Age-related macular degeneration (AMD) leads to progressive loss of central vision in the elderly. At a cellular level, there is aging of the retinal pigment epithelial (RPE) cells, and accumulation of lipofuscin that interferes with the proper functioning of RPE which eventually leads to apoptosis. Treatment depends on the stage of the disease. Wet AMD which has neovascularization is managed by local therapies such as laser photococoagulation and photodynamic therapy and is managed with injections of antivascular endothelial growth factor-based therapy. Unlike the wet AMD, an effective therapy does not exist for dry AMD and geographic atrophy. Cell replacement therapy has shown promise. This review discusses the opportunities in the various types of cell-based therapy, their limitations, and what is possible for India.

Key words: Age-related macular degeneration, embryonic stem cells, retinal pigment epithelial, stem cells

Age-related Macular Degeneration

Age-related macular degeneration (AMD) leads to progressive loss of central vision. AMD was believed to be a leading cause of blindness in elderly population only in the Western countries. However, a recent study suggested that the prevalence of AMD is similar in India.[1] AMD is classified into dry (nonexudative) and wet (exudative) AMDs. The dry AMD is more common (85%) than the wet AMD, with 10% of the dry forms progressing to wet AMD. AMD is believed to be caused by the primary failure of retinal pigment epithelial (RPE) cell functions.[2] RPE plays an important role in the survival of photoreceptor. However, during the aging process, the RPE undergoes senescence and its dysfunction leads to accumulation of lipofuscin. N-retinylidene-N-retinylethanolamine (A2E) is a major component of lipofuscin that interferes with the proper functioning of RPE and its apoptosis. The loss of RPE subsequently leads to the development of geographic atrophy (GA).[3] Amorphous deposits containing lipids and proteins called drusen accumulate between RPE and Bruch’s membrane (BM). The presence of drusen has been attributed to increased risk of AMD development. In addition, the drusens lead to the detachment of RPE from BM, which is considered to induce choroidal neovascularization (CNV) observed in wet AMD.[4]

Strategies for Management of Age-related Macular Degeneration

Preventive strategies

Since AMD is a multifactorial disease, which has both genetic and environmental components, the patients are stratified based on the known risk factors. Those individuals who have risk alleles in genes such as complement factor H, ARMS2, and vascular endothelial growth factor A (VEGFA) have been predicted to develop AMD compared to those who do not carry the risk alleles. In addition, smoking and diet low in antioxidants have been attributed to the progression of AMD in individuals with known risk alleles. Hence, cessation of smoking and diet rich in antioxidants have been advocated as preventive measures in high-risk individuals.[5,6] The results of the studies on the efficacy of antioxidant therapy have been controversial. A recent review suggested that there is no role for antioxidants in AMD progression.[7] However, the age-related eye disease study suggested an increased reduction in risk of AMD progression in individuals consuming antioxidant and mineral supplements.

Treatment strategies for age-related macular degeneration

Several treatment strategies are available for treating wet AMD. The strategies that are widely employed as therapy for wet AMD include laser photococoagulation,[8-11] photodynamic
therapy,[12,13] and anti-VEGF therapy.[14-21] However, there is no effective therapy that exists with regard to dry AMD and GA. Several strategies are being attempted for treating dry AMD and GA such as drugs involved in reducing or blocking drusen formation,[22-24] reducing and eliminating inflammation.[25-27]

Cell Replacement Therapy as a Suitable Alternative

The initial surgical procedure for replacing diseased macula involved 360° retinal rotation to translocate the macula.[20] This surgery was always complemented with strabismus surgery to counterrotate the globe. The surgery resulted in reasonable visual outcome. However, complication of proliferative vitreoretinopathy was noted in 8%–18% of the cases. The recurrence of the disease in RPE was noted in new foveal regions owing to compromised photoreceptor function in the region in most cases of GA.[20] Recently, transplantation of RPE cells has emerged as a suitable alternative to conventional therapies.

Points to be Considered during Retinal Pigment Epithelial Transplantation

The success of RPE transplantation depends on the following conditions:

i. A renewable source of RPE cells
ii. Surgical method for transplanting RPE cells in subretinal region
iii. Survival and functioning of RPE in the transplanted region
iv. Restoration of retinal architecture
v. Improvement of visual acuity.

