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Ophthalmology

The promise of stem cells for age-related macular degeneration and other retinal degenerative diseases

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Transplanted cells can secrete numerous molecules that may exert a beneficial effect on the host retina and/or choroid even if they do not cure the underlying disease. Ideally, with a single transplant operation, many different pathways can be modified, which may reduce the chance of 'escape' associated with typical pharmacotherapy as well as the need for repeated drug administration. In addition, transplanted cells can replace dead cells (e.g. photoreceptors). Because of their pluripotency and unlimited proliferative capacity, stem cells seem to be a logical choice for starting material because they can be produced en masse safely and they can be induced to differentiate into ocular cells with potential for replacement and rescue therapy. Although preclinical studies demonstrate the feasibility of using embryonic stem cells and induced pluripotent stem cells for treating degenerative retinal diseases associated with abnormalities in the retinal pigment epithelium and/or photoreceptors, some issues may limit the use of stem cells in clinical practice. These issues include: immunogenicity of the cells, stability of cell phenotype (both inherent and environment-induced), the propensity to form tumors *in situ*, the abnormal microenvironment that can accompany degenerative disease and the synaptic rewiring that accompanies retinal degeneration. In the case of non-exudative age-related macular degeneration, cell transplants might prevent progression of geographic atrophy (through replacement of dysfunctional or dead RPE) and might even bring about some visual improvement in selected cases (through rescue of photoreceptors that are dying but not dead). Cell-based therapy may one day be sight-restoring for patients who are blind due to retinal degeneration of various etiologies. RPE transplantation is an attractive starting point for this sort of therapy as these cells can integrate with the host retina easily.

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Stem cells: definitions and classes [1]

Stem cells are unspecialized cells with the capacity for unlimited self-renewal, and each daughter cell has the capacity to remain a stem cell or to differentiate into more specialized, tissue- or organ-specific cells. Two transcription factors,

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Nanog and Oct4, are associated with helping to keep the cells in an undifferentiated state with the capacity for self-renewal. Different types of stem cells are considered below.

Human embryonic stem cells (hESCs)

In the blastocyst (three- to five-day-old, pre-implantation-stage embryo), the inner cell mass gives rise to the entire body of the organism, for example, brain, heart, lung, skin, sperm, eggs. hESCs are derived from the inner cell mass of the blastocyst. hESCs are pluripotent, which means that they can form all lineages of the body (ectoderm, mesoderm, endoderm). (Totipotent stem cells can form all lineages of the organism (including placenta).) hESCs can be obtained without destruction of the embryo [2].

Adult (somatic) stem cells

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, corneal limbal stem cells give rise to corneal epithelium [3,4], and adult Muller stem cells may be a source of photoreceptors [5,6]. Adult stem cells are multipotent, which means they can form multiple cell types of one lineage (e.g. retinal progenitor cell). Adult stem cells are present in many organs and tissues, for example, brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium and testis. Adult stem cells reside in a specific area of each tissue, termed a 'stem cell niche'. Some types of adult stem cells are pericytes. Adult stem cells may remain quiescent for long periods until activated by a normal need for more cells to maintain tissues, or by disease or injury.

Induced pluripotent stem cells (iPSCs)

Adult (somatic) cells can be reprogrammed to an embryonic state using somatic nuclear cell transfer [7]. Nuclear transfer may be more effective at establishing the ground state of pluripotency than factor-based reprogramming, which can leave an epigenetic memory of the tissue of origin that may influence efforts at directed differentiation for applications in disease modeling or treatment [8]. Adult cells also can be genetically reprogrammed to an embryonic stem cell-like state by being forced to express transcription factors using retroviruses or lentiviruses [9–11].

