, inner limiting membrane seems to be a potential barrier for the migration and integration of the cells. Subretinal injection provides more close contact of transplanted cells to the retinal tissue; however, it is difficult to perform and is associated with higher risk. Intravenous route of administration is safest, however; it requires a higher number of cells to be injected. In addition, the cues that would allow the MSCs to migrate to the retinal site of injury may not be profound in systemic conditions such as diabetes and age-related macular degeneration, where the MSCs may be recruited to other sites of injury and may not be available in sufficient numbers to fix the retinal injury.

Several studies with respect to “homing” of MSCs to specific organs have revealed that the process is orchestrated by a complex association of chemokines, chemokine receptors, cell adhesion molecules, and proteolytic enzymes [73]. More studies on the abovementioned factors in the normal and degenerating retina are essential for gaining insights and devise potential methods for systemic delivery of MSCs. Some studies have also recommended delivering MSCs with specific cocktails of growth factors or genetic modification for increased neurotrophic factors [8]. However, it must be noted that some conditions such as glaucoma may not be conducive for such modifications, since higher than optimal level of neurotrophic factors can lead to apoptosis in these conditions, owing to the increased expression of low-affinity proapoptotic neurotrophic factor receptors.

In an open-label phase 2a proof-of-concept clinical trial in patients with secondary progressive multiple sclerosis with visual pathway involvement, [27] found that the intravenous administration of autologous bone marrow-derived MSCs improved several neuro-ophthalmic parameters including visual acuity and visual-evoked responses. The clinical trial suggested that the procedure was safe and led to visual improvement in subjects owing to some neuroprotection [27].

8.7 Embryonic/Fetal Stem Cells

8.7.1 Neural Stem Cells

Neural stem cells are self-renewing multipotent cells that give rise to neurons, glia, and oligodendrocytes. Several studies have reported isolation and culture of neural stem cells, and they vary to a great extent with respect to the source of the neural stem cells and the culture protocols [46, 53, 134, 162]. Although listed under embryonic or fetal stem cells for the purpose of this chapter, neural stem cells have been identified in both fetal and adult forebrain structures. Initial research established the

possibility of isolation of neural stem cells from the adult hippocampus and maintaining them in long-term cultures without compromising their self-renewal and differentiation properties [4, 5, 44, 45, 113]. These cells have been shown to differentiate into retinal neurons in the presence of specific growth factors and culture matrices. In addition, NSCs, when transplanted into the neonatal fetal retina, have been shown to integrate into the retinal tissue and expressed pan-neuronal and glial markers [71, 99, 156]. However, these cells did not show a retinal morphology nor they expressed retinal markers. Another study showed that subretinal transplantation of the NSCs led to an efficient migration of cells to nerve fiber layer as well as the optic nerve. The study also showed that these NSCs were capable of penetrating the lamina cribrosa layer of the optic nerve [54].

However, in one study, the intravitreal transplantation of NSC after optic neuropathy did not improve the retinal function even though the transplanted cells differentiated and integrated into the inner retinal layers [52]. This observation was confirmed by another study which utilized a mice model lacking RGC which showed that the transplanted NSCs integrated well and also expressed β III-tubulin+ markers, however, did not form mature and functional RGCs [101].

A study by [99] on subretinal transplantation of human fetal neural stem cells isolated from the brain of medically terminated fetuses of 16–20 weeks gestation into RCS rats showed that the cells migrated and survived in the retina [99]. In addition, the procedure revealed substantial improvement in the visual parameters. Analysis of retinal histology showed significant improvement in the treated eyes. It is confirmed that the transplanted cells do not transdifferentiate into retinal phenotypes; however, it is hypothesized that the transplanted cells could provide trophic support thereby preventing further retinal degeneration. It was also suggested by further studies that the protection of the photoreceptors could be orchestrated by NSC-directed phagocytosis of photoreceptor outer segments, which is usually compromised due to RPE degeneration in the RCS rats [28] and also by the induction of CNTF expression by Muller glia [87].

