Enhancing RPE Cell-Based Therapy Outcomes for AMD: The Role of Bruch’s Membrane

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Age-related macular degeneration (AMD) is the leading cause of legal blindness in older people in the developed world. The disease involves damage to the part of the retina responsible for central vision. Degeneration of retinal pigment epithelial (RPE) cells, photoreceptors, and choriocapillaris may contribute to visual loss. Over the past decades, scientists and clinicians have tried to replace lost RPE cells in patients with AMD using cells from different sources. In recent years, advances in generating RPE cells from stem cells have been made and clinical trials are currently evaluating the safety and efficiency of replacing the degenerated RPE cell layer with stem cell–derived RPE cells. However, the therapeutic success of transplantation of stem cell–derived RPE cells may be limited unless the transplanted cells can adhere and survive in the long term in the diseased eye. One hallmark of AMD is the altered extracellular environment of Bruch’s membrane to which the grafted cells have to adhere. Here, we discuss recent approaches to overcome the inhibitory environment of the diseased eye and to enhance the survival rate of transplanted RPE cells. Our aim is to highlight novel approaches that may have the potential to improve the efficacy of RPE transplantation for AMD in the future.

Introduction

Age-related macular degeneration (AMD) is the major cause of blindness in the elderly in the developed world. The disease is multifactorial and affects the macular region of the eye, which is responsible for central vision (Fig.). Changes to Bruch’s membrane and the choriocapillaris frequently occur as AMD develops and are associated with degeneration of the retinal pigment epithelium (RPE). Irreversible structural damage to other retinal layers may also occur as the disease progresses. Worldwide, 30 to 50 million individuals, and up to one-third of the people older than 75 have some form of AMD. The incidence of AMD is increasing in European countries, the United States of America, and Japan. Also, as life expectancy increases, the number of patients suffering from age-related diseases, such as Alzheimer’s and AMD, is likely to rise.

AMD can be classified into the dry and the wet form of the disease (Fig.). Dry AMD often results in gradual loss of central vision accompanied by atrophy of RPE cells. In wet AMD, new blood vessels from the choroid (choroidal neovascularization) may leak, resulting in macular edema and hemorrhage. Although the wet form of the disease only accounts for about 10% of total AMD cases, most available treatments target this form of the disease. Currently, the most widely used treatment for wet AMD involves administration of antibodies against vascular endothelial growth factor to prevent the formation of new blood vessels and to cause those already established to regress. Other available treatments include surgical excision of choroidal neovascular membranes, photodynamic therapy, and radiotherapy.

RPE cells, critical to the integrity of the outer retina, are often lost relatively early during the development of AMD. RPE cells transport nutrients from the choriocapillaris to the photoreceptors and
they phagocytose shed outer segments. They are also involved in maintaining relative immune privilege within the eye as part of the blood–retina barrier. RPE cells are subject to many stresses caused by the absorption of scattered light, and due to their phagocytic function. As RPE cells age, the efficiency of phagocytosis and subsequent recycling and degradation of waste declines and this may lead to a build-up of toxic waste that is deposited beneath the RPE cells on Bruch’s membrane. Together with other Bruch’s membrane abnormalities this may lead to the dysfunction and ultimately the death of the RPE cells. For patients with RPE loss due to AMD, but in whom the photoreceptors and choriocapillaris remain relatively intact, the possibility of transplanting healthy RPE cells to prevent secondary loss of photoreceptors and potentially keep or restore vision has received much recent attention.