Although iPSCs are pluripotent stem cells, iPSCs and ESCs do differ in some important ways. iPSCs have the theoretical advantage of not being rejected by the patient from whom they are derived (vs. ESCs, unless the ESCs were harvested from the patient as an embryo), but abnormal gene expression in some cells differentiated from iPSCs (both via a retroviral and episomal approach) can induce a T-cell-dependent immune response in a syngeneic recipient [12]. Expression of these antigens is a reflection of epigenetic differences (e.g. DNA methylation) between iPSCs and ESCs [8,13–17]. Some evidence indicates that continuous passaging of iPSCs may help attenuate these differences [18], although iPSCs may retain a greater risk for tumor formation (e.g. due to p53 suppression) than ESCs. Nonetheless, there may be risks associated with using either ESCs or iPSCs that have been extensively passaged. Extensive passaging has been associated with alterations in the X-inactivation apparatus in ESCs and iPSCs, and these changes are linked to processes that may induce tumor formation such as upregulation of X-linked oncogenes, downregulation of tumor suppressor genes, accelerated growth rate *in vitro*, and poorer differentiation *in vivo* [19,20]. In preclinical models, if tumors, such as teratomas (a tumor with tissue or organ components derived from all three germ layers), are going to develop, they usually do so within three to six months of transplantation.

The therapeutic potential of iPSCs has been demonstrated in animal models of sickle cell anemia [21] and Parkinson's disease [22]. However, these cells contain multiple viral vector integrations that make them unsuitable for human clinical trials. The use of genome-integrating viruses can cause insertional mutagenesis and unpredictable genetic dysfunction [23,24]. The oncogenic properties of some transcription factors (e.g. *c*-Myc) also create safety concerns. Some progress to improve the safety of iPSCs has occurred. Modified protocols that do not require *c*-Myc, Sox-2 and/or Klf4, for example, have been described [25–29]. Also, mouse iPSCs can be created without viral vectors using expression plasmids rather than an integrating vector [30–33]. Other vector-free methods, using modified synthetic mRNA [34], recombinant proteins that can penetrate the plasma membrane of somatic cells [35,36], or exposing somatic cells to ESC-conditioned media [37] have been used to reprogram cells to pluripotency, which may be safer than using viral vectors to induce reprogramming [34]. Additional progress in this area may usher in the era of truly personalized regenerative medicine.

Human iPSCs might be used to study disease pathogenesis, for high-throughput screening to identify small molecule therapy, as well as for cell-based therapy for regenerative medicine [38,39]. In the case of disease models in which the phenotype is associated with X chromosome inactivation or genomic imprinting, however, one must verify the epigenetic status of the PSCs because with increasing time in culture, epigenetic and transcriptional aberrations have been documented in genes subject to X chromosome inactivation and genomic imprinting [20,40]. (Genomic imprinting is a phenomenon in which monoallelic gene expression occurs in a parent-of-origin-specific manner and can occur in the germline ('gametic') or in the post-implantation embryo ('somatic') in association with spreading of gametic imprints [41].) This phenomenon can result in gradual derepression of genes normally subjected to X chromosome inactivation [40].

Several ocular tissues have been derived from stem cells (Table 1).

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Table 1. Stem cells as sources of ocular tissue

Stem cell	Stem cell-derived ocular tissue
Limbal stem cell	Corneal epithelium [4,42]
Trabecular meshwork progenitor cell	Trabecular meshwork cells [43]
Embryonic and/or induced pluripotent stem cells	Retinal ganglion cells [44–46] Retinal pigment epithelial cells [47–54] Photoreceptors [50–52,55–57]

Embryonic vs. adult vs. induced pluripotent stem cells for cell-based therapy

Embryonic, adult and reprogrammed (including nuclear transfer, cell fusion, or genetic manipulation to create a pluripotent cell) stem cells each have advantages and disadvantages as therapeutic modalities (Table 2). Although each of these donor cell lines, unless manipulated, harbors disease-causing genes of the donor, this fact may not have practical significance. In the case of diseases such as age-related macular degeneration (AMD), for example, the time needed to redevelop retinal pigment epithelium (RPE) and photoreceptor damage after cell transplantation might exceed the expected life span of the recipient, who might be in the eighth or ninth decade of life. As tissues derived from ESCs can be rejected even if there is only a single minor histocompatibility antigen mismatch between donor and recipient [58], control of the immune response may be an important aspect of stem cell therapy even if iPSCs are used [59]. Detailed consideration of the immunology of stem cell transplants is beyond the scope of this review, but differentiated progeny of ESCs express MHC class I antigens [60,61]. Several different strategies to circumvent immune rejection of transplanted stem cells have been explored [58,59,62–73]. The role of the immune suppressive nature of the subretinal space as well as the inherent immunological properties of the transplanted tissue (e.g. photoreceptors or RPE cells) in mitigating this requirement is not clear at this time [74].

