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Concise Review

Recent Progress in Photoreceptor Cell-Based Therapy for Degenerative Retinal Disease

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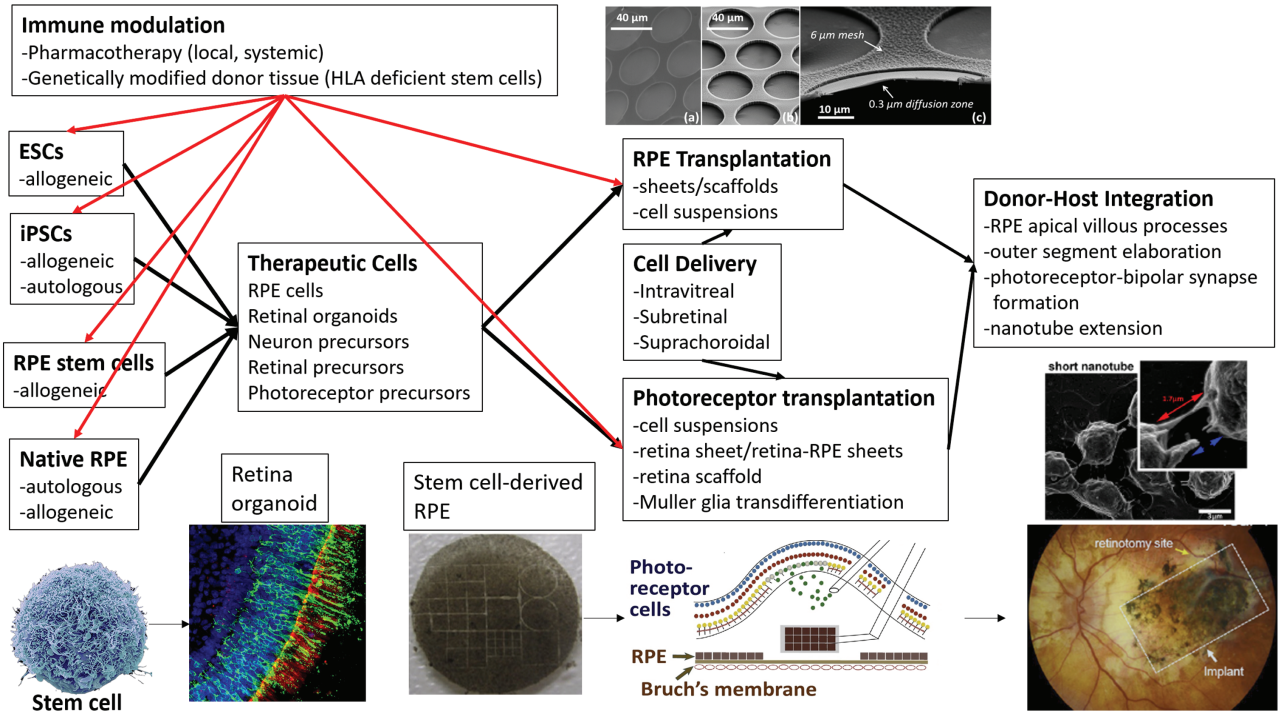
Abstract

Age-related macular degeneration and retinitis pigmentosa are degenerative retinal diseases that cause severe vision loss. Early clinical trials involving transplantation of photoreceptors as treatment for these conditions are underway. In this review, we summarize recent progress in the field of photoreceptor transplantation, including some pertinent results regarding photoreceptor manufacture, photoreceptor transplantation, mechanisms of donor–host cell integration such as material transfer and photoreceptor transplant immunology. We conclude by proposing several approaches that may provide a rational basis for selecting a vision restoration strategy (eg, donor–host synapse formation vs donor–host nanotube formation) and improved transplant efficiency.

Key words: geographic atrophy; macular degeneration; induced pluripotent stem cells; embryonic stem cells; cell transplantation; retina; retinal pigment epithelium; retinitis pigmentosa.

Graphical Abstract



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Significance Statement

This review summarizes recent clinical and preclinical studies involving photoreceptor transplantation as treatments for age-related macular degeneration and retinitis pigmentosa. Unresolved issues involving transplant immunology, cell delivery, cell manufacture, and mechanisms of sight restoration are explored in detail. We propose areas in which additional research could help accelerate progress in cell-based therapy for blinding retinal disease.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the industrialized world among persons aged 55-years and older.[1] By 2040, ~288 million persons worldwide will have AMD.[1] In the advanced stages of the disease, patients lose central vision due to the growth of abnormal blood vessels (termed choroidal new vessels) under the central retina (termed the macula), which leads to impairment of reading, driving, recognizing faces, and living independently. This outcome affects ~10%-15% of patients with AMD and can be treated reasonably well with intravitreal injections of agents that interfere with vascular endothelial growth factor (VEFG) signaling.[2] The other form of advanced AMD causing central visual loss involves atrophy of the macular photoreceptors and the subjacent retinal pigment epithelium (RPE) and choriocapillaris and is termed geographic atrophy (GA). In the US, ~1.2 million patients are projected to have AMD-GA by 2033 with an annual incidence of 160 000.[3] Retinitis pigmentosa (RP) comprises a group of inherited retinal degenerative diseases characterized by progressive photoreceptor death and profound visual loss, in many cases total blindness.[4] The global prevalence is approximately 1:2000.[5] In most RP patients, the pathogenic mutation affects photoreceptors primarily, but in some cases (eg, MERTK mutation) the pathogenic mutation is expressed in the RPE with secondary photoreceptor degeneration.[6]

Recently, medications that block activation of complement factor 3[7,8] or complement factor 5[9] have been approved by the US Food and Drug Agency (FDA) to treat AMD-GA. These therapies do not seem to restore lost vision but delay the rate at which AMD-GA progresses. Gene therapy is available to treat a rare cause of Leber Congenital Amaurosis,[10-12] and numerous clinical trials using mutation-specific gene therapies as well as mutation-agnostic gene therapy[13] for different forms of RP are underway. In addition, retinal prostheses are available to treat patients with profound visual loss due to photoreceptor degeneration.[14-16]

Cell-Based Therapy

An alternative approach to treat the late-stage causes of AMD-associated blindness involves cell-based therapy to replace damaged RPE and/or photoreceptors. Photoreceptor transplantation offers the potential for sight restoration, which would be beneficial for patients with AMD-GA involving the fovea as well as for patients with other causes of photoreceptor-related blindness such as RP. In principle, photoreceptor precursors can improve vision by producing trophic factors that *rescue* native photoreceptors and/or by *replacing* lost photoreceptors. A variety of cell sources might be used (Table 1). Photoreceptor rescue also might be achieved using retinal or neural progenitor cells, fibroblast-derived induced pluripotent stem cells (iPSCs), bone marrow-derived stem cells, or umbilical cord mesenchymal stem cells. Early-phase clinical trials exploring all these possibilities are underway (Table 2).[17,18] This review is focused on recent progress in photoreceptor transplantation as a *replacement* strategy for the treatment of AMD-GA and RP.