Hence, the most important criteria for successful RPE transplantation are the source of RPE cells.

Source of Retinal Pigment Epithelial Cells for Transplantation

The RPE cells in the adult retina are postmitotic and hence cannot be utilized for transplantation purposes. The ideal characteristics of cells for transplantation are as follows:

(i) Available in sufficient numbers, preferably renewable,
(ii) autologous or less immunogenic, and
(iii) ethical and less complex.

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 have been used in one of the two therapeutic strategies: (i) Source of neurotrophic support and (ii) source of functional cells during cell replacement therapies.

Source of Neurotrophic Support

Neural stem cells

Neural stem cells (NSCs) are multipotent cells isolated from both adult and fetal brain tissues that can give rise to neurons, glia, and oligodendrocytes. Transplantation of the NSCs isolated from aborted fetuses of 16–20 weeks of gestation into the subretinal space of postnatal day 21 Royal College of Surgeons (RCS) rats revealed that the transplanted cells could survive for at least 7 months in the retina without any evidence of tumor formation.[30] In addition, the treatment revealed sustained visual acuity and luminance sensitivity improvement. Further studies in this line confirmed that the transplanted cells rescue the degenerating cells predominantly by providing trophic support and do not transdifferentiate into retinal phenotypes. In addition, NSC-directed phagocytosis of photoreceptor outer segments has also been contemplated as the reason for the increased visual acuity.[31] Recently, human central nervous system stem cell (HuCNS-SC), a Current Good Manufacturing Practices (cGMP) compliant adult NSC, has been developed by Stem Cells Inc. (Newark, CA, USA) along with HuCNS-SC which has been developed by the Retina Foundation of the Southwest (Dallas, TX, USA). Recently, a clinical trial with HuCNS-SC was conducted on patients with GA secondary to AMD and the results are awaited.

Adult mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent stem cells initially derived from bone marrow. Currently, several adult as well as fetal tissues have been shown to harbor these stem cells.[32-45] Of the available sources, bone marrow-derived and human umbilical tissue-derived MSCs have been used in treating outer retinal degenerations in animal models. Studies involving subretinal as well as intravitreal transplantation of bone marrow-derived MSCs showed increased survival of RPE and photoreceptors with sustained retinal function in treated versus the sham-treated control in RCS rats and mouse model of retinitis pigmentosa (RP).[46,47] A study by Wang et al. suggested that bone marrow-derived MSCs injected through intravenous route have the ability to home into the sites of retinal injury.[48] A study on mouse model of laser-induced CNV suggested that MSCs that are genetically modified to secrete pigment epithelium-derived factor were shown to be a source of long-term antiangiogenic factor, thus providing an alternative for repeated intravitreal injections of anti-VEGF in wet AMD-associated CNV.[49] Another source of MSC that has been proven to be effective in the RCS rats is from human umbilical tissue. Subretinal injection of the umbilical tissue-derived MSCs revealed better visual outcomes in the treated eye compared to the control.[50] It is hypothesized that MSCs, like in other neural degenerations, have paracrine effect which rescue the degenerating cells. These findings subsequently led to a Phase I/II clinical trial by Janssen Research and Development (Philadelphia, PA, USA) utilizing cGMP compliant cell line of human umbilical tissue-derived stem cells (CNTO 2476). In this clinical trial, the cells were delivered to the subretinal space using a catheter-based delivery system. The results are yet to be published.

Bone marrow-derived hematopoietic stem cells or bone marrow stem cells

Li et al. showed that sodium iodate-induced RPE damage in C57Bl6 mice led to an active migration of bone marrow stem cell (BMSC) to the subretinal space and it was hypothesized that these BMSCs can orchestrate retinal repair.[51] Atmaca-Sonmez et al. showed that green fluorescent protein (GFP)-labeled allogeneic BMSC delivered intravenously into the mouse model of sodium iodate-induced RPE damage, migrated and survived in subretinal spaces with loss of RPE sparing the normal subretinal sites. It was suggested that these GFP-positive cells also expressed RPE65 an RPE-specific marker but did not show morphological or functional attributes of RPE.[52] There are at least four clinical trials that are ongoing utilizing allogeneic BMSCs to treat AMD [Table 1]. The results of these studies are awaited.
**Source of Functional Cells during Cell Replacement Therapies**