The commercial version of the neural stem cells has been developed by StemCells Inc. (Newark, CA, USA) and called as HuCNS-SC. Recently, a clinical trial has been initiated by StemCells Inc. in partnership with the Retina Foundation of the Southwest (Dallas, TX, USA) for dry AMD. In this clinical trial, the HuCNS-SC is provided as a subretinal injection in patients with geographic atrophy secondary to AMD. The study has been completed and the results are yet to be published.

8.7.2 Fetal Retinal Progenitor Cells

Retinal progenitor cells are multipotent progenitors that are isolated from dissociated embryonic or neonatal retina. These cells have the potential to give rise to almost all the retinal cells. Several studies have been carried out with transplantation of fetal retinal tissues either as whole retinal sheets or as dissociated cells in various animal models [33, 85, 88, 141]. Almost all the studies have found that the fetal retinal tissue can successfully engraft and can differentiate into photoreceptors.

Some studies have shown spontaneous ganglion cell activity posttransplantation of fetal retinal tissue in retinal degeneration model which is attributed to the partial functional integration of graft with host retina [127–129]. However, unlike the animal studies, the studies on humans with retinitis pigmentosa did not show evidence of visual improvement post-fetal retinal tissue transplants. This was mainly attributed to the failure of the graft to survive in the host retina for an appropriate period of time and also the inefficient transplantation methods involved in the whole fetal retinal transplant.

8.7.3 Embryonic Stem Cells

Embryonic stem cells are pluripotent stem cells that are derived from the inner cell mass of the blastocysts. These cells have the potential to self-renew indefinitely for a longer period and also can theoretically differentiate into almost all the cells that exist in the body except for the extraembryonic tissues. One of the most important advantages of the embryonic stem cells over other multipotent stem cells is that these cells can be coaxed to differentiate into specific lineages by recapitulating normal developmental mechanisms. Most of the differentiation protocols involving the ESCs are designed based on the knowledge and information available in developmental biology. With respect to the retinal tissue, the protocols involve activation and inhibition of signaling pathways known to be modulated during early optic cup development. In most protocols, the ESCs are differentiated in the presence of inhibitors of Wnt and BMP/Nodal pathways to orient the cells toward cells that are akin to the progenitors found in the optic cup. Culturing the cells in the presence of DKK1 (Wnt inhibitor) and Lefty (Nodal inhibitor) conditions seems to propel the ESCs toward early retinal progenitor phenotypes which express most eye field genes such as Pax6, Rx, Six3, Six6, Lhx2, and Chx10 [110, 178]. In some studies, the inclusion of insulin-like growth factors (IGF) seems to increase the efficiency of this differentiation [82]. In addition, studies have also emerged, where the recombinant endogenous inhibitors and growth factors are replaced with small molecules modulating the signaling pathways thus making the process less expensive and clinically more feasible [111]. Interestingly, Meyer et al. devised a strategy which did not employ any signaling pathway modulators included in the culture. This protocol involved culturing human ESCs (hESCs) in the absence of bFGF for promoting its differentiation and involved manual selection of optic vesicle-like embryoid bodies and their preferential differentiation toward retinal cells [102].

This study proved the fact that cells within the embryoid bodies during differentiation modulate the original developmental signaling pathways to instruct retinal differentiation which can be enriched by manual selection without the need for any extraneous factors. Although different protocols showed that the retinal progenitors can be efficiently generated from the ESCs and also proved that these retinal progenitors in the presence of specific factors can give rise to mature retinal cells such as photoreceptors, RGCs, and RPE, it was in 2011 that a study showed that a three-dimensional retinal tissue can be generated in a culture dish. Using an ultralow

adhesion round-bottomed, 96-well plate and Matrigel as cell matrix, [36] showed that ESCs could form clusters or aggregates that autonomously generate retinal primordial structures. These aggregates in long-term cultures could form stratified neural retinal tissue akin to that seen *in vivo* during retinal development. This study provided the first evidence for the possibility of *in vitro* retinogenesis from ESCs [36].