During the past several years, some important developments in photoreceptor cell transplantation have occurred

1. Photoreceptor manufacture: Retinal organoids may serve as vehicles for high throughput screening of mammalian photoreceptor rescue strategies as well as a source of photoreceptors for transplantation.[17] Methods are being developed to promote endogenous retinal neuron production via Muller glial (MG) cell transdifferentiation with limited photoreceptor production but generally more robust bipolar and retinal ganglion cell production.[19,20]
2. Photoreceptor transplantation: Human embryonic stem cell (ESC)- and iPSC-derived retinal sheets[21-26] and suspensions[27-29] have been transplanted into preclinical models of RP and in some cases have demonstrated recovery of light sensitivity.
3. Material transfer: Photoreceptor transplants can establish meaningful non-synaptic connections with host photoreceptors (via photoreceptor nanotubes) that can promote host photoreceptor survival in pre-clinical models, including nonhuman primates (NHPs).
4. Photoreceptor transplant immunology: HLA-matched allogeneic photoreceptor grafts exhibit reduced immune cell infiltration and improved graft survival in preclinical RP models, but it has been established that when immune recognition of the graft occurs, it may not be evident clinically.

We will summarize briefly the background and progress in each of these areas and propose areas in which additional research could help accelerate progress in cell-based therapy for blinding retinal disease. Familiarity with the anatomy and physiology of the RPE and retina is presumed in what follows. (see Figure 1 in the accompanying article on RPE transplantation for an overview of relevant anatomy.) Although preliminary studies in humans have been reported,[30-32] photoreceptor cell replacement therapy has not yet advanced to the state of RPE replacement therapy although much has been

Table 1. Some sources of photoreceptors for transplantation.

Photoreceptor cell sources	
Precursor cells	Neuron precursor cells
	Retinal precursor cells
Stem cells	Induced pluripotent stem cells
	Embryonic stem cells
Muller glial cell transdifferentiation	

Table 2. Clinical studies of retinal transplantation.

Study Title	Clinicaltrial.gov ID	Cell type	Disease target	Trial design	Results
Production of patient autologous induced pluripotent stem cell-derived retinal cells for AMD	NCT05991986	iPSC from somatic cells	AMD	Observational	None
Generation of induced pluripotent stem cell (iPSC) lines from skin fibroblast cells of participants with AMD	NCT03372746	iPSC from skin fibroblast	AMD	Observational	None
Safety study of allogenic hiPSC-retinas in RP (hiRERP)	jRCTa050200027	Allogenic iPSC-retinal sheets	RP	Interventional	None
Safety of repeat intravitreal injection of human retinal progenitor cells (jCell) in adult subjects with RP	NCT04604899	Retinal progenitor cells	RP	Phase II	R
Safety and efficacy of intravitreal injection of human retinal progenitor cells in adults with RP	NCT03073733	Retinal progenitor cells	RP	Phase II	R
Clinical study to assess safety and efficacy of subretinal injection of human neural progenitor cells for treatment of RP	NCT04284293	Retinal progenitor cells	RP	Phase I/IIa	None
First-in-human phase I/IIa, open-label, prospective study of the safety and tolerability of subretinally transplanted human retinal progenitor cells (hRPC) in patients with RP	NCT02464436	Retinal progenitor cells	RP	Phase I/IIa	None
Phase I/II study of the safety and preliminary efficacy of human CNS stem cells (HuCNS-SC) subretinal transplantation in subjects with geographic atrophy of AMD	NCT01632527	Human CNS stem cells	RP	Phase I/IIa	None
Long-term follow-up study of the phase I/II safety and preliminary efficacy of human CNS stem cells (HuCNS-SC) subretinal transplantation in subjects with geographic atrophy of AMD	NCT02137915	Human CNS stem cells	AMD	Phase I/IIa	None
Study of HUCNS-SC subretinal transplantation in subjects with GA of AMD (RADIANT)	NCT02467634	Human CNS stem cells	AMD	Phase II	None
Safety study in retinal transplantation for dry AMD	NCT00346060	Fetal retinal tissue	AMD	Phase 2	None
Safety study in retinal transplantation for RP	NCT00345917	Fetal retinal tissue	RP	Phase 2	None
Long-term safety of UC-MSC transplantation in patients with RP	NCT05786287	Umbilical cord mesenchymal stem cells	RP	Observational	None
Autologous bone marrow-derived stem cells transplantation for RP (RETICELL)	NCT01560715	Bone-marrow-derived stem cells	RP	Phase 2	None
Autologous bone marrow-derived stem cells transplantation for RP	NCT01068561	Bone-marrow-derived stem cells	RP	Phase 1	None
Role of UC-MSC and CM to inhibit vision loss in RP phase I/II	NCT05909488	Umbilical cord mesenchymal stem cells	RP	Phase 2/3	None
Autologous bone marrow-derived CD34+, CD133+, and CD271 + stem cell transplantation for RP	NCT02709876	Bone-marrow-derived stem cells	RP	Phase 1/2	None

achieved since the early pre-clinical experiments of the late 20th century.[17]

Photoreceptor Manufacture

Photoreceptors and photoreceptor precursors can be isolated from ESCs, stem cell-derived retinal organoids, and fetal tissue.[33,34] ESC-derived retina organoids can be induced to develop photoreceptor populations enriched in cones.[28] One also can manufacture retina sheets from human ESCs and iPSCs.[35-40] From a practical standpoint, it seems most likely that human iPSC-derived 3-D retinal organoids will provide the best source both with regard to the number of photoreceptors that can be generated and also with regard to issues concerning the use of fetal and embryonic tissue.[38,41-45] Currently there is a preference to use cells enriched in photoreceptor precursors or retinal sheets instead of retinal progenitor cell donor material.[21,26,46,47] Transplanted photoreceptors might not need to form synapses with host retinal bipolar cells, as they may be able to rescue remaining viable host photoreceptors via material transfer (see below).