**Human fetal retinal pigment epithelial**

The first RPE transplant in AMD patients involved human fetal RPE cultured *in vitro*. The *in vitro* cultured human fetal RPE cells were transplanted as a patch into the subretinal space under the retina. However, 50% of the patients lost their vision after surgery. In another case report, a patient underwent RPE transplantation with human fetal RPE cells cultured *in vitro*, although capable of phagocytosis of rod photoreceptor outer segments, is considered to lack enzymes involved in retinoid visual cycle. Global gene expression revealed significant similarity to human fetal RPE. In both scenarios, the transplantation led to rejection of graft and no significant visual improvement.[60] In the pursuit of autologous cells for transplantation, iris pigment epithelial (IPE) cells obtained by peripheral iridectomy surgery was expanded *in vitro* in culture followed by subretinal transplantation of the cells. The results showed visual acuity improvement in approximately 80% of the patients with minimal complications. However, the procedure of obtaining IPE cells itself was considered to be complicated, and the IPE cells *in vitro* are not capable of phagocytosis. In the study, it was established that introduction of the pluripotency factors, namely, Oct4, Sox2, Klf4, and cMyc, is sufficient to induce pluripotency in somatic cells. The protocols for deriving iPSCs from somatic cells are currently employed to generate hESC-derived RPE cells for the treatment of AMD. The recent clinical trials utilizing hESC-derived RPE cells are in progress worldwide.

**Induced pluripotent stem cells**

hESCs, although renewable and has the potential to differentiate into RPE, suffer from limitations such as immunogenicity and related ethical issues. In 2006, the autologous and ethical source of pluripotent stem cells was discovered by Takahashi and Yamanaka. In this study, it was established that introduction of the pluripotency factors, namely, Oct4, Sox2, Klf4, and cMyc, is sufficient to induce pluripotency in somatic cells. The cells that are reprogrammed through the pluripotency factors are referred to as induced pluripotent stem cells (iPSCs). These reprogrammed cells are shown to be similar to ESCs with respect to their morphology, immunocytochemical, and differentiation properties. The global genetic profiles of these cells are mostly similar to hESCs. However, they do not have the limitations that are associated with the hESCs, such as the ethical issues and immune rejection. Various sources of cells including peripheral blood monocytes, NSCs, and primordial germ cells have been used for reprogramming. Both viral-based and nonviral strategies have been widely employed. The nonintegrative strategies by means of Sendai viruses and episomal vector transfection are currently employed to generate iPSC lines. With respect to the protocols for deriving RPE from the iPSC lines, several studies have successfully employed the protocols already established in hESCs on most iPSC lines. Almost all of these studies provide evidence that iPSCs have potential similar to hESCs in terms of RPE differentiation.
Clinical Trials Using Pluripotent Stem Cell-Derived Retinal Pigment Epithelial 

The clinical trials and their outcomes are shown in Table 1. The first clinical trial using hESC-derived RPE cells was performed by Advanced Cell Technology (Santa Monica, California, USA) in 2011. This Phase I/II clinical trial was carried out to understand the safety and efficiency of hESC-derived RPE transplantation on advanced dry AMD and Stargardt’s disease (clinical trial registration number: NCT01345006 and NCT01344993). The preliminary results of the clinical trial established that the subretinal injection of 5 x 10^6 hESC-derived RPE cells in two patients, one with dry AMD and the other with Stargardt’s disease, did not lead to teratoma or immune rejection. The clinical trial suggested that hESC-derived RPE cells are safe and lead to marginal improvement in visual acuity. Recently, the medium to long-term safety and efficiency of the clinical trial outcomes was published. Dose escalation study revealed that 1.5 x 10^6 RPE cells were well tolerated in most patients with increased visual acuity from 16 to 25 letters over a period of 3–12 months posttransplantation. Although the clinical trial did not lead to any adverse events, the use of immunosuppressive drugs has been looked into as one of the disadvantages of the procedure. A commentary by Zhang et al. suggested that future clinical trials should include optimization of dose and duration of immunosuppressive regimen necessary during the subretinal procedure and inclusion of a larger cohort to decipher both safety and efficacy of the procedure. In addition, Sunness, 2015, suggested that the patients who are recruited in the clinical trial should undergo microperimetry analysis and low-vision training before the subretinal procedure to authenticate the absolute visual improvement of the procedure and to remove the bias that can exist among the individuals. The authors recently published a reply indicating that the microperimetry analysis was in fact carried out on some patients and suggested that it did not lead to any significant difference in the reported results. However, they noted merits in the comments provided by Zhang et al. and agreed that the incorporation of the suggestions in future clinical trials will provide an unbiased assessment of the treatment. The findings of this clinical trial were reproduced and reported recently by an independent trial conducted in Korea.