**RPE Cell Transplantation in Animal Models**

Culture techniques for human RPE cells were established in the early 1980s. RPE cells can be harvested as single cells or as monolayers. The first transplantations of cultured human RPE cells were performed into monkey eyes in the mid-1980s. RPE cells have also been grafted into the eyes of other animals, including rats and rabbits. The therapeutic potential of RPE transplantation was highlighted in experiments where RPE cells were grafted into the eyes of the Royal College of Surgeons (RCS) rat. The RCS rat has been used widely as a model of retinal degeneration for decades. The degeneration is caused by a naturally occurring mutation in the c-mer proto-oncogene tyrosine kinase (MERTK) gene that codes for a protein essential for the phagocytosis of outer segments. Although this animal model can be used to evaluate the survival and function of RPE cell grafts it does not show typical hallmarks of AMD. Li and Turner published the first successful transplantation of rat RPE cells into young neonatal and adult Sprague Dawley rats as well as RCS rats. The cells integrated into the host RPE cell layer and repopulated denuded parts of Bruch’s membrane. In 1989, another group reported the successful grafting of rat RPE cells into the subretinal space of the RCS rat with reversal of their inherited incapability to phagocytose outer segments. In 1997, Castillo et al. described the transplantation of adult human RPE cells into the RCS model to prevent retinal degeneration. In addition, transplantation of the human RPE cell lines, ARPE19 and h1RPE7 into the RCS model can stop the progression of retinal degeneration. As well as assaying phagocytosis of outer segments after transplantation, the ability of RPE cells to prevent retinal degeneration has been assessed functionally, for example by measuring the effects on the pupillary light reflex and by electroretinography. Additionally, Schwann cells, fetal human RPE cells, neonatal rat RPE cells, olfactory ensheathing cells, human central nervous stem cells, and other cell types have been transplanted into the RCS rat.

**Transplantation of Stem Cell–Derived RPE Cells**

In addition to the above mentioned cell types, stem cell–derived RPE cells have been used in RPE transplantation studies. Human embryonic stem cells (HESCs) are pluripotent cells harvested from the inner...
cell mass of day-5 human blastocysts that are leftover from in vitro fertilizations. HESCs possess the potential to differentiate into every cell type of the body and they are essentially immortal because they can be expanded in culture without differentiation. HESC-derived RPE cells were first derived from an ESC/stromal cell (PA6) coculture system inducing neuronal differentiation. RPE cells have also been derived from mouse ESCs. Several other studies have been published where RPE cells were derived from HESCs using a spontaneous differentiation protocol. HESC-derived RPE cells express many RPE cell markers including MERTK and the 65-kDa RPE-specific protein (RPE65), and the cells are able to perform the functions of RPE cells such as phagocytosis of rod outer segments or latex beads.

Besides random differentiation, RPE cells have been derived from HESCs through embryoid body formation/neural differentiation. These cells express RPE-specific markers and phagocytose outer segments. Stem cell–derived RPE cells behave more like fetal RPE cells compared with adult RPE cells. The cells express genes that are essential for the function of RPE cells and that are normally not found in RPE cell lines.

HESC-derived RPE cells have been used for transplantation into rat and mouse models of retinal degeneration and the cells were able to rescue visual function. Lu et al. transplanted HESC-derived RPE cells subretinally into rat and mouse models of retinal degeneration and Stargardt’s disease. A few cells survived for over 220 days and they maintained some visual function. Additionally, no teratoma formation or any other pathological reaction was observed. HESC-derived RPE cells are currently being used in human clinical trials and first results were published in 2012. HESC-derived RPE cells were transplanted as a cell solution into one patient with Stargardt’s disease and one patient with dry AMD. Although the cells did not form a functional monolayer in the patients, slight improvements in visual acuity in both patients was observed. However, it should be noted that the measured visual acuity in retested AMD patients can sometimes improve over time due to changes in eccentric fixation, where a slightly different part of the retina is used to view central visual targets. This mechanism could also explain why there was some improvement in the fellow eye, which received no RPE cells. Thus, careful experimental design and cautious interpretation of results are essential to be sure that any change in visual acuity is truly due to an RPE transplant. Yet the preliminary findings are certainly suggestive and the results of later phase studies are awaited with interest.

In addition to HESCs, human induced pluripotent stem cells (iPSCs) have been used to derive RPE cells. iPSCs are reprogrammed somatic cells that share many similarities with HESCs. They are also pluripotent and can be expanded in culture to a theoretically infinite degree. RPE cells were derived from iPSCs using the spontaneous differentiation method. Additionally, iPSCs have been used to derive RPE cells through embryoid body formation/neural differentiation or through directed differentiation. The iPSC-derived RPE cells also expressed several RPE markers and fulfilled RPE functions as well as being able to replace RPE cells after transplantation. Most recently, RIKEN and the Foundation for Biomedical Research and Innovation, Japan, jointly applied to conduct a clinical trial using iPSC-derived RPE cells to treat AMD, so active development of this approach appears likely in the near future.

The use of stem cells in regenerative medicine carries the risk of tumor formation and immune rejection. Because of its immune privilege, cells transplanted into the eye may survive for longer than in other parts of the body. However, xenografts of RPE cells can still be targeted and destroyed by the recipient and in animals, immunosuppression did not help to protect RPE allografts from destruction by the recipient’s immune system.

