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Retinal Repair with Induced Pluripotent Stem Cells

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Abstract

Retinal degenerations like age-related macular degeneration (AMD) and other inherited forms such as Stargardt's disease and retinitis pigmentosa, and optic neuropathies including glaucoma and ischemic optic neuropathy, are major causes of vision loss and blindness worldwide. Damage to retinal pigment epithelial cells (RPE) and photoreceptors in the former, and to retinal ganglion cells' (RGCs') axons in the optic nerve and their cell bodies in the retina in the latter diseases leads to the eventual death of these retinal cells, and in humans there is no endogenous replacement or repair. Cell replacement therapies provide one avenue to restoring function in these diseases, particularly in the case of retinal repair, although there are considerable issues to overcome, including the differentiation and integration of the transplanted cells. What stem cell sources could be used for such therapies? One promising source is induced pluripotent stem cells (iPSCs), which could be drawn from an individual patient needing therapy, or generated and banked from select donors. Here we review developing research on the use of iPSCs for retinal cell replacement therapy.

Introduction

The retina is an outgrowth of the central nervous system (CNS) and because of its direct accessibility for visualization and drug delivery, it provides an optimal opportunity to examine stem cell biology and therapeutics. The light-sensitive retina lies in the back of eye, is approximately 30-40 mm in diameter and 0.5 mm thick in humans, and accommodates 5 broad classes of neurons: photoreceptors, horizontal cells, bipolar cells, amacrine cells, and retinal ganglion cells (RGCs). The cell bodies of these neurons are elegantly arranged in 3 layers, the outer nuclear layer which contains cell bodies of both photoreceptors, rods and cones; the inner nuclear layer containing the cell bodies of the bipolar, horizontal and

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amacrine cells as well as the Muller glia, and the ganglion cell layer containing the cell bodies of RGCs and displaced amacrine cells. Synapses lie between each cell layer in the outer and inner plexiform layers. Light stimulates the photoreceptors, which then synapse to the other interneurons, which activate the RGCs. RGC axons combine to form the optic nerve, which then carries all of the visual information to the brain. In the center of the retina lies the macula, with the fovea positioned in the center. The fovea contains the highest density of cone photoreceptors in the retina, and is responsible for our central, high-acuity vision [1].

Just behind the retina lies the retinal pigment epithelium (RPE) The RPE is composed of a monolayer of pigmented cells, and serves many important roles in the retina. RPE cells' tight junctions contribute to the blood-retina barrier, and RPE cells are responsible for transporting nutrients from the blood to the photoreceptors, and waste products in the opposite direction [1]. RPE cells also phagocytose the outer segments of the photoreceptors, and they harbor essential enzymes responsible for regenerating visual pigments needed by the photoreceptors to convert photons of light into chemical signals[2]. Loss of RPE is associated with hereditary or age-related retinal degenerations such as age related macular degeneration (AMD), Stargardt's disease or retinitis pigmentosa (RP). More than 40 million people suffer from AMD worldwide and it is a leading cause of blindness in people over 60 years old. The death of RPE cells is associated with loss of photoreceptors in the macula and eventual loss of vision. The cellular atrophy that accompanies AMD is normally irreversible, and unfortunately other than delaying the disease process by supplements, medications or surgery, there are no treatments to recover lost cells or completely prevent ongoing damage to remaining cells. Therefore, cell replacement therapy and regenerative medicine creates a new window of hope for treatment of retinal degenerative conditions through a number of potential avenues, by replacing lost cells, by supplying neuroprotective molecules to at-risk cells, and by improving disease models in the laboratory to help us better understand the pattern and cause of these diseases [3-6].

Sources of Cells for Retinal Repair

Primary Retinal Cells and Retinal Progenitor Cells (RPCs)

It has long been attractive, even before the current era focused on stem cells, to consider the transplant of fully differentiated photoreceptors and RPE cells, whether from a patient's fellow eye, or from human donors. In pre-clinical models, animal data suggests that newly differentiated rod photoreceptors may integrate better after cell transplant than RPCs [7]. In the outer retina, similar data suggests that primary RGCs integrate better and receive more retinal synapses than RPC-derived RGCs [8]. Early work in human trials demonstrated that transplanted neural tissue can survive in human patients without immunosuppression, and without apparent inflammation or rejection, and suggested the possibility of vision improvement after implanting retina with RPE [9-11].