The first clinical trial on utilizing iPSCs for AMD was initiated by Masayo Takahashi, RIKEN, Japan, in September 2014. However, the study was suspended in March 2015. The study reported mutations in the second patient’s iPSCs that were not detectable in the patient’s original fibroblasts. The mutations included three single-nucleotide variations and three copy-number variants. It is not definitely known whether the reprogramming process induced the iPSC abnormalities, although iPSCs often acquire mutations and epigenetic and chromosomal changes in culture. The current report on the trial revealed that the trial protocol was modified to utilize allogeneic iPSC cells instead of autologous iPSC lines. Toward this, an iPSC cell line bank is being created at the center for iPSC research and application at Kyoto University, Japan. These iPSC lines are being created from human leukocyte antigen (HLA) typed peripheral blood and cord blood samples. It is expected that the RPE differentiated from the HLA-matched allogeneic iPSCs could be relatively less expensive and could be used without immunosuppressive therapy, thereby being advantageous than the hESC-RPE.

Current Scenario on Cell-based Therapy for Retinal Degeneration in India

With respect to the clinical trials conducted for retinal degenerations in India, the first long-term safety study of transplanting human fetal neuroretinal cells was carried out by Dr. Taraprasad Das, from L.V. Prasad Eye Institute, India. In this Phase I clinical trial, fetal neuroretinal cells isolated from 14–18-week gestation were transplanted into subretinal space into 14 patients with advanced RP. A constant dose of cells (0.6 million cells in 150 µl) in suspension was injected into the subretinal space. The study did not observe any detrimental effect at least for 40-month posttransplant except for a retinal detachment in one patient. However, the status of the Phase II study is not known. Currently, a clinical trial is being carried out by the All India Institute of Medical Sciences, where patients with AMD and RP are treated with intravitreal injections of autologous bone marrow-derived stem cells under the Clinical Trial Registry of India CTRI/2010/091/000639. The results of this trial are yet to be published. With regard to the pluripotent stem cells, there are a few reports available on the transplantation of hESCs in several degenerative conditions and emphysematous chronic obstructive pulmonary disease (COPD) hESCs in the treatment of emphysematous COPD: A case report. These reports are rudimentary and inconclusive on the actual safety and efficacy of these pluripotent stem cells. Recently, Mariappan et al. established the feasibility of generating RPE cells from Indian hESC line BJNHem20. With respect to the use of iPSCs in ophthalmology, there has been a single study utilizing mouse iPSC to generate retinal progenitors and RPE. However, there are no reports available on the differentiation of human iPSC lines to retinal cells from India.

Conclusion: Toward Pluripotent Stem Cell-based Clinical Trials in India

Fig. 1 shows the step that needs to be taken toward clinical trials for AMD in India. Collective efforts from reputed ophthalmic institutes are required for feasibility of clinical
trials for AMD in India. Efforts are required to characterize hESC lines that are generated in India (BJNhem19 and 20 and KINDI and KINDII) for their potential to differentiate into RPE. In addition, preclinical studies on animal models of AMD are required to confirm the feasibility of the approach before the clinical trials on the patients become a reality [Fig. 1]. The alternative approach would be to join hands with Steven Schwartz of Advance Cell Technology and initiate the clinical studies in India through proper channel. This approach will hasten the clinical work in India as the earlier approach has some bottlenecks. The vital step of formulating the guidelines for conducting research and clinical trials utilizing pluripotent stem cells in India has already been clearly laid down by the Department of Biotechnology and the Indian Council of Medical Research which ensures ethical and unbiased evaluation of these procedures. With a clear regulatory guideline in place and progress made in the pluripotent stem cell-based research, we can expect the clinical trials to initiate in the near future.

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Conflicts of interest

There are no conflicts of interest.

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