A more challenging approach to photoreceptor restoration involves transdifferentiation of MG into photoreceptors. After damage, MG, which are endogenous retinal glial cells, can be stimulated to de-differentiate, proliferate, and act as neural progenitors.[48] Experimentally induced MG transdifferentiation generally involves induced expression of transcription factors typically expressed during fetal retinal development, eg, Ascl1 and Atoh1.[19,49] While synapses between newly formed retinal neurons and pre-existing retinal neurons seem to occur, most of the cells generated have been retinal bipolar or ganglion cells. The neurogenic potential of MG may be suppressed by nuclear factor kappa B (NFkB) signaling,

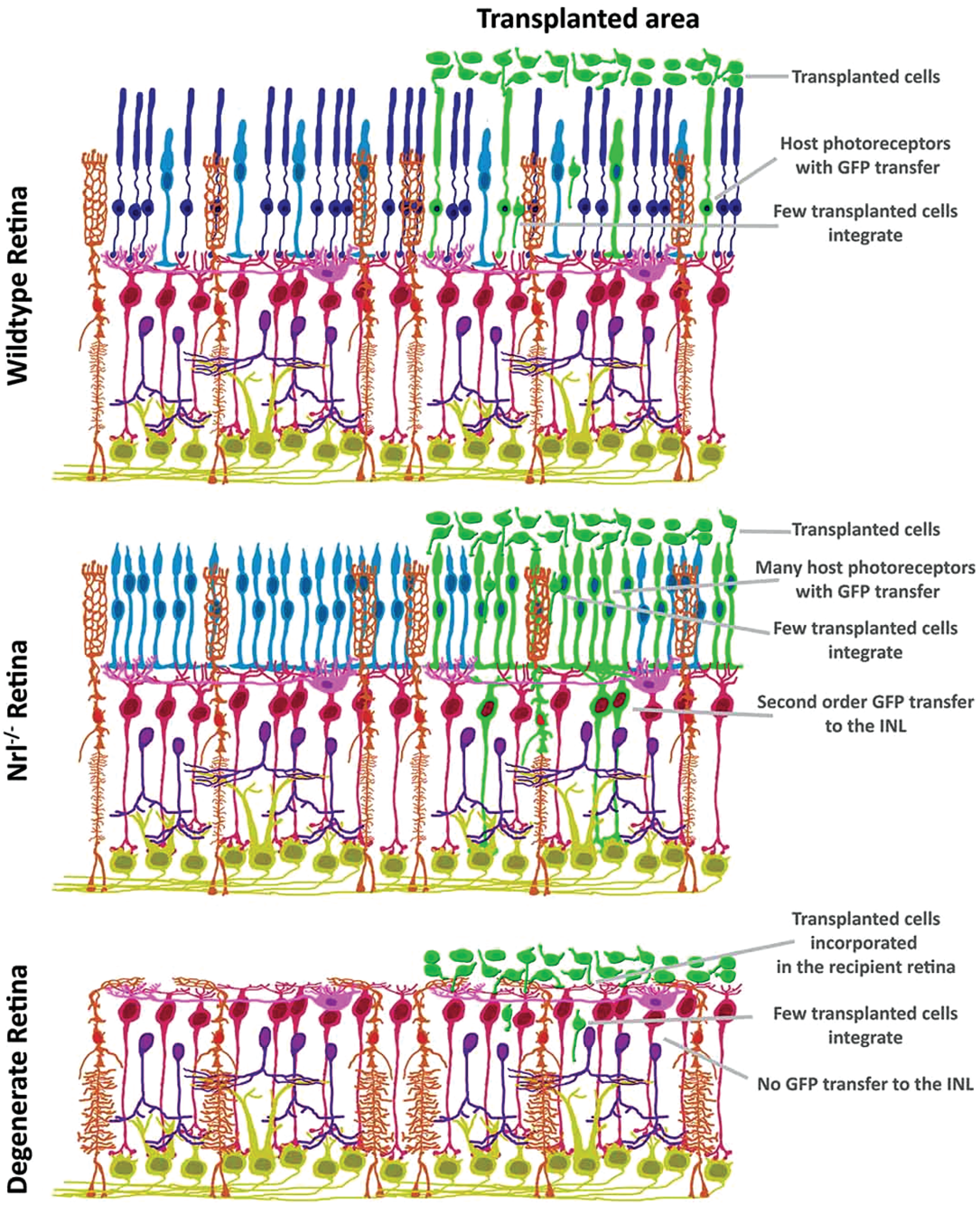

Figure 1. Variability in green fluorescent protein (GFP) patterning is dependent on mutant background. In contrast to the restricted outer nuclear layer (ONL)-GFP signal observed in wild-type background at 21 days following transplant (top), the NRL$^{-/-}$ hybrid-cone only recipient (middle) exhibits robust GFP signal that extends to Müller glia and bipolar neurons. In contrast, no GFP material exchange has been reported in the degenerating retina (bottom), suggesting a photoreceptor-to-photoreceptor modality of intercellular communication. Reproduced with permission from Nickerson et al[133].

which is a typical response to retinal injury.[50] Inhibition of NFkB signaling as well as inhibition of histone deacetylase (eg, using trichostatin A) may help to promote neurogenesis from endogenous MG.[51] (Injury to fish retina (eg, zebrafish) induces MG transdifferentiation to retinal neurons more readily than in mammals probably because the inflammatory response is transient in fish whereas it is sustained in mammals.[52]) It seems likely that clinically useful production of endogenous photoreceptors via MG transdifferentiation will require sequential expression of transcription factors as well as epigenetic modifications that recapitulate the events occurring during fetal development.[20,53,54] Fujii et al[55] reported rhodopsin-positive cell production by intravitreal injection of transforming growth factor (TGF)-beta inhibitor, bone morphogenetic protein inhibitor, glycogen synthase kinase 3 inhibitor, and gamma-secretase inhibitor in N-methyl-N-nitrosourea-treated and rd10 mice. (The rd10 mouse has a mutation found in some human patients with RP, and nitrosourea causes photoreceptor damage with relative preservation of the inner retinal neurons.) In the rd10 mice, production of rhodopsin-positive cells (presumed similar or identical to rod photoreceptors) was associated with improvement in the scotopic ERG, and improved cone photoreceptor survival also was documented. While this approach

cannot be translated directly into patients (eg, due to the timing of the injections), it does provide evidence that transdifferentiation might be an effective approach for endogenous cell replacement therapy.