Therapeutic strategies for cell-based therapy: replacement vs. rescue

Replacement

Replacement therapy is an approach to regenerative medicine in which cells that have died or are dysfunctional are replaced by healthy cells. For example, in retinitis pigmentosa (RP), photoreceptors die. Replacement therapy for RP could involve transplantation of cells that can integrate with the host retina and function as photoreceptors. Replacement retinal therapy is sight-restoring.

To be useful for cell replacement therapy, stem cells must *proliferate* extensively to generate sufficient quantities of material to serve as a 'universal donor'. In addition, they must *differentiate* into the desired cell type(s). hESC-derived RPE can spontaneously dedifferentiate to non-RPE-like cells and spontaneously redifferentiate into RPE-like cells, indicating phenotypic instability [47]. The cultures may not retain a stable phenotype after 5–8 passages. ESCs and iPSCs vary in their tendency to differentiate into cells of a given lineage [8,75].

What defines a 'differentiated' RPE cell? Bharti *et al.* [76] have summarized several potentially important features of differentiated RPE cells such as proper expression of signature genes, microRNA and appropriate physiology (e.g. transepithelial resistance) and anatomy (e.g. proper distribution of ion channels).

What defines a photoreceptor cell? Gene expression profiling has been used to determine how closely ESC-derived retinal cells resemble normal retina, the developmental stage of the ESC-derived cells (relative to fetal retinal cells), and whether there are significant contaminating non-retinal cells [77]. These studies indicate that some minimal contamination with non-retinal cells (e.g. RPE, ciliary epithelium) can occur, but that undifferentiated, pluripotent cells decline with time in culture, which may mean that a longer duration differentiation protocol may minimize the risk of teratoma formation. Some features of photoreceptor differentiation rely on interactions with surrounding cells. Interaction of photoreceptors with RPE is crucial for foveal development

Table 2. Embryonic vs. adult vs. induced pluripotent stem cells for cell-based therapy

Cell type	Advantages	Disadvantages
Embryonic stem cell	Pluripotent (can form all lineages of the body: ectoderm, mesoderm, endoderm)	Likely to be rejected (if donor is allogeneic, unmatched)
	Grown relatively easily	Harbors disease-causing genes of donor
Adult stem cell	Multipotent (can form multiple cell types of 1 lineage, e.g. retinal progenitor cell)	Relatively hard to harvest
	Not rejected if transplanted into donor	Harbors disease-causing genes of donor
Induced pluripotent stem cell	Pluripotent	May retain epigenetic features of cell type of origin
	Grown relatively easily	Harbors disease-causing genes of donor
	Probably not rejected if transplanted into donor	

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[78]. Interaction with Muller cells via zonula adherens (crumbs homolog 1 protein) is important for normal outer retinal organization [79].

The retinal and subretinal microenvironment can influence the differentiation and functionality of transplanted cells, including expression of developmental markers and markers of proliferation [48,55,80,81]. The recipient's microenvironment can also influence transplanted cell survival. For example, although human iPSC-derived RPE survive 4 months in RCS rats (xenograft) [53,54], abnormalities in Bruch's membrane may prevent transplanted hESC-derived RPE from surviving and differentiating long-term in AMD eyes [82]. Because Bruch's membrane is derived from mesoderm, there is no expectation that hESC- or iPSC-derived RPE will manufacture Bruch's membrane. Abnormalities in RPE of AMD eyes might prevent transplanted ESC-derived photoreceptor transplants from surviving. Also, cone survival depends on rod survival [83–85]. Typical RP is characterized by early rod photoreceptor death. Therefore, it might be best to transplant a mixture of rods and cones to achieve improvement in cone-mediated visual function.