RPCs, during normal retinal development, clearly have the capacity to differentiate into all the cells of the retina [12], but currently it is difficult, experimentally and politically, to garner enough RPCs from human embryos to pursue this approach. Although many groups continue to focus much of their attention on human RPCs, muller glia, and RPE progenitors [13-17], large cell-banked supplies of lines from these sources have not be demonstrated and could prove more difficult to generate. Thus between limited cell or tissue supply, and the excitement about the prospects for stem cell-derived products, primary retinal cells have not been pursued much further.

Human Embryonic Stem Cells (hESCs)

hESCs are undifferentiated cells derived from the inner cell mass of the blastocyst. They are characterized by the ability to proliferate indefinitely without differentiating, and the capacity to differentiate into all cell lineages. The discovery of hESCs in 1998 was a breakthrough in the field of regenerative medicine [18]. Since then there has been a leap of progress in generating retinal cells from hESCs, including differentiating and purifying hESC-derived retinal progenitor cells, photoreceptors, RPE and RGC-like cells [19-28]. Futhermore, hESC-derived RPE and photoreceptor cells successfully integrate into the retina, express specific retinal markers, and enhance visual function in pre-clinical animal models [29-37]. Finally, trials have begun in human retinal degenerations (Stargardt disease and AMD) with hESC-derived RPE, raising the exciting possibility of translating these therapies into human use [38].

Induced Pluripotent Stem Cells (iPSCs)

In 2006 it was first reported how to reprogram adult somatic mouse cells and 2007 human cells to a hESC-like state by introducing four factors, (OCT3/4, KLF4, SOX2, C-MYC) into somatic cells [39, 40]. Over the subsequent years this approach has been refined both through altering the vectors used, e.g. with other viruses, mRNA, or even pharmacologic agents [41-47], as well as through the specific genes used. For example, OCT4, SOX2, NANOG, and a different gene LIN28 are as effective at cellular reprogramming [48].

There are many promises of iPSCs—that they may allow for personalized treatment with a patient’s own cells; that they should be safe from the ethical and immunological concerns related to hESCs; that they will allow cells and tissues from patients with specific diseases to be recapitulated and studied in a laboratory dish. Some of these promises are already demonstrating fruition throughout the body—what about progress towards diseases in the eye? Recently, the Japanese Ministry of Health, Labor and Welfare has approved the world’s first clinical trials involving iPSCs, to try to restore vision in AMD patients by transplanting iPSC-derived RPE cells (http://www.riken.jp/en/pr/press/2013/20130730_1/). In this review we will focus on advances in generating RPE cells, photoreceptors and RGCs, and discuss the implications of bringing these to human trials.

Generating RPE from iPSCs

Of all the retinal cells, there has been the most progress in generating functional retinal pigment epthilial (RPE) cells from pluripotent cells (Table 1). Two major methods of generating human iPSC-derived RPE (hiPSC-RPE) include forming embryoid bodies or more commonly, as a monolayer by allowing the hiPSC to overgrow as multiple layers in the dish. In either case, cells are then allowed to spontaneously differentiate by removing the mitogen fibroblast growth factor (FGF) from their maintenance media [49-54] or by supplementing with certain retinal differentiation-inducing factors and proteins such as Wnt, nodal and bone morphogenic protein (BMP) signaling pathway inhibitors: DKK-1, lefty A and Noggin, respectfully, which are the three most commonly used. In addition, insulin growth factor 1 (IGF-1), retinoic acid (RA), activin, bFGF, nicotinamide, SB431542, B27 and N2 have been demonstrated to help induce RPE fate [55-59].

Pigmentation of RPE cells is crucial for absorbing scattered light entering the eye and maintaining visual function [2]. Depending on the protocol followed and cells used, pigmented cells usually appear around 2-6 weeks after induction in culture. These cells may be cultured a few additional weeks until they form colonies large enough to be manually picked and expanded. Recently, a simpler technique for a less labor intensive and purer hiPSC-RPE outcome was proposed, by whole-plate serial passaging of hiPSC-RPE, as opposed to manually selecting hiPSC-RPE [60]. Briefly, pluripotent cells propagated with blebbistatin were allowed to spontaneously differentiate on matrigel by removing FGF from the media. Once RPE sheets with pigmented cells formed (at around 50 days) the whole plate was passaged on a fresh matrigel plate and maintained in RPE media. To further purify the culture a second passage was done after two additional weeks. 50 days after the second passage, functional pigmented RPE cells formed monolayers and non-pigmented cells were not observed. The RPE cells after passaging had a purity of 98%-99% as indicated by flow cytometry for Mitf and RPE65 markers.