Photoreceptor Transplantation

The developmental stage of the donor cell significantly affects the outcome of photoreceptor transplantation, as post-mitotic cells can yield better outcomes than retinal progenitors.[56-59] Allo- and xenograft photoreceptor transplants can partially restore vision in preclinical models of RP and can generate rods as well as cones.[21,26,47,56,59-72] Initially, this restoration was thought to be due entirely to the establishment of synaptic connections between donor and host neurons. Additional research demonstrated that, in many cases, most of the function restoration probably was due to the transfer of cytoplasmic material, including phototransduction proteins, between donor and recipient photoreceptors through material transfer.[73-77] At less advanced stages of degeneration, in which some host photoreceptors remain, donor photoreceptors can transfer endogenous molecules to recipient photoreceptors through material transfer.[73-75,77] In settings where essentially all the photoreceptors have died, however, donor photoreceptors seem to form synaptic connections with host inner retinal neurons (Fig. 1).[21,24,29,46,67,72,78-80] (Even in the latter setting, however, there may be some surviving host photoreceptors.[26,67,81,82]) The presence of native photoreceptors does not preclude donor-host synapse formation. Gasparini et al[59] transplanted human organoid-derived cone precursor-enriched cell suspensions into a murine model in which dysfunctional cones rapidly degenerate, and rods are largely unaffected. During the ensuing 6 months, the transplanted cones integrated into the host ONL, established functional synapses with host bipolar cells, and extended shortened outer segments.[59] Although synaptic architecture exhibits significant species differences that might limit the ability of functional synapses to form in xenografts,[82] some studies show that xenografts can establish functional synaptic contact with host retina.[26,29,59,67,69,71,72] Intact outer limiting membrane (OLM), gliosis, and high levels of chondroitin sulfate proteoglycan also can impair transplanted photoreceptor precursor tissue integration with host retina,[62,71,72,83-86] which may underlie the finding that the efficacy of synapse formation between donor photoreceptors and host bipolars depends on the host "environment," ie, (1) the cause of the host photoreceptor degeneration and (2) the stage of the degenerative process.

Although glial barriers can impair photoreceptor integration, several studies have demonstrated that transplanted photoreceptors exhibiting host integration are intimately associated with host MG, forming adherens junctions even in xenografts.[59,67,72] This finding as well as the time required for photoreceptor transplants to express proteins consistent with terminal differentiation (including those associated with synapse formation) and other second-order events such as bipolar neurite and Muller cell process extension into the graft, suggests that donor–host interactions are required for maturation, proper morphology, and full function of the transplant.[59,87]

As is the case for RPE transplantation, dissociated photoreceptor cell injections are relatively simple and minimally invasive, but cell survival can be suboptimal (Table 3). Vitrectomy with excision of the posterior hyaloid face and creation of a "pre-bleb" with balanced salt infusion into the subretinal space limits the efflux of transplanted cells from the subretinal space into the vitreous cavity.[72] Unfortunately, subretinal delivery of a cell suspension tends to result in a gravitationally dependent distribution of the transplanted cells rather than uniform coverage of the transplant area. (This outcome might be mitigated by strict prone head positioning after surgery.) In contrast, retinal sheet delivery provides better cell organization, but the surgical technique is more complex and requires specialized instruments. An intermediate solution involves transplantation of polymeric scaffolds designed to carry cells as an organized graft (Fig. 2).[88-90] The choice of polymeric material (eg, degradable vs non-degradable) and scaffold structure/modulus is critical, and a number of options have been studied.[91,92] Subretinal implantation of poly(glycerol sebacate) membranes, for example, has been associated with photoreceptor degeneration and MG activation.[93] Stiff, non-degradable materials can be associated with fibrosis.[94] Thus, the development of scaffolds to support cell delivery requires in vitro as well as in vivo testing.

Other potentially beneficial approaches include transplantation of combined retina-RPE sheets[22] and retinal sheets with genetically engineered bipolar cell deficiency (to remove an integration barrier between donor photoreceptors and host bipolar cells).[80,95] Although retinal sheets lacking

Table 3. Photoreceptor transplant organization.

Cell suspension	Organized		Co-transplant with RPE
	Scaffold	Hydrogel	

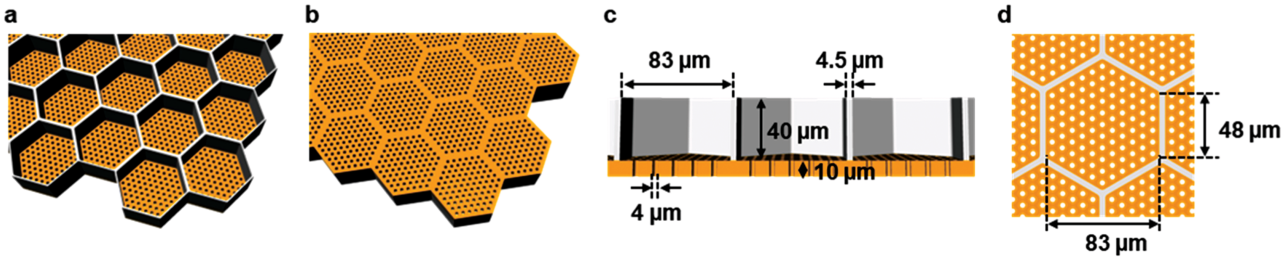

Figure 2. Schematic illustration of the honeycomb-shaped microscaffold showing (a) a tilted top view, (b) a tilted bottom view, (c) a cross-sectional view, and (d) a top view, respectively. The hexagonal prism-shaped cell capture wells (white in color) are designed to have a large volume (ie, 40 μm in depth and 48 μm in length for each side of the hexagon) for capturing and retaining both RPE and PR cells in the wells, and the cylinder-shaped fluid channels (orange in color) are tailored to be narrow enough (ie, ~4 μm) to prevent the seeded cells from migrating through the channels while still supporting cell functions during scaffold degradation. Reproduced with permission from Lee et al[134].

an inner nuclear layer tend to exhibit photoreceptor rosettes (and do not integrate well with host RPE), these rosettes seem to function satisfactorily and do not require, for example, 9-*cis*-retinal supplementation.[80,95] In the case of cones, visual pigments may be recycled via MG.[96,97] Gasparini et al[59] demonstrated successful cone photoreceptor cell suspension engraftment although the host exhibited a well-structured ONL largely devoid of cones. Ribeiro et al[67] demonstrated that cones dissociated from human iPSC-derived retina organoids not only survived but also established functional synapses with host ON and OFF bipolar cells in an immune-deficient mouse RP model. Ribeiro et al. posited that 2 features were responsible for the observed rescue: (1) improved photoreceptor graft maturation with the formation of rudimentary outer segments, possibly achieved via the establishment of a niche of a large number of transplanted cells thus mimicking the environment of a retinal sheet, and (2) formation of new synapses between donor human cones and host murine bipolar cells. These reports also demonstrate that the synaptic rewiring that accompanies retinal degeneration[98-100] may not preclude some degree of cell transplant-induced vision restoration in AMD-GA and RP patients with advanced disease.