Although RPE cells derived from embryonic stem cells seem to be relatively safe in terms of tumor risk post transplantation, it has been reported that these cells can dedifferentiate into neural rosettes. In the latest human clinical trial, the transplanted HESC-derived RPE cells did not appear to cause any serious side effects, although data from only two patients have been published so far.

In addition, iPSC-derived RPE cells lead to a more rapid host cell rejection after subretinal transplantation compared with HESC-derived RPE cells. This rejection might be due to the reprogramming of the somatic cells into iPSCs using integrating viruses. The use of nonintegrative episomal viruses to reprogram cells generates pluripotent cells that are less immunogenic and more similar to HESCs. In addition, the use of a Sendai virus directed mRNA or proteins to reprogram the cells might overcome immunogenic problems. Recently, Hou et al. found a way to induce pluripotency in mice somatic cells using chemical compounds alone.
of these compounds are already used in clinical trials. The researchers are now trying the same approach on human cells.\textsuperscript{102} To overcome the stem cell–associated tumor risk human fibroblasts have been differentiated directly into RPE-like cells by overexpressing RPE-specific transcription factors.\textsuperscript{103} Also this approach uses retroviruses to overexpress the transcription factors which might lead to complications in vivo. Patient-derived iPSCs might overcome the host immune response but they will still carry AMD susceptibility loci that might lead to AMD features post transplantation.\textsuperscript{96} Targeted gene repair to genetically correct AMD associated mutations prior to transplantation might overcome this problem.\textsuperscript{85}

### Primary RPE Cell Transplantation into Human Patients

Following encouraging results in animal models, grafting of RPE cells was tested in human patients using a variety of techniques. In some studies, healthy autologous RPE from the periphery of the eye was transplanted into the macular region of the same eye to replace the diseased foveal RPE cells. This autologous transplantation involved either RPE cells alone or a patch of RPE cells together with the underlying choroid.\textsuperscript{104–115} In the first of these studies, Peyman et al.\textsuperscript{116} transplanted RPE cells into the eyes of two patients with advanced AMD. For one of the two cases visual improvement was achieved for several months, but the other did not show any positive effects. Improved instrumentation to minimize damage of healthy tissue as well as improvements in the delivery method of RPE cell suspensions or sheets have made RPE cell transplantation somewhat easier and safer, but the technique is still difficult, time-consuming, and may lead to retinal detachment during the harvesting of peripheral RPE cells.\textsuperscript{116–118} To date, a total of more than 30 homologous and 230 autologous RPE transplantations have been reported.\textsuperscript{119}

A comparison between the grafting of a RPE cell suspension and transplantation of an RPE sheet did not find significant differences with some improvement in visual acuity observed.\textsuperscript{107} However, transplantation of a uniform layer has proven difficult because the grafts tend to wrinkle up or contract after grafting, forming thin patches or multilayered folds.\textsuperscript{120} On the other hand, RPE cell suspensions alone may not survive in the diseased environment of an aged Bruch’s membrane,\textsuperscript{121} and the therapeutic success of transplantation of RPE cell solutions may be limited if few cells adhere to diseased Bruch’s membrane or fail to survive for long enough to show a positive effect. The rejection rate due to inflammation seemed to be a major problem in some studies and this may be a bigger problem in wet than dry AMD as this type of the disease involves a greater compromise of the retinal–blood barrier.\textsuperscript{122} The extracellular environment to which the cells have to adhere plays an important role in the survival of the transplanted RPE cells.\textsuperscript{123–129} It has been shown that RPE cells undergo apoptosis if they do not adhere fast enough.\textsuperscript{125,127} The lack of adhesion can be explained through molecular changes on Bruch’s membrane that result from age-related deposition of anti-adhesive molecules and a decline in normal integrin ligands that would promote the attachment of the transplanted cells.