Yields of iPSC-RPE cells, critical to considering larger scale preparation of cells towards cell banks for human trials, also differs according to signaling molecules added to the culture media. One of the highest reported yields, ~60% Mitf-positive cells in 60 days, was superceded by adding SHH, RA, Noggin and bFGF at specific time points [56]. Another study published a yield of ~63% Pmel17-positive cells in 14 days [61] by combining nicotinamide, which has been shown to enhance differentiation to RPE [22], with retinal inducing factors IGF1, DKK1, Noggin and bFGF which sped up neural/eye field gene expression by day 4. This was hypothesized to occur from the inhibitory effect of nicotinamide on poly (ADP-ribose) polymerase (PARP), which controls cell death after neural induction of pluripotent cells [62]. By day 14, sheets of RPE expressing specific RPE genes were observed. Furthermore, adding activin and SUS402 slightly increased RPE gene expression and down-regulated retinal marker Rx expression by day 10. The hormone vasoactive intestinal peptide (VIP) led to an increase in pigmentation at an earlier time but was not cost-effective to use. Compared to controls, cells cultured in the RPE differentiation factors mentioned above showed significantly increased levels of RPE marker genes Mitf, Tyrosinase, Tyrp2, PEDF, BEST1 and Pmel17 by quantitive polymerase chain reaction (qPCR) analysis [61]. The search for factors that will increase yields and purity of RPE cells from iPSCs continues; advances in defining these signals may impact the quality of cells that are eventually moved towards human testing.

Differentiating iPSCs in the absence of animal products or feeder layers may be crucial for clinical application [51, 63, 64]. Towards this end, hIPSC-RPE cells were cultured on various proteins and substrates that resemble that of the human RPE extracellular matrix (ECM), hypothesizing that the ECM proteins would be recognized by RPE integrin receptors and thereby support their differentiation and survival [51]. To study the effects of different ECM molecules on RPE cell differentiation, different plates were coated with laminins-111 and 332, collagens I and IV, fibronectin and vitronectin, which are all described to be part of the ECM protein composition present in Bruch's membrane, in addition to testing gelatin (denatured collagen) and Matrigel (Becton Dickenson). iPSCs were cultured until day 7; after which the FGF used in the media was removed to induce spontaneous differentiation. The total area of pigmented cells on Matrigel was significantly higher than all other substrates except for mouse laminin-111, which showed similar results. The ability of mouse laminin-111 to support generation and maintenance of iPSC-RPE and hESC-RPE for multiple passages indicates that a single purified ECM protein may be able to replace mouse fibroblast feeder layers or Matrigel.

Taken together, considerable progress has been made in the generation of iPSC-RPE. These cells have not only been shown to express key genetic markers such as Mitf, OTX2, bestrophin 1 (BEST1), ZO1, PEDF, LRAT, PEML17 and CRALBP, but they also have been shown to perform key phenotypes in functional assays, such as phagocytosis of photoreceptor outer segments, ion transport, and secretion of basal levels of VEGF, which is necessary to maintain the underlying blood supply in the choriocapillaries [49, 50, 52, 58, 60, 65]. To what degree these phenotypic functional assays are important in predicting the utility of the cells after transplant in human clinical testing may be determined in the coming years, possible in part because of the number of different stem cell-derived RPE products entering human trials.

hiPSCs to photoreceptors

Recent data have demonstrated significant advances in generating rod and cone photoreceptors from pluripotent cells. hESCs have been successful in deriving photoreceptor cells, as well as integrating in to host retina and to an extent restoring vision in mouse models [21, 28, 34].