Material Transfer

Neurons can communicate with each other via synapses, gap junctions, and material transfer. Material transfer involves the exchange of proteins, messenger RNA, micro RNA, and even organelles between cells and is mediated by extracellular vesicles as well as nanotubes.[101-103] Transplanted rods as well as cones can conduct material transfer with host photoreceptors.[57,73-77] Post-mitotic rods and cones engage in material transfer more effectively than photoreceptor precu rsors.[57,73,75,76]

Extracellular Vesicles

Extracellular vesicles contain messenger RNA, micro RNA, lipids, and proteins and are secreted from virtually all cells.[101] Their cargo can be delivered to nearby cells as well as to distantly located cells via the bloodstream.[104] Extracellular vesicles include exosomes, microvesicles, and apoptotic bodies, each with distinct biogenesis pathways and distinct size, contents, and surface proteins.[101] Exosomes form via the fusion of multivesicular bodies (derived from endosomes) with the cell membrane and subsequent release into the extracellular space. Microvesicles form via the outward budding of the plasma membrane. Apoptotic bodies are released via membrane blebbing of cells undergoing apoptosis. Exosomes from different cells contain distinct contents. Those derived from RPE,[105] for example, have different contents from those derived from retinal progenitor cells.[106] Extracellular vesicle cargo also may vary depending on cell passage number. Extracellular vesicles can pass through the inner limiting membrane.[107] Extracellular vesicles can exhibit anti-inflammatory properties.[108] Extracellular vesicles do not seem to mediate material transfer between photoreceptors, whereas nanotubes do (see below).[102] Photoreceptor-derived extracellular vesicles seem to contain cargo that differs from the cytoplasmic composition of the cells from which they are derived.[102] In vitro, photoreceptors release bioactive extracellular vesicles that are taken up predominantly by non-receptor populations.[102] In vivo, it seems that MG takes up these vesicles.[102] One clinical trial is focused on exosome delivery as a rescue strategy (https://www.reneuron.com).

Photoreceptor Nanotubes

Cellular material transfer also can occur via the extension of photoreceptor nanotubes, which have a neurite-like structure and function as a cytoplasmic bridge between cells (Figs. 3 and 4). Photoreceptor nanotubes contain microtubules and are distinct from photoreceptor axons and dendrites. The nanotubes resemble immature neurites with pre-synaptic markers scattered along the length of donor cell protrusions.[103,109] The outgrowth and material transfer through photoreceptor nanotubes are regulated by Rho GTPase-dependent actin remodeling and can be promoted with the use of ROCK inhibitors such as Y-27632[103] and inhibited with cytochalasin D and latrunculin, which block actin polymerization.[102] As was noted for exosomes, mRNA as well as protein transfer can be mediated by photoreceptor nanotubes; even mitochondria can be transferred.[103] Transfer of both membrane and cytoplasmic materials requires healthy, viable donor cells and does not arise from the uptake of debris.[102]

The extent of material transfer via photoreceptor nanotubes seems to be related to the number of surviving donor photoreceptors, but, in addition, recipient photoreceptors differ in their capacity to support material transfer from a given pool of donor photoreceptors.[57,103] The OLM, a continuous band of heterotypic adherens junctions involving the photoreceptor inner segment and Muller glia cell membranes, may serve as a barrier that prevents donor photoreceptor nanotubes from making physical contact with recipient photoreceptors. This finding may underline the phenomenon that pharmacological disruption of the OLM in wild-type retina by treatment with alpha-amino adipic acid or zonula occludens-1 protein knockdown increases the amount of material transfer observed by 2-3 fold, suggesting that the structures serve as physical barriers, preventing nanotube contact with host neurons.[110,111] Gliosis also may inhibit material transfer[62] as can hyaluronan and methylcellulose hydrogel.[112] In addition, the capacity for material transfer may be lower in humans into rodent xenografts than in allogeneic transplants.[34,57,113]

It may be that transplanted rods restore glucose transport and reactivate dormant cones via material transfer.[63] In preclinical models of RP, material transfer correlates with host photoreceptor survival in vivo.[102,103] As noted above, material transfer is more likely to occur in degenerating retinas with remaining host photoreceptors than in models of end-stage retinal degeneration.[73-77] Whether nanotube expression is a transient phenomenon or a stable feature of the transplants is not clear at this time. In some recipients, both material transfer and synapse formation between donor photoreceptors and host bipolars can occur.[57] Thus, material transfer and synaptic integration need not be mutually exclusive mechanisms of vision restoration.

Photoreceptor Transplant Immunology

Normally, human ESC- and iPSC-retina exhibit little HLA class I antigen and beta-2 microglobulin expression and very little, if any, HLA class II antigen.[78] Exposure to interferon-gamma increases the expression of these antigens.[78] Although human ESC- and iPSC-retina exhibit low immunogenicity, enzymatically dissociated single cells derived from these