Several studies have fine-tuned the protocols for differentiating hESC-derived retinal progenitors to RPE and other retinal neural cells such as rod and cone photoreceptors and RGCs [6, 18, 21, 49, 56, 57, 65, 76, 81, 89, 97, 175]. Among these, ESC-derived RPE cells are the best characterized. In most studies, the hESC-derived RPE had global genetic profiles similar to fetal RPE cells with all the functional attributes established by *in vitro* assays such as rod outer segment phagocytosis, PEDF and VEGF secretion, and analysis of activities of the enzymes involved in visual cycles. In addition, several preclinical studies established that subretinal transplantation of hESC-derived RPE significantly improves the visual acuity in a variety of retinal degeneration models such as RSC rats (model of AMD) and *Evol4* and *ABCA4* knockout mice (models of Stargardt disease) [138]. The most recent clinical trial establishes subretinal transplant of hESC-derived RPE to be safe and effective for AMD and Stargardt disease (expanded under clinical trials section).

With respect to the rod and cone photoreceptors, although, several studies have reproducibly observed that *in vitro* differentiation of these cells from hESCs is possible; however, very few studies have shown that hESC-derived photoreceptor precursors survive, migrate, and integrate into retinal degenerative models. Most studies have found limitations with respect to the outer segment formation of the photoreceptors and with their synaptic connections. It is also suggested that the success of transplantation may depend on the specific stage of the differentiated photoreceptors [92] and also on the recipient host retinal environment [51, 118, 119, 169, 170].

Although these studies demonstrate the feasibility of hESC-based retinal cell transplantation for retinal degenerative diseases, the use of hESCs poses significant limitations that need to be addressed. As already stated, hESCs is confounded with ethical issues and immunogenic barrier, which has been recently addressed by a different set of stem cells which are reprogrammed from somatic stem cells – the induced pluripotent stem cells (iPSCs).

8.8 Reprogrammed Cells

8.8.1 Induced Pluripotent Stem Cells

In 1962, the study by Sir John Gurdon provided the first evidence that fully mature somatic cells could be reprogrammed to their earlier developmental stage. In this experiment, the nucleus of the egg of the frog was replaced with the nucleus of a mature intestinal cell, which allowed normal tadpole to develop [55]. This provided the proof that the major factors responsible for the somatic reprogramming are present in the cytoplasm of the oocyte. In addition, isolation and maintenance of ESCs

by [96] and subsequent work by others on the molecular biological aspects of ESCs allowed the possibility of establishing the factors that are involved in the maintenance of ESCs in the pluripotent state [96]. Based on the abovementioned studies, in 2006, nearly 42 years later, Takahashi and Yamanaka hypothesized that introduction of the factors that are involved in maintaining the ESCs in a pluripotent state might also be capable of reprogramming somatic cells into a pluripotent state. They initiated the study with a set of 24 factors and, by careful titration of different combinations, identified that a combination of 4 factors Oct4, Sox2, Klf4, and cMyc (now addressed as Yamanaka factors) is sufficient to induce pluripotency in adult fibroblasts. These somatic cells which are reprogrammed are named induced pluripotent stem cells (iPSCs) [155]. These reprogrammed cells are similar to ESCs in the fact that they can theoretically be differentiated into any type of cells following the same protocols as established for the ESCs. However, they do not have the limitations that are associated with the hESCs, such as the ethical issues and immune rejection. The iPSCs showed morphology similar to ESCs, express pluripotent and other cell surface markers, and exhibit similar genetic and epigenetic profiles.

Several studies have emerged in the field of iPSC pertaining to different sources of somatic cells that are amenable to reprogramming and also in terms of the methods employed for delivering the pluripotency factors. These studies confirmed that the iPSCs can be reproducibly generated from almost all kinds of somatic cells including complex cells such as lymphocytes and primordial germ cells [11, 17]. In addition, several methods of reprogramming such as viral/nonviral, integrative/non-integrative, and mRNA/protein-based protocols have been developed for delivering the pluripotent factors into the somatic cells. Of these, skin tissues and peripheral blood have been reported in numbers much higher than other sources. Recently, non-integrative approaches such as episomal plasmid-based delivery and Sendai viral-based methods have been widely employed as reprogramming method of choice [9, 43, 91, 94, 140].