Similar protocols have been implemented on hiPSCs leading to comparable outcomes [55, 57, 58, 66-70]. Methods in generating hiPSC-photoreceptors usually involve growing iPSCs as embryoid bodies in suspension for a few weeks in neural induction media containing N2 and B27 supplements, with additional retinal cell fate-inducing factors (e.g. Noggin, Lefty A, DKK1, IGF1) and then plating on an adherence substrate for several months (Table 1). In some studies photoreceptor-inducing factors such as taurine, retinoic acid (RA) and sonic hedgehog (SHH) are added [56, 58, 68] until cells express photoreceptor markers such as CRX, Nrl, opsin, rhodopsin, and recoverin. Culturing iPSCs in neural induction media without such retinal fate-inducing factors has also resulted in a small percentage of cells that express early (CHX10) and mature (opsin and recoverin) photoreceptor markers after 80 days of culture [54]. Other methods such as adherent monolayer culture may also produce iPSC-photoreceptors [66, 67]. Recently it was demonstrated that by culturing hiPSCs with a specified ECM, these cells formed a self-organized neuroepithilium, generating cells expressing rod photoreceptor markers as early as 4 weeks after induction[67]. Clumps of hiPSCs were plated on 1% Matrigel and then, after one hour, the adherent cells were covered with 2% Matrigel diluted in neural differentiation media, thereby providing a 3-dimensional environment for further differentiation. Matrigel-free media was changed every other day and cells were supplemented with taurine, RA, FGF and SHH from day 10. After 2 days the cells expressed Pax6, Rx and CHX10 and interacted with the ECM forming a polarized neuroepithelium, which subsequently lost its integrity after the fifth day and grew as a monolayer. By day 10 around 60% of the cells expressed the pan-photoreceptor marker CRX and at 4 weeks 36% of cells expressed rod specific markers Nrl and rhodopsin [67]. Thus, as was shown for hiPSC-RPE induction, hiPSC-photoreceptor induction is sensitive to both substrate and soluble signaling molecules.

Another tantalizing area of research involves deriving retinal cells like photoreceptors from patients with retinal degenerations, to study the patient's own retinal cell-like progeny in the laboratory dish. For example, fibroblasts from patients with specific retinal degenerative diseases such as retinitis pigmentosa (RP), Leber's congenital amaurosis (LCA) and Stargardt's disease have been successfully reprogrammed to iPSCs and then differentiated to photoreceptor-like cells [63, 68]. Interestingly, iPSC-photoreceptors from patients with RP were found to die off after day 120 in culture. Antioxidant vitamins a-tocopherol, ascorbic acid, and b-carotene, which have been clinically tested on patients with RP and AMD, were added at day 120, to determine whether countering oxidative stress could preserve photoreceptor survival or differentiation in these cultures. There was a marked increase in rhodopsin-positive cells only in cells with the RP9 mutation treated with a-tocopherol [68], suggesting there may be some specificity in whether antioxidant therapy could benefit a particular subset of genetically identifiable patients. Thus these disease models are like to prove extremely beneficial in understanding disease processes, creating disease models specific for every patient, and tailoring the treatment based on drug screening results in iPSC-derived retinal cell cultures.

Generating RGCs from iPSCs

Unlike the photoreceptors that give a single synapse to interneurons, transplanted RGCs are required to not only integrate in the retinal ganglion cell layer but also develop very long axons that connect to the optic nerve and develop connections with the brain. Because of that, considering cell replacement therapy for RGCs bears the most challenge out of all retinal cells. Nevertheless, significant progress has been made in generating RGC-like cells from stem cell populations (Table 1). For example, hESCs can generate RGC-like cells by adding FGF2 and Shh to the differentiating cells, but less than 2% of these cells expressed RGC-specific markers such as ATH5, BRN3B, RPF-1, Thy1, and ISLET1, and intravitreal transplantation of these cells into mouse retina was not promising [24]. hESC-RGC-like cells have also been induced by adding Noggin, DKK1 and DAPT as well as over-expression of Math5. Cell progeny elongated neurites and expressed specific retinal genes such as Brn3b, Islet-1, and Thy1.2. Although these cells survived post-transplantation, they failed to integrate into the host retina [71]

iPSCs have also been examined for an ability to generate RGCs [72]. In one study, embryoid bodies were exposed to neural and retinal induction factors including B27, N2, insulin, transferrin, sodium selenite, fibronectin, FGF and noggin. After 35 days, iPSC-derived retinal progenitor cells were cultured for 10 additional days in conditioned media from E14 rat retinal cells. At the end of this differentiation process, cells expressed the markers ATH5

(~26%), BRN3b (~14%), and RPF1 (~12%) [72]. Thus extrinsic signaling molecules can be used to enhance RGC-like cell differentiation from hiPSCs.