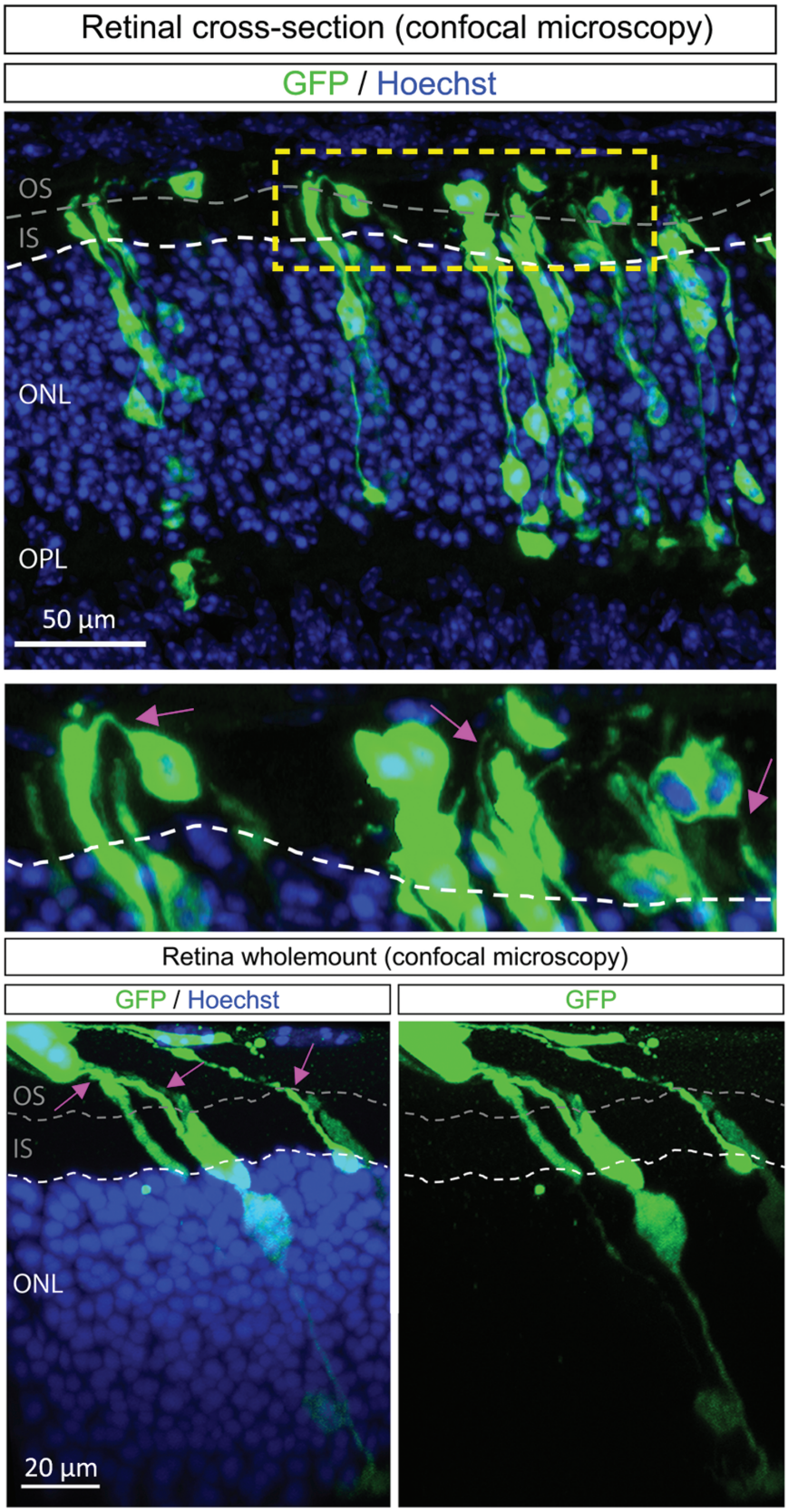

Figure 3. Green fluorescent protein (GFP)-labeled acceptor photoreceptors are connected to transplanted donor photoreceptors via cell protrusions. Top: Maximum intensity projection of a confocal micrograph of a cryostat section from a Nrl$^{-/-}$ recipient retina 21 days after transplantation with Nrl::GFP donor photoreceptors, showing protrusions (pink arrows) connecting donor and acceptor photoreceptors. Bottom: 3D reconstruction of confocal microscopy images of whole-mounted retinas from C57BL6/J recipients 21 days after transplantation with Nrl::GFP donor photoreceptors (top layer) shows that donor and GFP + acceptor photoreceptors appear to be attached via protrusions. 3D reconstruction shows protrusions (pink arrows) and GFP labeling in acceptor cells. Reproduced with permission from Ortin-Martinez et al[103].

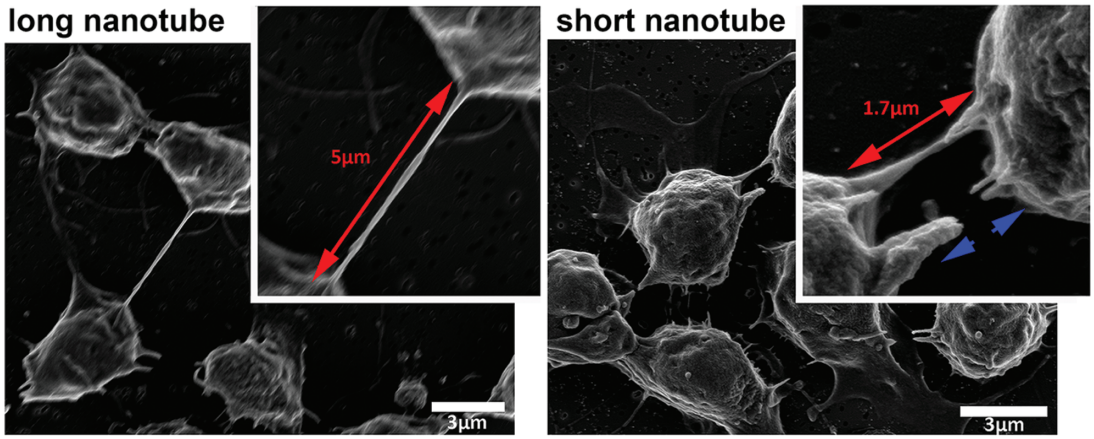

Figure 4. Representative scanning electron micrograph images with digitally enhanced microphotographs of cultured P8 Nrl.Gfp+/+ photoreceptors. Red arrows indicate photoreceptor nanotube connections between neighboring photoreceptors, while blue arrows indicate broken photoreceptor nanotube connections ($N = 3$ cultures). Scale bar = 5 µm. Reproduced with permission from Kalargyrou et al[102].

preparations, but not semi-dissociated human ESC- or iPSC-retina, partially activate allogeneic peripheral blood mononuclear cells from an allogeneic host.[78] In vitro, human ESC- and iPSC-retina suppress activation of allogeneic lymphocytes (eg, CD4 helper T cells, CD8 cytotoxic T cells, CD11b macrophage/monocyte, NK group 2A cells), and these tissues strongly suppress interferon-gamma secretion by peripheral blood mononuclear cells.[78] Secretion of TGF-beta by these retinal tissues partially accounts for this immunosuppressive effect.[78] Human ESC- and iPSC-retina also express CD47, which may render them resistant to phagocytosis.[78,114] It is important to note that human ESC- and iPSC-retina prepared in vitro lack retinal microglia and vascular cells, which may have an important influence on the immune properties of the graft.[78] Taken together, these data indicate that ESC- and iPSC-derived retina, but not enzymatically dissociated cell preparations derived from these tissues, have low immunogenicity and also have immune suppressive properties. Furthermore, despite some attractive features of cell suspension transplantation (eg, widespread cell delivery to the subretinal space, minimally invasive surgery), aggregated cells may have a survival advantage.

In a normal physiological state, the subretinal space is a relatively immune privileged site,[115] and photoreceptors and RPE cells are relatively immune privileged tissues.[116,117] In a disease state (eg, AMD or RP) the immune privilege of the subretinal space is likely to be compromised,[118] and, unless autologous cells are transplanted, the transplanted cells are likely to be subjected to immune surveillance. Thus, despite the immune privileged and immune suppressive nature of the tissue, subretinal photoreceptor precursor and ESC- and iPSC-retina xenografts can be rejected in the absence of systemic immunosuppression.[72,78,119-121] MHC-matched allogeneic grafts exhibit reduced immune cell infiltration and better graft survival (Figure 5).[122,123] Clinical signs of immune rejection in this setting can include mild vasculitis, generalized retinal edema, presence of hyper-reflective material in the vitreous and on the inner retina visualized with OCT, and a transient increase in the subretinal transplant volume.[72] However, signs of immune recognition may not be evident clinically although peripheral blood mononuclear cell proliferation assays using host lymphocytes and donor tissue may provide useful information in this regard.[123]

Future Directions

Better methods to monitor host immune surveillance of the graft might improve allogeneic transplant survival. Clinical evaluation with fundus examination, fluorescein angiography, and currently available OCT technology are important but are not likely to detect the initial stages of rejection—a time when intervention with more aggressive immunosuppression might be indicated. Laboratory studies monitoring the activation of host peripheral blood mononuclear cells probably should be done at regular intervals since the nature of the immune response to the graft seems to vary over time. It might be helpful to develop imaging technology that permits the identification of immune cells (eg, by labeling host immune cells with markers that can be visualized with OCT or with fundus autofluorescence), which could facilitate early detection of an immune reaction to the graft. Most elderly patients do not tolerate sustained immunosuppressive agents well, and these approaches might minimize exposure to these agents. In addition, it might be useful to systematically compare different methods of donor tissue preparation, as described in the Cell Manufacturing section, to identify a method that is least immunogenic while maximizing the likelihood of donor-host integration, if such a manufacturing process exists. In the case of scaffold-based cell delivery, it may be possible to incorporate anti-inflammatory molecules into the scaffold (ie, scaffold as a sustained delivery device) to minimize the host response to the graft.