Moreover, in wet AMD, surgical approaches face the problem that choroidal new vessels have grown from the choroid through Bruch’s membrane into the retina and have altered the environment of Bruch’s membrane in the process. Prior to transplantation of RPE cells, choroidal neovascular membranes can be excised and RPE cells can be seeded on the denuded area.\textsuperscript{130,131} After subretinal choroidal neovascular removal, the endogenous RPE layer displays a certain wound healing ability but the RPE cells are not able to cover the exposed deeper layers of the diseased Bruch’s membrane completely and progressive atrophy of the choriocapillaris frequently occurs.\textsuperscript{132,133}

Binder et al.\textsuperscript{104,105} showed that autologous macular transplantation led to some visual improvement after choroidal neovascular removal. The clinical study comparing membrane excision alone with simultaneous RPE transplantation found a small improvement in visual acuity for near but not for distance.\textsuperscript{104} Other studies showed that the debride-ment of Bruch’s membrane followed by transplantation of autologous RPE cells led to a repopulation of the cleaned area of Bruch’s membrane and a prevention of photoreceptor loss.\textsuperscript{33} In 2007, McLaren et al.\textsuperscript{111} reported that their autologous transplantation after choroidal neovascular excision led to visual function improvements and that the graft survived until the 6-month time point. However, surgery-associated complications remained high.\textsuperscript{114}

In addition to age-related changes to Bruch’s membrane that can at least in part be held responsible for a failure of survival of grafted RPE cells, mechanical damage due to the transplantation itself may result in poorer adhesion of the graft.
that are used to excise choroidal neovascular membranes in wet AMD can result in damage or removal of the basement membrane of Bruch’s membrane. \(^{134}\) The RPE basement membrane is the best substrate for transplanted RPE cells and its removal has serious consequences for RPE survival, as has been shown \(\textit{in vivo}.\) \(^{125,124,127,135,136}\)

In addition to using autologous RPE cells, allotransplantation has been performed in human patients. \(^{122,137–140}\) In one study, grafted fetal RPE cells resurfaced the denuded Bruch’s membrane after removal of choroidal neovascular membranes and the cells survived for up to 3 months in the five patients. \(^{137}\) However, at the 12-month follow-up, most of the patients reported further visual loss compared with the pre-operative vision. \(^{137}\) In non-exudative AMD, fetal RPE cell suspension grafts showed positive effects and stabilized visual acuity. However, in disciform lesions and in dry geographic atrophy AMD, the transplants did not lead to visual improvement. \(^{122,140}\) Transplantation of allogeneic fetal RPE grafts is associated with a high rejection rate without immunosuppression, especially in wet AMD cases. \(^{138}\) Autologous transplantation of a sheet of adult RPE cells has also been studied in one clinical trial. \(^{139}\) Although no improvements in visual acuity were observed, the adult RPE monolayer was not rejected by the host and it appeared healthy up to 1 year after transplantation. \(^{139}\)

### Enhancing the Adhesion and Survival of Transplanted RPE Cells

As mentioned above, transplanted RPE cells show limited adhesion and survival after transplantation into human eyes. \(\textit{Ex vivo}\) models played an important role in determining the survival rate of RPE cells after transplantation. By comparing the attachment rate of RPE cells seeded on Bruch’s membrane from young and old donors, researchers have demonstrated that aged Bruch’s membrane does not support adhesion, survival, differentiation, and function of grafted RPE cells. \(^{15,123,127–129,141}\) Age-related debris in all layers of Bruch’s membrane might account for the lack of adhesion. \(^{126}\) That RPE cells behave differently on Bruch’s membranes from differently aged donors has further been evaluated with gene expression profiles; RPE cells seeded on aged Bruch’s membrane upregulate 12 genes and downregulate eight compared with younger membranes. \(^{142}\)

Sugino et al. \(^{143}\) compared the fate of fetal and HESC-derived RPE cells on aged and AMD Bruch’s membrane. Both cell types were able to resurface parts of the membranes after 3 weeks. However, even after successful adhesion of the grafted RPE cells, the changes on Bruch’s membrane hindered the formation of a functional monolayer of RPE cells. \(^{143}\) This study emphasizes that stem cell–derived RPE cells behave similarly to fetal RPE cells in a diseased Bruch’s membrane environment and new approaches are required to overcome the inhibitory environment to ensure RPE cell resurfacing of Bruch’s membrane.