With respect to the retinal differentiation, several studies have employed the protocols already established in ESCs to aid retinal differentiation with iPSC cells. Most of the studies provide evidence that iPSCs have potential similar to ESCs in terms of retinal cell differentiation. The reports with regard to differentiation of iPSC-derived retinal progenitors toward RPE, photoreceptors, and retinal ganglion cells are encouraging. [22, 114] have established the possibility of differentiating iPSCs to RGCs [22, 114]. While the former studies utilize an approach wherein the environmental cues that promote normal RGC histogenesis are adapted in RGC differentiation, the latter study utilized overexpression of exogenous transcription factor MATH5 to achieve the differentiation. Nonetheless both the studies provide substantial proof that iPSCs can be used as an autologous source for generating RGCs. The study by [115] also revealed that in addition to expressing the differentiation markers, the iPSC-derived RGCs when transplanted intravitreally migrate and integrate into the GCL layers of a rat model of glaucomatous neuropathy [115]. In addition, the study also provided substantial evidence that these iPSC-derived cells do not cause teratoma, establishing the safety of the procedure. Transplantation of iPSC-derived RPE and photoreceptors is also well documented and shows safety

and efficacy profiles similar to those observed with the ESCs. Recently, a clinical trial utilizing iPSC-derived RPE cells has been initiated in Japan for treating patients with AMD.

8.9 Stem Cell-Based Clinical Trials in Retinal Degenerative Diseases

A data search for stem cell-based clinical trials in retinal degenerative diseases in the clinical trials registry databases of the National Institute of Health (<https://clinicaltrials.gov/>), European Medicines Agency (<https://www.clinicaltrialsregister.eu>), Indian Council of Medical Research (<http://www.ctri.nic.in/>), and Japanese Ministry of Health (<http://www.umin.ac.jp/>) reveal that there are at least 37 clinical trials registered (Table 8.2). Thirty-two of the 37 clinical trials are either ongoing or completed. Figure 8.3 shows the distribution of these clinical trials in relation to the source of stem cells that are transplanted and the disease of interest. hESC-RPE and BMSCs are the most predominant source of stem cells, and macular degeneration and retinitis pigmentosa are the most common diseases where the stem cell-based clinical trials are currently being conducted.

The US Food and Drug Administration granted orphan drug designation to hESC-derived RPE cells for Advanced Cell Technology, Inc. (Santa Monica, CA, USA) in 2010, for initiating the phase 1/2 clinical trials to treat patients with SMD. In addition, in 2011, an approval in same lines was received from the committee for orphan medicinal products of the European Medicines Agency. The results of this clinical trial have been recently published [139]. This study provided the first evidence of long-term safety and graft survival in dry AMD and SMD patients who underwent subretinal transplantation of hESC-RPE. The study reported no serious adverse events that could directly be associated with the hESC-RPE. The common drawbacks attributed to transplantation of cells derived from hESC such as teratoma formation, immune rejection, and risk of differentiation into unwanted cellular phenotype were not noted in the study. However, the authors report complications with systemic immunosuppression in older patients and recommend modified immunosuppressant protocols. Although the predominant aim of the study was to establish the safety of the procedure, the authors also reported that they could appreciate a visual improvement in 8 out of 18 patients. Overall, the study suggested that the subretinal transplantation of hESC-RPE was relatively safe and was effective in the cohort tested.

This study was received more positively, as reflected by the news and commentaries published. However, the following concerns were raised on the outcome:

1. The true visual improvement posttransplantation versus the spontaneous improvement during the course of the disease must be distinguished.
2. Insufficient size of the cohort (9 AMD and 9 SMD) to draw either safety or efficacy of the treatment.
3. Actual duration and dose of the immunosuppressive regimen.

Table 8.2 Stem cell-based clinical trials for retinal diseases registered under FDA/CTRI/Japanese Ministry of Health