Improved and more extensive imaging also might be useful to identify appropriate hosts, particularly for photoreceptor grafts. Disruption of the OLM and thinning of the ONL are both readily identified with high-resolution OCT imaging (Fig. 6). These anatomic features seem to favor donor cell integration with host bipolars. When the ONL is reasonably well preserved, one might attempt visual restoration/preservation using a material transfer (ie, rescue) rather than a cell replacement strategy. (The number of cells required to achieve long-term material rescue may be different, ie, greater, than the number required to achieve cell replacement.[59,67]) In RP, for example, there is relative preservation of foveal cones until the advanced stages of the disease. In this setting, there will be a substantial loss of adjacent extrafoveal rods and disruption of the OLM, conditions that might favor

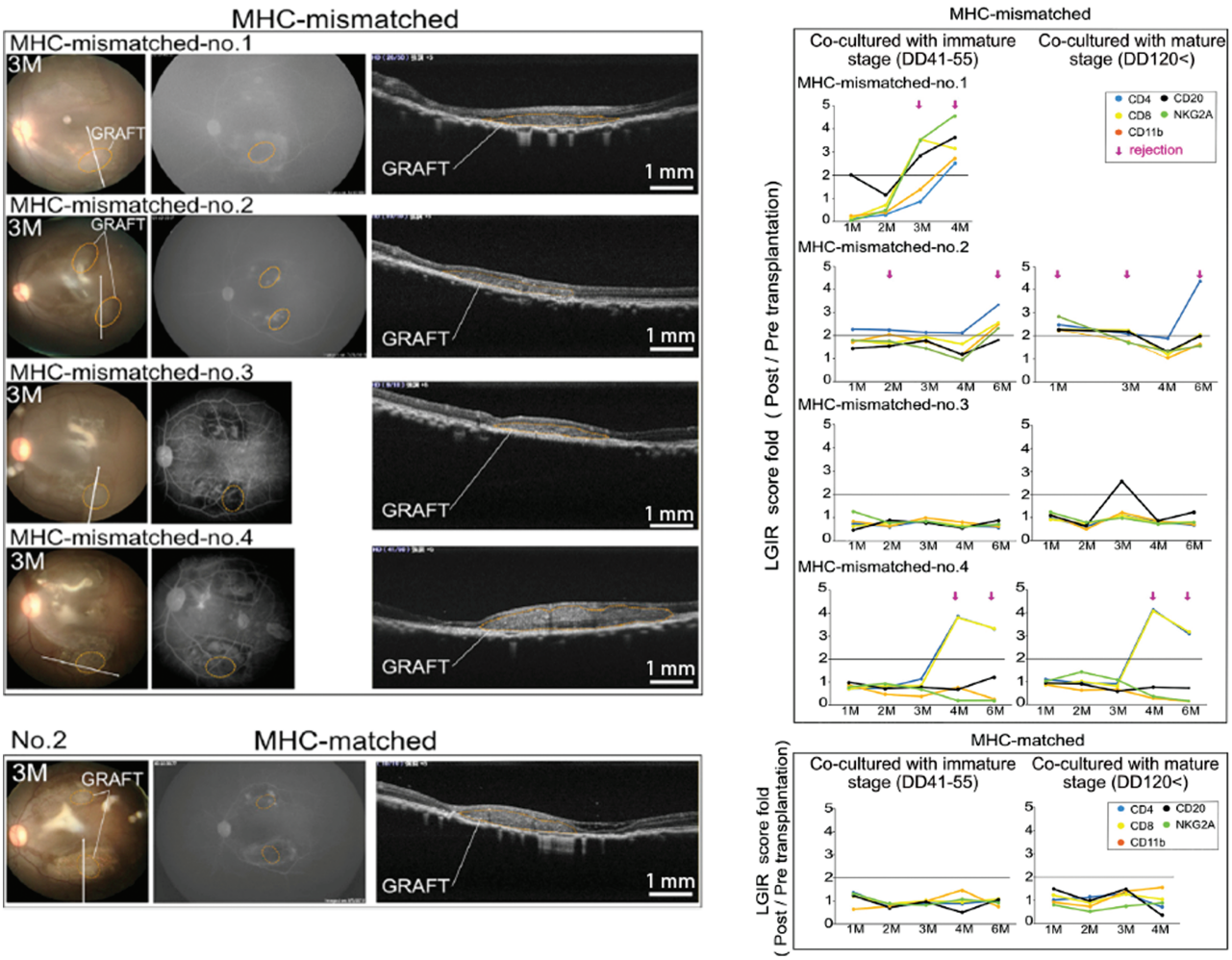

Figure 5. Lymphocyte graft immune reaction (LGIR) tests detected subclinical rejection in repeated MHC-mismatched transplantation. Left photographs: In vivo images of the color fundus photographs, FAG, and OCT images of the eyes that received a second transplantation 3 months after the first transplantation. Orange circles indicate graft areas. Each OCT image shows the sectional view of the line indicated by the white arrow on the color fundus image. Right graphs: Line plotting results for LGIR tests following transplantation. Vertical lines indicate post/pre-transplantation LGIR scores. Horizontal lines indicate months after transplantation. Magenta arrows show the time points when the post/pre-LGIR scores were above 2-fold in 2 or more cell types after transplantation (subclinical rejection). LGIR test, lymphocyte graft immune reaction test; DD, differentiation day; FAG, fluorescein angiography; OCT, optical coherence tomography. Scale bars: 1 mm. Reproduced with permission from: Uyama et al[123].

donor cell integration with host bipolars if transplants are placed extrafoveally. (Preservation of extrafoveal rods could improve the survival of foveal cones in this setting.[124]) In RP, the parafoveal RPE may remain functional[125] so such transplants might derive metabolic support from subjacent RPE. In AMD-GA, atrophy typically spreads centripetally from an extrafoveal to a foveal location, but in contrast to RP, the extrafoveal RPE are likely to be atrophic. In this setting, one might contemplate the exploitation of a material transfer rescue approach to subfoveal cones as the OLM and subfoveal RPE, although damaged, may be partially preserved whereas extrafoveal OLM, photoreceptors, and RPE will be disrupted.[126,127] In both cases, one should expect the immune privilege of the subretinal space to be abrogated, which likely will necessitate some method to address immune surveillance.