In addition to differentiation and survival, replacement therapy requires that the transplanted cells *integrate* into the surrounding tissue after transplantation. Targeted disruption of glial reactivity and disruption of the outer limiting membrane may improve integration of transplanted cells with the inner retina [86–88]. The developmental age of the donor cells may be crucial for successful integration with host retina [89], but it is not clear that this is the case [90]. Synaptic reorganization of the retina occurs in association with photoreceptor degeneration in RP [91]. This reorganization might limit the extent of functional photoreceptor integration with the host. Presumably, if the transplanted cells have differentiated and integrated appropriately, they also will function physiologically in the host tissue.

Rescue

The term *rescue* refers to the preservation and, in some cases, restoration of function of host tissue that is destined to die or malfunction due to an underlying disease. Effective retinal rescue therapy is sight-preserving, and it also may be sight-restoring to the degree that dying cells, which cannot support vision, can return to normal physiological function as a result of the transplant. For example, degenerating photoreceptors may first lose their outer segments, and thus become inefficient transducers of light energy. Rescue therapy might restore photoreceptor physiology with elaboration of outer segments and corresponding recovery of lost vision.

To be useful for cell-based rescue therapy, transplanted cells must elaborate needed trophic factors and not proliferate in an uncontrolled manner. Stem cells can be used for rescue therapy. In a preclinical model of glaucoma, for example, intravitreal somatic neural stem cells [92] and bone marrow-derived mesenchymal stem cells [93] can substantially reduce retinal ganglion cell death. In preclinical models of degenerative retinal diseases such as RP, bone marrow-derived mesenchymal stem cells and hESC-derived RPE rescue photoreceptors [94–96].

Combined replacement and rescue

RPE cell transplants are an attractive starting point for cell-based combination replacement and rescue therapy in the eye because hESCs and iPSCs can be induced to differentiate into RPE relatively easily, and one can generate large quantities of cells with a stable and appropriate genotype and phenotype [47,53,97–101]. In addition to the relative ease of producing differentiated RPE from stem cell progenitors, RPE cells integrate easily with host photoreceptors, and RPE cells elaborate trophic substances that support photoreceptors [82,102,103]. There is abundant evidence for RPE transplant efficacy in preclinical models [74]. Diseases in which RPE cells appear to be targeted primarily include Best disease [104,105] and some forms of RP [106,107], and secondarily include Stargardt macular dystrophy [108,109] and age-related macular degeneration (AMD) [110,111]. However, in the case of AMD eyes, survival and proper differentiation on submacular Bruch's membrane may be problematic [82].

Stem cell treatment of retinal degenerative disease

Stem cell therapy has been effective in preclinical models of retinal degenerative disease, including models of RP and Stargardt macular dystrophy (Table 3).

Stem cells are being used in human clinical trials to treat degenerative retinal diseases, including Stargardt macular dystrophy, AMD and RP (Table 4). These studies represent early efforts in this area, and there are no phase III studies underway at this time. Two of the studies have published preliminary results, and these studies are considered in greater detail below.

Stargardt macular dystrophy

Stargardt macular dystrophy is the most common macular dystrophy of childhood [122]. Currently, there is no proven treatment for this condition although gene therapy (ClinicalTrials.gov identifier: NCT01367444; Sponsor: Oxford BioMedica) and nutritional supplementation (NCT01278277; Sponsor: Catholic University of the Sacred Heart (Rome)) are under study. Experiments in an animal model of Stargardt disease indicated that hES-derived RPE could rescue photoreceptors [99]. Schwartz *et al.* [123] reported that four months after subretinal transplantation of hESC-derived RPE into a patient with advanced Stargardt macular dystrophy, visual acuity improved from hand motions before surgery to 20/800 (Table 4). There was no improvement in the unoperated fellow eye. Clinical exam disclosed the presence of pigmented cells at the transplant site, and these cells seemed to proliferate during the four-month period of observation. Optical

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Table 3. Retinal stem cell therapy: preclinical models