Can intrinsic regulation, for example via the transcription factors that normally guide RGC development, also be harnessed to induce RGC differentiation from hiPSCs? The fraction of Brn3-positive RGC-like cells derived from hiPSCs was recently increased further to about 33% [73] by supplementing the hiPSCs with retinal-inducing factors Dkk1, LeftyA, Noggin and DAPT during the embryoid body phase, then dissociating the cells and plating them on PDL/laminin substrates and transducing them with lentivirus carrying Math5 and/or Sox4 genes, which are required for RGC formation during devellopment [74, 75]. The increased yield of RGC-like cells after dual transduction with both Math5 and Sox4 was accompanied by evidence for normal physiologic generation of action potentials in response to current injection, suggesting that these are appropriately electrically active neurons. Future work examining their ability to integrate after transplantation, and to extend axons towards or down the optic nerve, will be critical steps in considering such approaches for cell replacement therapies.

Although generating iPSC-RGCs able to integrate and replace lost RGCs in diseases such as glaucoma is an important goal [8, 76, 77], other possible applications of stem cells could be borne out by transplanting iPSCs to serve a neuroprotective role. For example, by transplanting iPSCs capable of secreting neurotophic factors, RGCs might be protected from the insults in optic neuropathies like glaucoma [78].

iPSCs Recapitulating Retinal Development

Development of the retina occurs in a chronological order facilitated by genes that are expressed at certain time-points throughout retinal morphogenesis. Pluripotent cells have demonstrated the ability to mimic the major stages in early eye and retinal development at comparable timelines to the human retina [54]. For example, in one set of experiments [20], pluripotent cells were suspended as free-floating aggregates and supplemented with neural induction media to form neural rosettes. After about 10 days these aggregates started co-expressing the early eye-field markers Rx and Pax6, while pluripotent markers oct4 and noggin were concurrently lost. Around 2 weeks later, optic vesicles expressing Mitf and Pax6 started to appear. The outer layer of the optic vesicle continued expressing the early RPE marker Mitf and formed the RPE. As the RPE matured it started expressing Bestrophin 1 (BEST1) and RPE65. The inner part of the optic vesicle gave rise to the neural retina, confirmed by down regulation of Mitf and up-regulation of early neural retina marker CHX10. The neural retina gave rise to all 5 classes of neurons; of note, photoreceptor-like cells expressed Crx, recoverin and opsins, and RGC-like cells expressed Brn3. Thus not only can iPSCs generate individual retinal cell types, but they may also generate whole tissues that share many key characteristics with the normal retina. Of course, much work remains to be done, to characterize these tissues for cell-cell connectivity, optimize differentiation, and consider neurite growth and integration.

Transplanting hiPSC-Derived Retinal Cells

The derivation of specific retinal cell types from iPSCs is, on its own, a major step forward, for example for the study of human disease phenotypes in the lab. However, there is significant promise in the use of hiPSC-derived retinal cells for cell replacement therapy. In pre-clinical models, such cells have already demonstrated promise. For example, iPSC-photoreceptors have been shown to successfully integrate into host rhodopsin-/- mouse retina, a model for RP [79]. ERG and functional anatomy studies showed improved electro-retinal function and significantly increased inner nuclear layer c-Fos expression at 21 days post-transplantation, a marker for light-induced retinal electrical activity. Similarly, transplanting hESC-RPE cells to the sub-retinal space of Royal College of Surgeons (RCS) rats, a genetic model of RPE degeneration, showed cell survival at 10 weeks [23] and in one study up to 30 weeks [80]. iPSC-RPE have only demonstrated short-term survival when transplanted into RCS mice thus far [52]. Thus for diseases including AMD and RP, pre-clinical studies are promising, although many rounds of optimization and further characterization are certainly ahead. Also, when transplanting retinal cells it is important to note that the RPE and photoreceptor layers are dependent on one another, meaning that in degenerative diseases affecting both the RPE and the photoreceptor layer [81], transplantation of just one of these two cell types may not repair the damaged retina. For example, replacement of the RPE or photoreceptors to treat AMD will be largely ineffective in the absence of the other cell type, or of a functional choroidal blood supply.