Having identified a preferred vision restoration strategy, one might wish to provide adjunctive therapy to facilitate success. In select cases, could exposure of the subretinal space to chondroitinase, for example, increase the chance for donor-host synaptic integration? Could transient exposure to appropriate agents (eg, antibodies to disrupt the OLM; ROCK inhibitors to foster nanotube extension), increase the likelihood of host photoreceptor rescue by fostering nanotube conduits between donor and host? ROCK inhibitors also might reduce iatrogenic retinal detachment-induced synaptic disruption between native photoreceptors and bipolar cells.[128-132]

Additional developments in scaffold technology may further increase the chance of photoreceptor transplant success. As mentioned above, the scaffold might serve 2 purposes: (1) a platform to deliver an organized layer of cells that will cover a precisely defined surface area; (2) a drug delivery system to promote cell survival, integration, and immunosuppression. RPE scaffolds are at a relatively advanced state of development, but photoreceptor scaffolds, whose clinical utility is unproven but plausible, are at an early stage.

While gene therapy is elegant and proven effective for some forms of RP and allied conditions, it seems unlikely to be sight-restoring in most conditions and generally will require a different treatment for each mutation (of which there

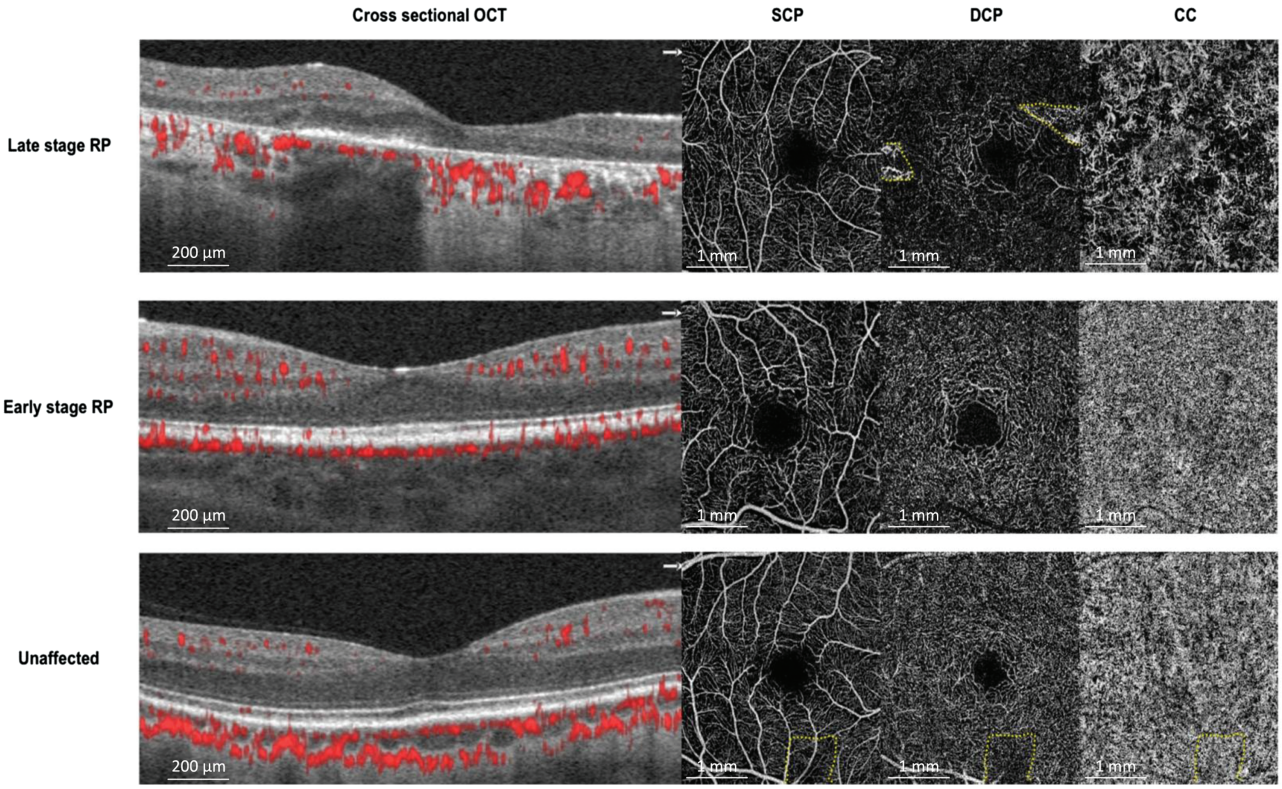

Figure 6. Cross-sectional optical coherence tomography (OCT) with angio flow (denoted in red) and 3 × 3 mm en face optical coherence tomography angiography (OCTA) of the superficial capillary plexus (SCP), deep capillary plexus (DCP), and choriocapillaris (CC). Top—images from a 28-year-old man with severe center involving retinitis pigmentosa (RP1 mutation). There is diffuse loss of vasculature in the DCP and CC. Middle—images from an 18-year-old woman with mild center sparing retinitis pigmentosa (IMPDH1 mutation). The vasculature in SCP, DCP, and CC appear grossly preserved. Bottom—images from a 44-year-old male control without retinitis pigmentosa. Dotted yellow lines denote artifacts from segmentation errors (top) and a vitreous floater (bottom). Reproduced with permission from Ong et al[135].

are ~3000 in the case of RP) unless a generic molecular approach (eg, MG transdifferentiation) can be developed. By contrast, cell-based therapy, while likely more invasive than gene therapy, offers the possibility of generic therapy (ie, one treatment for many different diseases) and sight restoration. These features of cell-based therapy are compelling, and it seems likely that cell-based therapy will have increasing importance as a treatment for photoreceptor blindness during the next decade.

Conflict of Interest

Marco A. Zarbin: Consultant for Boehringer Ingelheim, EdiGene, Genentech/Roche, Illuminare, Life Biosciences, Novartis Pharma AG, Perfuse Therapeutics, Seeing Medicines, Tamarix Pharmaceuticals, Tenpoint Therapeutics; Equity: NVasc. The other authors declared no potential conflicts of interest.

Author Contributions

V.K., O.G.G.M., M.A.Z.: conception and design, manuscript writing, data analysis, final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

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