Source cell	Funding source	Location	Ocular condition	Route of administration	Status	Published	Identifier
ADSC	CCHOHCBA PRF	Russia	Glaucoma	Sub-Tenon	Ongoing	No	NCT02144103
ADSC	Bioheart Inc.	USA	AMD (dry)	Intravitreal	Withdrawn	No	NCT02024269
BMMNCs	Chaitanya Hospital	India	RP	Not provided	Ongoing	No	NCT01914913
BMSC (autologous)	AIIMS	India	AMD (dry) and RP	Intravitreal	Ongoing	No	CTRI/2010/091/000639
BMSC (autologous)	Al-Azhar University	Egypt	AMD	Intravitreal	Ongoing	No	NCT02016508
BMSC (autologous)	Retina Associates of South Florida, Al Zahra Hospital, Dubai	USA, UAE	Retinal disease; AMD; hereditary retinal dystrophy; optic nerve disease; Glaucoma	Retrolubar; Sub-Tenon intravenous intravitreal intraocular	Ongoing	No	NCT01920867
BMSC (autologous)	University of Sao Paulo	Brazil	Glaucoma	Intravitreal	Ongoing	No	NCT02330978
BMSC (autologous)	University of Sao Paulo	Brazil	RP	Intravitreal	Completed (phase 1)	No	NCT01068561
BMSC (autologous)	University of Sao Paulo	Brazil	RP	Intravitreal	Completed (phase 2)	[142]	NCT01560715
BMSC (autologous)	University of Sao Paulo	Brazil	AMD and SMD	Intravitreal	Ongoing	No	NCT01518127

BMSC (autologous)	University of Sao Paulo	Brazil	Ischemic retinopathy	Intravitreal	Ongoing	No	NCT01518842
BMSC (autologous)	Clinical University Hospital	Spain	RP	Intravitreal	Ongoing	No	NCT02280135
BMSC (autologous)	Stem Cells Arabia	Jordan	RP	Intravitreal	Ongoing	No	NCT02709876
BMSC (autologous)	University of California	USA	Retinopathy	Intravitreal	Ongoing	No	NCT01736059
hESC-RPE	AIRM ^a	UK	SMD	Subretinal	Completed	[137, 139]	NCT01469832
hESC-RPE	AIRM ^a	USA	SMD	Subretinal	Completed	[137, 139]	NCT01345006
hESC-RPE	AIRM ^a	USA	SMD	Subretinal	Ongoing	[137, 139]	NCT02445612
hESC-RPE	AIRM ^a	USA	AMD (dry)	Subretinal	Ongoing	[137, 139]	NCT01344993
hESC-RPE	AIRM ^a	USA	AMD (dry)	Subretinal	Ongoing	[137, 139]	NCT02463344
HESC-RPE	CHA Biotech	Korea	SMD	Subretinal	Ongoing	[145]	NCT01625559
hESC-RPE	CHA Biotech	Korea	AMD (dry)	Subretinal	Ongoing	[145]	NCT01674829
hESC-RPE	Southwest Hospital	China	AMD and SMD	Subretinal	Ongoing	No	NCT02749734
hESC-RPE	Chinese Academy of Sciences	China	AMD (dry)	Subretinal	Ongoing	No	NCT02755428
hESC-RPE	Cell Cure Neurosciences Ltd.	Israel	AMD (dry)	Subretinal	Ongoing	No	NCT02286089
hESC-RPE	Pfizer, UCL	UK	AMD (wet)	Subretinal	Ongoing	No	NCT01691261
hESC-RPE	University of California, Los Angeles	USA	Myopic macular degeneration	Subretinal	Withdrawn	No	NCT02122159
hRPC	ReNeuron Ltd.	USA	RP	Subretinal	Ongoing	No	NCT02464436
hRPC	jCyte Inc.	USA	RP	Intravitreal	Ongoing	No	NCT02320812

(continued)

Table 8.2 (continued)

Source cell	Funding source	Location	Ocular condition	Route of administration	Status	Published	Identifier
HSC (autologous)	University of Calgary	Canada	Neuromyelitis optica	Intravenous	Ongoing	No	NCT01339455
HUCNS SC	Stem Cells Inc.	USA	AMD (GA)	Subretinal	Completed	No	NCT01632527
HuCNS SC	Stem Cells Inc.	USA	AMD (GA)	Subretinal	Terminated	No	NCT02467634
HuCNS SC	Stem cells Inc.	USA	AMD (GA)	Subretinal	Terminated	No	NCT02137915
iPSC-RPE	Riken	Japan	AMD (wet)	Subretinal	Ongoing	[47]	UMIN000011929
MSCs (autologous)	Tianjin Medical University	China	Neuromyelitis Optica	Intravenous	Ongoing	No	NCT02249676
MSCs (autologous)	Mahidol University	Thailand	RP	Intravitreal	Ongoing	No	NCT01531348
UTSC	Janssen Research & Development	USA	AMD (GA)	Subretinal	Ongoing	No	NCT01226628
UTSC	Centocor Inc.	USA	RP	Subretinal	Terminated	No	NCT00458575