Another important obstacle to overcome is the purity of iPSC-retinal cells, as the remaining undifferentiated cells in the culture could lead to teratoma formation. Purifying the transplanted cells could be done either by positive selection [70] or negative selection [79]. For positive selection of rod photoreceptors, iPSC-photoreceptors underwent fluorescent activated cell sorting (FACS) by constructing a lentivirus expressing GFP from the human inter-photoreceptor retinol binding protein (IRBP) promoter, a photoreceptor-specific gene expressed early in development [82]. In these experiments, retinal cells derived from iPSCs as well as hESCs were infected with IRBP-GFP for 4 to 8 weeks after starting a differentiation protocol. 100% of GFP-positive cells also expressed CRX and the majority were positive for NRL, AIPL1 or rhodopsin, supporting the use of the IRBP-GFP approach for selection. Around 10% of live iPSC cultures expressed IRBP-GFP before FACS. After FACS more than 90% of the cells expressed IRBP-GFP, all were positive for CRX, and most were positive for recoverin [70, 82]. To test how well these cells survived and integrated in the retina, FACS-sorted IRBP-GFP-positive cells were transplanted into the sub-retinal space of adult wild-type mice. After three weeks, iPSC-photoreceptors were identified in the sub-retinal space, and also migrated into the outer nuclear layer and expressed Otx2, recoverin and rhodopsin. However, cell survival was less in FACS-sorted cells [70].

In an example of using negative selection, a magnetic bead-based cell sorting system was employed to remove unwanted pluripotent SSEA-1-expressing cells [79]. Cells went through the depletion process twice, and were then transplanted onto rhodopsin-knockout RP mice. At 21 days, 60-80% of mice receiving heterogeneous iPSC transplants formed teratomas, only 20% of mice that received cells that underwent one depletion cycle formed teratomas, and none of the mice that were injected with twice-depleted iPSC-RPE cells developed tumors [79]. Thus both positive and negative selection may improve purity of cells used, and purity of cells used may influence both positive outcomes like cell integration, and negative outcomes like tumor formation. Clearly the method of selection (FACS, magnetic bead-based), and markers used in selection will influence the efficacy in cell replacement therapies. Selection may also influence the outcomes of laboratory-based experiments on differentiation or cell function, and should be considered in the interpretation of such studies.

Conclusions

Since the discovery of human embryonic stem cells in 1998 and human induced pluripotent stem cells (iPSCs) in 2006, the eye has been an attractive organ to study the efficiency and long-term safety of generating and transplanting cells. The attraction of using hiPSCs to generate patient-specific cell replacement therapies for the retina is strong, but there are numerous challenges still to overcome. Improvement in hiPSC differentiation, transplantation and integration for maximal therapeutic efficacy will likely require a number of cycles back and forth between early phase human trials and additional laboratory investigation and optimization. Whether hiPSCs will prove better for some disease therapies than others will remain an empirical question and deserves appropriate laboratory and human testing to determine. Nevertheless, the prevalence of retinal diseases and the significant worldwide morbidity of these diseases motivates moving stem cell-derived retinal cell therapies into human trials as fast, as safely and as ethically as possible.

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Table 1

A summary of salient experimental features from recent stem cell-to-retina data.