The interaction of RPE cells with Bruch’s membrane is mediated mostly via \(\beta 1\)-containing integrins, which bind to a variety of extracellular ligands including laminin, fibronectin, vitronectin, and collagen IV. \(^{144–146}\) Further studies have shown that laminin and fibronectin facilitated the attachment of RPE cells and prevented RPE apoptosis most effectively. \(^{125,147}\) The upper-most layers of Bruch’s membrane are rich in these two extracellular matrix molecules \(^{148,149}\) and exposure of deeper layers of the membrane will result in disturbed adhesion of RPE cells. Therefore, it is important to enable the RPE cells to cope with the extracellular environment that they will encounter after transplantation. \(^{147,150}\)

One approach to enhance the binding of transplanted RPE cells in the pathological environment is to provide the RPE cells with a more favorable substrate. However, \(\textit{in vivo}\), the addition of poly-L-lysine in combination with RPE translocation did not show any positive effects, \(^{114}\) although this failure might have been due to a permeabilization of the grafted RPE cells by the poly-L-lysine itself. Nevertheless, \(\textit{in vitro}\), the alteration of the extracellular matrix (ECM) composition of Bruch’s membrane through the addition of conditioned media helped the attachment of seeded RPE cells to aged Bruch’s membrane. \(^{126,151–153}\) Another approach is to increase the surface levels of integrins. It has been shown that uncultured RPE cells from aged donors fail to adhere, survive, or function on Bruch’s membrane. \(^{124,127,135,154}\) Thus, Gullapalli et al. \(^{155}\) tested the effect of long-term culturing on the adhesion rate of the RPE cells. The long-term culture increased the expression levels of \(\alpha\)-integrins, which has a positive effect on the adhesion of RPE cells to Bruch’s membrane and its components. \(^{155}\) In addition to long-term culturing of the RPE cells, genetic manipulation to overexpress integrins can be used to enhance RPE adhesion. As an example, overexpression of the integrin \(\alpha 6\beta 4\) led to an increased binding and proliferation of RPE cells on all layers of Bruch’s membrane.
membrane. Manipulation of this integrin through site directed mutagenesis disrupted the adhesion and proliferation of the RPE cells on Bruch’s membrane.\textsuperscript{156} Additionally, our group has shown that overexpression of \( \alpha 9 \) integrin in ARPE19 cells enhanced the adhesion of the cells to Bruch’s membrane explants from donors with wet AMD.\textsuperscript{147}

As well as changing the integrins exposed on the surface of cells, the activation state of these cell surface integrins can be controlled from within the cell (inside-out signaling). This mechanism promotes the cellular binding to extracellular ligands and the linkage of the integrins to the cytoskeleton.\textsuperscript{157} Several researchers have investigated the role of integrin activation in improving cell adhesion, migration, and neurite outgrowth.\textsuperscript{158–163} In addition, it has been shown that inhibitory factors can influence the activation state of integrins and decrease the ability of the cells to interact with their extracellular environment.\textsuperscript{159,162,163} It is therefore possible to change the expression profile of integrins or their activation state to promote the adhesion of RPE cells to pathological Bruch’s membrane prior to transplantation. It has been shown in ARPE19 cells that the addition of manganese as a broad integrin activator\textsuperscript{164} or the administration of the monoclonal antibody TS2/16\textsuperscript{165,166} enhances the adhesion as well as migration of the cells to Bruch’s membrane components as well as in our studies with Bruch’s membrane explants.\textsuperscript{147} Furthermore, recent unpublished data from our laboratory shows that the application of manganese as well as the overexpression of kindlin-1, an intracellular binding partner, and activator of integrins\textsuperscript{162,167–169} enhances the adhesion, spreading as well as migration of primary rat RPE cells on Bruch’s membrane components and rat Bruch’s membrane explants (Heller JP, et al. IOVS. 2011;52:ARVO E-Abstract 936). Upregulation of anti-adhesive molecules such as tenasin-C has been reported, particularly in wet AMD.\textsuperscript{147,170,171} The application of manganese, as well as the overexpression of kindlin-1 or \( \alpha 9 \) integrin, in primary rat RPE cells can overcome the inhibitory effects of TN-C and the chondroitin sulfate proteoglycan aggrecan and promote the adhesion, spreading and migration on these pathological Bruch’s membrane components (Heller JP, et al. IOVS. 2011;52:ARVO E-Abstract 936). Hence, in an environment where inhibitory molecules are more abundant than pro-adhesive molecules like laminin and fibronectin, the modulation of RPE cell integrins could potentially enhance the survival rate of transplanted RPE cells.\textsuperscript{147,150} Therefore, this approach might overcome age-related pathological changes on Bruch’s membrane as well as surgical damage caused by the excision of choroidal neovascular membranes.