Abbreviations: FDA Food and Drug Administration, *CTRI* Clinical Trials Registry India, *CCHOHCBA* PRF Central Clinical Hospital with Outpatient Health Center of Business Administration for the President of Russian Federati, *AIMS* All India Institute of Medical Sciences, *ARM* Astellas Institute for Regenerative Medicine, *UTCL* University College London, *USA* United States of America, *UAE* United Arab Emirates, *UK* United Kingdom, *AMD* age-related macular degeneration, *RP* retinitis pigmentosa, *SMD* Stargardt macular degeneration, *GA* geographic atrophy, *NCT* National Clinical Trial, *UMIN* University Medical Information Network

^aPreviously known as Advanced Cell Technology Inc. and Ocata Therapeutics

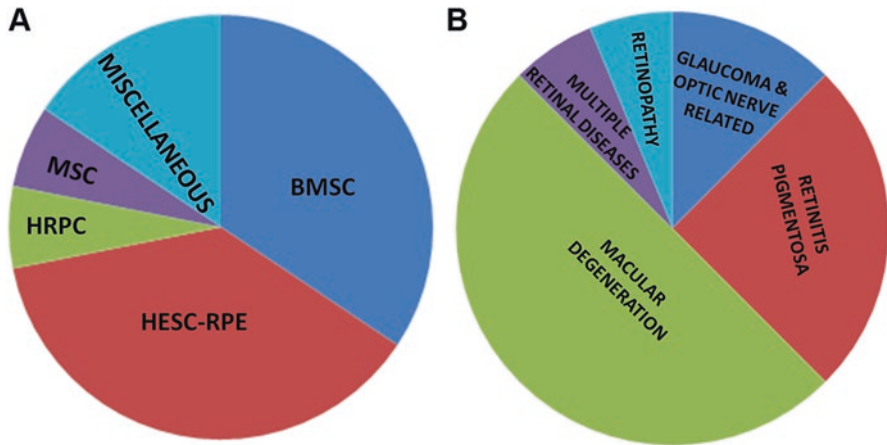


Fig. 8.3 Graphical representation of the distribution of the clinical trials. (a) Distribution in relation to the source of stem cells. (b) Distribution in terms of retinal diseases

- The need and possibility of transplanting RPE along with photoreceptors for actual visual improvement have been suggested, since most photoreceptors degenerate during the course of disease which might dampen the visual improvement parameters.

Sunness [154] suggested that microperimetry and low-vision training should be performed before the trial to evaluate the actual visual improvement due to the sub-retinal transplantation of hESC-RPE [154]. The authors of the clinical trial, although agreed with the potential of microperimetric analysis concluded that the microperimetric analysis would not have changed the results [137]. In addition, they agreed on other points provided in the commentary by [176] and its incorporation in future clinical trials will provide an unbiased and improved assessment of the treatment [137, 176]. A similar clinical trial was also initiated in South Korea by CHA Biotech (Seoul, South Korea) in collaboration with Ocata Therapeutics, and the results obtained in this study confirmed the earlier findings that hESC-RPE transplantation was safe without any evident adverse events [145].

Other prominent clinical trials with reported outcome are available on the safety and efficacy of intravitreal injection of autologous BMSCs in a range of retinal diseases, including advanced RP [116, 144] dry AMD, SMD, and retinal vascular occlusions. The first clinical study was reported from the University of Sao Paulo, Brazil, by [144] wherein the safety and beneficial effect on RP-associated macular edema were assessed. The trial suggested that the procedure was safe; however, the visual parameter did not improve significantly. This study provided an impetus for the phase 2 study incorporating questionnaire-based evaluation of a cohort of 20 patients. The phase 2 study revealed an improvement in the quality of life of the patient; however, the effect was transient and was not evident after 1 year of injection [142–144]. The second clinical study utilizing BMSC for retinopathy was

carried out at the University of California, Davis. The preliminary clinical findings from the phase 1 patients was published recently. This study which had the results of six patients with 6 months follow-up [116] reported that the autologous BMSCs were well-tolerated post-intravitreal injection. In addition, the treated eye showed evidence of improvement over the baseline. Similar, to the study by the University of Sao Paulo, the effect was transient in this trial as well.