Cell source	Method	Culture Condition	Type of Cells Generated	Factors Tested	Markers Detected	Time to Photoreceptors	Time to RPE Pigmentation	Functional Tests	Transplantation Tested?	Comparison to hESC	Reference
Human Fibroblasts	Adherent Culture	Matrigel	RPE	B27, N2, Noggin, DKK1, IGF1, bFGF+- Nicotineamide, Activin, SU5402, VIP	Pmel17, nitf, tyrosinase, Tyrp2, PEDF, bestrophin	N/A	14 days	Phagocytosis	No	hiPSCs less efficient	61
Human Fibroblasts	Adherent Culture	PDL/ Laminin	RPE	Nicotineamide, Activin, NIC, SB431542	Mitf, OTX2, bestrophin, RLBPI	N/A	4 weeks	Phagocytosis, ion transport, membrane potential, polarized VEGF secretion	No	N/A	65
Human Fibroblasts	Adherent Culture	Defined conditions, Matrigel or VN-PAS Synthemax	RPE	Blebbistatin and removal of FGF	Mitf, RPE65	N/A	25-30 days	Phagocytosis	Yes; assessed survival and phagocytosis	Similar	60
Human RPE cells	Embryoid body	Suspension then gelatin	RPE	Removal of FGF	Bestrophin, ZO1, TIR, OTX2, Pax6	N/A	2-3 weeks	Phagocytosis	No	N/A	49
Human Fibroblasts	Adherent Culture	Defined conditions, tested gelatin, Matrigel, laminin, collagen-I, -IV, elastin	RPE	Removal of FGF	PEDF, Mitf, OTX2, PMel17, ZO1, bestrophin	N/A	5 weeks	phagocytosis (on hESCs)	No	iPSCs pigmented at signifcantly lower frequencies	51
Human Fibroblasts	Adherent Culture	MEFs then gelatin	RPE	Removal of FGF	RLBP, MERIK, Mitf, OTX2, PMEL17, RPECs, ZO1, PEDF, ATP1B1, bestrophin	N/A	4 week	In-vitro and in-vivo phagocytosis	Yes, RCS rats	hiPSC-RPE rejected by host retina while hESC survived 10 weeks	52
Mouse and Human Fibroblasts	Embryoid body	Suspension then PDL/laminin, fibronectin	RPE, photoreceptors	DKK1, Lefty A, (RA and taurine for phoreceptor cultures)	MITF, PAX6, RX, CRX, recoverin, RHO	120	40 days	None	No	Similar	57
Human Fibroblasts	Adherent Culture	MEFs or Ks27s	RPE	Removal of FGF	Mitf, OTX2, tyrosinase, Typ1, SILVER, ZO1, BEST, and after 6 months RPE65	N/A	20-30 days	Phagocytosis	No	Similar	50
Human Fibroblasts	Embryoid body	Suspension then PDL/laminin, fibronectin	RPE, photoreceptors	Small molecules CKI-7 and SB-431542 (RA and taurine for	CRALBP, Mitf, RPE65, ZO1, CHX10, CRX, recoverin, RHO	120-140	40 days	Phagocytosis of latex beads	No	Similar	58

Cell source	Method	Culture Condition	Type of Cells Generated	Factors Tested	Markers Detected	Time to Photoreceptors	Time to RPE Pigmentation	Functional Tests	Transplantation Tested?	Comparison to hESC	Reference
				photoreceptor cultures							
Human Fibroblasts	Embryoid body	Suspension then laminin	RPE, photoreceptors	Removal of FGF; N2, B27	Mitf, RPE65, ZO1, bestrophin, CHX10, CRX, recoverin, opsin	80	35 days	None	No	Similar	54
Human Fibroblasts	Adherent Culture	MEFs then Matrigel	RPE	Removal of FGF	RPE65,ZO1	N/A	3-4 weeks	Phagocytosis (only on hESCs)	No	hESCs appear to resemble fetal RPE more than hiPSCs	53
Human Fibroblasts from RP, LCA, Usher syndrome and LHON patients	Adherent Culture	Feeder-free Matrigel	RPE	Noggin, bFGF, RA, Shh	MITF, RPE65, CRALBP, BEST, PAX6, OTX2, ZO1	N/A	35 days	None	No	N/A	56
Fibroblasts of RP patients	Embryoid body	Suspension then PDL/ laminin/ fibronectin	RPE, photoreceptors	Lefty A, SB-431542, DKK1, taurine, RA	PAX6, RX, MITF, recoverin, rhodopsin	60-110 days	30 days	None	No	N/A	68
Fibroblasts from normal, RP, LCA, and Stargadt patients; also iris pigment epithelium	Embryoid body	Suspension then Synthemax for xeno-free and Matrigel for feeder-free	RPE, photoreceptors, retinal ganglion cells	B27, n2, noggin, DKK1, IGF1, DAPT, FGF	PAX6, RX, MITF, ZO-1, recoverin, rhodopsin, CHX10, NRL, BRN3b, NF200	90 Days	before 60 days	None	No	N/A	63
Human Fibroblasts	Adherent Culture	Feeder-free Matrigel	RPE, photoreceptors, retinal ganglion cells	B27, N2, noggin, DKK1, IGF1	OTX2, Crx, Nrl, arrestin, recoverin, Trb2, rhodopsin and Pax6, ZO1, Hu C/D, BRN3,	2 months	Not mentioned	None	Yes, subretinal transplant of FACS sorted IRBP-GFP-expressing photoreceptors	Similar	70
Human Fibroblasts	Embryoid body	Suspension or Matrigel for feeder-free and Synthemax for xeno-free cultures	RPE, neural retina	B27, N2, heparin (no heparin in xeno-free)	PAX6, RX, MITF, CHX10, OTX2, BEST1, ZO1, PEDF, recoverin, CRX, BRN3	60 days	1 month	None	No	Compared feeder, feeder-free and xeno-free; all similar results	64