To overcome the problems of forming a functional monolayer post transplantation, RPE cells can be grafted as a polarized monolayer growing on a scaffold. This can circumvent the problems of detachment and redifferentiation of the RPE cells as well as avoiding the low attachment rate of the grafted RPE cells. Natural membranes including biologically-derived basal lamina, amniotic membrane, Descemet’s membrane and lens capsule have been used as substrates for RPE cells.\textsuperscript{121,172} Additionally, artificial Bruch’s membrane substrates including parylene films,\textsuperscript{173,174} plasma polymers,\textsuperscript{175} polyamidoamides,\textsuperscript{176} polyester membranes,\textsuperscript{177} polyimide,\textsuperscript{178} and modified polytetrafluoroethylene\textsuperscript{179} have been proposed.\textsuperscript{179} However, the use of some types of artificial membranes carries the risk of biological contamination and disease transmission.\textsuperscript{172} Encouraging results have been described by the group of Peter Coffey at the University of Ophthalmology in London, UK and the University of California at Santa Barbara and his collaborators using a coated, nonbiodegradable polyester membrane with HESC-derived RPE cells.\textsuperscript{61,72} A clinical trial of these approaches is planned to commence in the near future.

In a recent study, Diniz et al.\textsuperscript{173} compared the survival of 10,000 cells transplanted as a suspension versus 2700 cells seeded on a parylene membrane. The cells grafted as a monolayer survived better, and the cells in solution often formed aggregates or clumps in the immunocompromised rat model. However, the transplantation of a cell solution is technically easier and causes less trauma and biocompatibility issues.\textsuperscript{173} Again, this emphasizes that the use of a RPE cell solution might be the easier approach to replace lost RPE cells in AMD. The results from our laboratory using primary rat RPE as well as ARPE19 cells suggest that in future transplantation studies the integrin abundance on the cell surface as well as the integrin activation state of stem cell–derived RPE cells should be altered prior to transplantation to ensure satisfying adhesion to and interaction with the aged and diseased Bruch’s membrane\textsuperscript{147} (Heller JP, et al. IOVS. 2011;52:ARVO E-Abstract 936).

### Conclusion

Although the results of many of the animal studies performed in models of AMD are encouraging, it is
important to remember that there is no model that fully replicates the human disease. All of the animal models used to study AMD have limitations and the results should therefore be interpreted with caution, particularly with regard to how they apply to the human condition. In particular, models differ with regard to the mechanisms of RPE cell loss, the importance of inflammation and the recruitment of other cells during the degenerative process. Better animal models of AMD are an important goal for future research.

Nevertheless, approaches using RPE cell transplantation to treat AMD are reaching a very exciting stage. However, the recent results from the Advanced Cell Technology–sponsored trial using HESC-derived RPE cells showed that no functional monolayer was formed by the transplanted cells. Additionally, signs of cell clumping were detected in one patient. More patients are needed in this trial to assess the potential of the transplanted cells to replace the diseased RPE cells. However, the low attachment and spreading rate of the transplanted RPE cells might be avoidable, either through the use of genetic engineering to overexpress integrins or integrin activators in the RPE cells, or through the use of RPE cells growing on scaffolds. Planned clinical trials will evaluate the effectiveness of RPE cell monolayers rather than cell suspensions. In addition, the clinical trial will evaluate the feasibility of this technique in human patients as compared with the easier grafting of a cell suspension. The clinical trial in Japan will show whether the use of iPSC-derived cells is a safe tool for regenerative medicine, and whether transplantation of iPSC-derived RPE cells can be a potential treatment for macular degeneration in human patients. In addition, several banks for iPSC lines are going to be established in many parts of the world, including the United States, Europe, and Japan. These banks will enable the use of HLA-matched cells for transplantation to reduce the risk of graft rejection by the patients. The results of these trials may well have important implications for future AMD therapy and are awaited with great interest.

Nevertheless, the transplantation of RPE cells alone might not be sufficient if AMD has progressed to a stage when photoreceptors are affected too. Simultaneous or sequential grafting of RPE cells together with photoreceptor precursor cells could potentially be used as a treatment for more advanced AMD as the transplantation of developing rods has been shown to be effective in several animal models of inherited retinopathies.

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