8.10 Other Imminent Clinical Trials

In Japan, the first clinical trial utilizing subretinal transplantation of iPSC-RPE in wet AMD was initiated in 2014. This was the first use of iPSC technology in a clinical setting after the regulatory approval by the Ministry of Health, Japan. The iPSC-RPE cells were transplanted as sheets of 1.3–3.0 mm into the subretinal space, without any surgical complications. Recently, a report published [47] revealed that the trial was suspended owing to the regulatory change in the clinical trial and suggested that the trial protocol is revised to use allogeneic iPSC-RPE cells for evaluation. It added that this change in the regulation might have been brought about in wake of the identification of mutations in the iPSC lines generated and its confirmation by whole genome sequencing that these mutations were acquired during the reprogramming process. It is stated that the Center for iPSC Cell Research and Application (CiRA) at Kyoto University, Japan, is creating a bank of iPSCs from HLA-typed peripheral blood and cord blood samples from normal individuals after obtaining their consent. These iPSC lines will serve as a renewable and well-characterized source of cells which will be differentiated into the cells of interest and transplanted after matching the patient's major HLAs which determines the transplant rejection. It is assumed that the RPE differentiated from the HLA-matched allogeneic iPSCs could be used with immunosuppressive therapy which would provide an advantage over the hESC-RPE. In addition, it will also eliminate the issues of genetic stability and the expense involved in generating and characterizing iPSCs from all the patients. In addition to the abovementioned trial, regulatory approvals have been provided for a number of related trials utilizing hESC-RPE for AMD, namely, the London Project (London, UK) and California Project to Cure Blindness (USA).

8.11 Hype About Stem Cell Treatments: Blooming Stem Cell Clinics

Although several clinical trials are ongoing, none of these are approved stem cell-based therapies for retinal degenerative diseases. The realm of the stem cell trials has been besieged by the hype created from the predicted gains to patients desperately looking for a cure for otherwise incurable diseases. In addition, the profit motive companies and clinicians associated with these companies have provided additional thrust even though the reproducibility in terms of safety and efficacy has not been

established. The community expectations have added to the woes by allowing these companies and clinicians to propel the hype further. A quick search on the internet reveals information on clinics throughout the world offering unscientific stem cell treatments. Several news articles refer to the stem cells as “magic bullets” or “only hope,” and this has created unquestionable faith in the potential of stem cells in the public which is exploited by these clinics. Almost there is no clinical evidence available on the effect of the treatments offered, and the results are published as patient testimonials. The analysis of individual patient testimonials is difficult since improvement cannot be directly attributed to the treatment since almost any disease has a variable course. So, it is an absolute need that a clinical trial is conducted for all these new treatments with a large number of patients to make sure that they are really effective even before considering them as a treatment option. In addition, any untoward incidence associated with the trials is reported without any bias.

8.12 Conclusion

Currently, stem cell-based therapies for retinal degenerative diseases are on the cusp between preclinical research and phase 1/2 clinical trials. Although the approaches involving endogenous activation of innate stem cells/progenitors in the degenerating retina are ongoing, its progress in the level of human studies is slow compared to the cell replacement therapies involving transplantation of exogenous stem cells. As already discussed, several clinical trials are ongoing, and the results of these are eagerly awaited in terms of their safety and efficacy. In addition, the requirement of an immunosuppression regimen versus an autologous approach, specific cell replacement versus paracrine effect, and the best route of administration needs to be compared to arrive at the best treatment options. Immense hope has been created by the success of the ongoing clinical trials on the therapeutic application of stem cells for retinal disease. An unbiased assessment of the results needs to be communicated, and an awareness must be created among the professionals in the ophthalmology field on institutions that might commercialize unproven stem cell therapy, who can then counsel their patients on the real state of the science.

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