Disease	Cell type	Delivery route	Effect
Rd1 and rd10 mouse [112]	BM-derived lineage-negative hematopoietic SCs	Intravitreal	Rescue photoreceptors (primarily cones)
Rhodopsin knockout mouse [94]	BM-derived MSCs	Subretinal	Rescue photoreceptors
Ischemic retinopathy [113]	Endothelial progenitor cells	Intravitreal	Vascular repair and reversal of ischemic injury
Rd1 [114], **mnd** [115] **and CRX$^{-/-}$** [116] **mouse**	Human ESCs	Intravitreal Subretinal	Replace photoreceptors
RPE 65$^{-/-}$ mouse	ESC-derived RPE	Subretinal	Rescue photoreceptors[a]
RCS rat [95]	BM-derived MSCs	Intravenous	Rescue photoreceptors and preserved retinal function
RCS rat [47,49,81,99,117]	Human ESC-derived RPE	Subretinal	Rescue photoreceptors and improved visual function
RCS rat [118]	Human neural progenitor cells	Subretinal	Rescue photoreceptors and improved visual function
RCS rat [54]	Human iPSC-derived RPE	Subretinal	Rescue photoreceptors and improved visual function
RCS rat [119]	Human umbilical cord-derived SCs	Subretinal	Rescued photoreceptors and improved visual function
Elov14 mouse [99]	Human ESC-derived RPE	Subretinal	Rescued photoreceptors
Ush2a mouse [120]	Forebrain-derived progenitor cells	Subretinal	Reversed mislocalization of cone pigment and prevented functional deterioration

SCs, stem cells; BM, bone marrow; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; iPSC, induced pluripotent stem cell.
[a] Teratomas formed in this study.

Table 4. Human stem cell trials for retinal disease

Disease	Phase	No. patients (no. cells transplanted)	Center (PI)	Sponsor
Stargardt macular dystrophy (NCT01345006)[a]	I/II	3 (5×10^4 hES-RPE) 3 (10^5 hES-RPE) 3 (1.5×10^5 hES-RPE) 3 (2×10^5 hES-RPE)	Jules Stein-UCLA (Schwartz) Wills Eye Hospital (Regillo) Moorfields Eye Hospital (Bainbridge)	Advanced Cell Technology
AMD-GA (NCT01344993)[a]	I/II	3 (5×10^4 hES-RPE) 3 (10^5 hES-RPE) 3 (1.5×10^5 hES-RPE) 3 (2×10^5 hES-RPE)	Jules Stein-UCLA (Schwartz) Wills Eye Hospital (Regillo)	Advanced Cell Technology
AMD-GA or CNV (NCT01518127)	I/II	10 (10^7 autologous bone marrow-derived SCs)	University of Sao Paulo, Brazil (Siqueira)	University of Sao Paulo
RP and cone-rod dystrophy (NCT01068561)	I/II	5 (10^4 autologous bone marrow-derived SCs)	University of Sao Paulo, Brazil (Siqueira *et al.* [121])	University of Sao Paulo

AMD, age-related macular degeneration; GA, geographic atrophy; CNV, choroidal neovascularization; RP, retinitis pigmentosa; hES-RPE, human embryonic stem cell-derived retinal pigment epithelium; SCs, stem cells.
[a] Allogeneic cell transplant.

coherence tomography (OCT) indicated that the pigmented cells were organized in a monolayer. OCT images of the retina overlying these cells did not demonstrate improved photoreceptor anatomy, and the subjacent choroid seemed unchanged also (Figure 1). There was no evidence of teratoma formation or immune rejection of the transplanted cells. This work is part of a Phase I/II open-label, prospective, multicenter study to determine the safety and tolerability of subretinal transplantation of hESC-derived RPE cells in patients with Stargardt macular dystrophy and AMD. As part of the treatment protocol, the patient received a seven-week course of tacrolimus and mycophenolate mofetil starting one week before surgery. Per protocol, at week 6 after surgery, tacrolimus was discontinued and mycophenolate mofetil was continued for an additional six weeks. Although the subretinal space is an immune privileged environment, this privilege is not absolute [74]. Furthermore, RPE cells express HLA Class II antigens. Thus, a period of immune suppressive therapy was prescribed to reduce the likelihood of immune rejection of the transplanted allogeneic hESC-derived RPE.

Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness in persons older than 55 years in the United States

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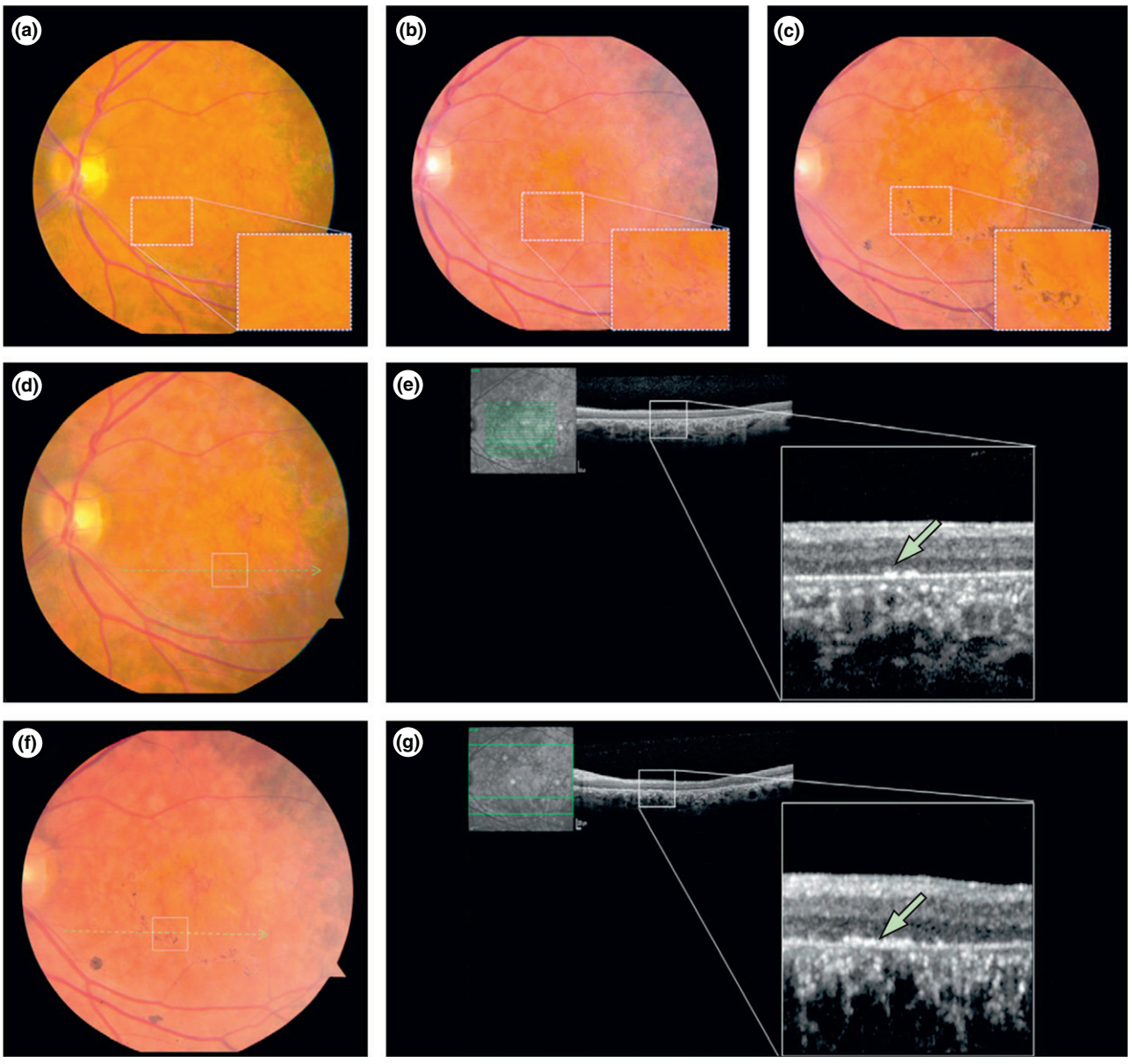

Figure 1. Images of the hESC-RPE transplantation site in the patient with Stargardt macular dystrophy. Color fundus photographs of the patient's left macula preoperatively and postoperatively (A–C). The region inside the rectangle bisects the border of the surgical transplantation site and corresponds to macular atrophy not included in the surgical injection. (A) Baseline macular color image with widespread RPE and neurosensory macular atrophy. (B) Color macular Image 1 week after hESC-RPE transplantation. Note the mild pigmentation most evident in the region of baseline RPE atrophy. This pigmentation increased at week 6 (C). (D–G) Color fundus photographs and SD-OCT images at baseline (D) and month 3 after transplant (F). Color images show increasing pigmentation at the level of the RPE from baseline to month 3. Registered SD-OCT images (E, G) show that increasing pigmentation is at the level of the RPE, normal monolayer RPE engraftment, and survival at month 3 (arrow) adjacent to region of bare Bruch's membrane devoid of native RPE. hESC, human embryonic stem cells; RPE, retinal pigment epithelium; SD-OCT, spectral domain optical coherence tomography.
Reproduced with permission from Schwartz *et al.* [123].

[124]. Patients can experience profound visual loss due to the growth of choroidal new vessels (CNVs) under the fovea or due to geographic atrophy (GA) involving the fovea. The latter probably is due to AMD-induced RPE death. Although there are effective treatments for CNVs [125], there is no proven therapy for GA currently. Several novel treatments are under study [125] as described in greater detail elsewhere in this issue.

Schwartz *et al.* [123] reported that four months after subretinal transplantation of hESC-derived RPE into a patient with GA, vision improved from 20/500 at entry to 20/200 by week 2 after surgery (Table 4). Visual acuity was 20/320 by week 6 and remained stable at the three-month follow-up visit. Of note, mild visual improvement was also noted in the unoperated fellow eye after surgery. This patient received

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tacrolimus and mycophenolate mofetil as described above for the patient with Stargardt disease.

Retinitis pigmentosa

Siqueira *et al.* [121] reported the results of a prospective phase I, nonrandomized open-label study of RP patients with best-corrected ETDRS visual acuity worse than 20/200 (Table 4). Three patients with RP and two with cone-rod dystrophy underwent intravitreal injection of autologous bone marrow-derived mononuclear cells with no adverse effects (and no documented benefit at 10-months follow-up).

Summary

Why should one develop cell-based therapy in the current era of pathway-based pharmacological therapy for retinal disease? Transplanted cells can secrete numerous molecules that may exert a beneficial effect on the host retina and/or choroid even if they do not cure the underlying disease [83,99,102,103,126]. Ideally, with a single transplant operation, many different pathways can be modified, which may reduce the chance of 'escape' associated with monotherapy as well as the need for repeated drug administration. In addition, transplanted cells can replace dead cells (e.g. photoreceptors). Because of their pluripotency and unlimited proliferative capacity, stem cells seem to be a logical choice for starting material because they can be produced en masse safely and they can be induced to differentiate into ocular cells with potential for replacement and rescue therapy. Although preclinical studies demonstrate the feasibility of using ESCs and iPSCs for treating degenerative retinal diseases associated with abnormalities in the RPE and/or photoreceptors, some issues may limit the use of stem cells in clinical practice. These issues include: immunogenicity of the cells, stability of cell phenotype (both inherent and environment-induced), the propensity to form tumors *in situ*, the abnormal microenvironment that can accompany degenerative disease and the synaptic rewiring that accompanies retinal degeneration. In the case of non-exudative AMD, cell transplants might prevent progression of geographic atrophy (through replacement of dysfunctional or dead RPE) and might even bring about some visual improvement in selected cases (through rescue of photoreceptors that are dying but not dead). Cell-based therapy may one day be sight-restoring for patients who are blind due to retinal degeneration of various etiologies. RPE transplantation is an attractive starting point for this sort of therapy because these cells can integrate with the host retina easily.

Conflicts of interest

Dr Zarbin has served as a paid consultant for Advanced Cell Technology, Alimera Sciences, Allergan, Celgene, Eli Lilly, Genetech, Iridex, Novartis and Pfizer. Together with the University of Medicine and Dentistry of New Jersey and his co-inventors, Dr Zarbin has patents pending regarding methods to improve cell-based therapy for retinal degenerative disease.

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