Chapter 4

Restoring Vision to the Blind: Stem Cells and Transplantation

The Lasker/IRRF Initiative for Innovation in Vision Science

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Introduction

Stem cells are primitive cells characterized by (1) a capacity for self-renewal, and (2) at least some level of potency, or ability to differentiate into other cell types. Human embryonic stem cells (ESCs), for example, have the capacity to differentiate into all of the cell types of the human body. Thus, as evident during development, a single cell can give rise to every tissue of an organism. One can easily imagine the immense potential that stem cells have for regenerative medicine and the treatment of disease, developmental defects, aging, and accidental tissue damage. Because of this potential, stem cell technologies aimed at the restoration of vision loss due to retinal disease or injury have emerged as a field of great clinical interest over the last decade.

Many diseases that result in vision loss are neural degenerations, which can lead to the death of retinal neurons and/or retinal pigment epithelial cells (RPE). A regenerative event is needed for the replacement of these lost cell types. Whereas endogenous stem cells have been identified in many adult tissues, including the adult human eye, endogenous human eye stem cells do not regenerate or replace retinal neurons and/or RPE, although there are current efforts to stimulate such an event (see Chapter 5: Endogenous Regeneration). Because of this, the potential of other stem cell sources that can be transplanted into the eye (or that can generate cell populations to be transplanted) is being evaluated. The source of these cells varies, but most often includes either stem cells isolated at various stages of development or induced pluripotent stem cells (iPSCs).

The purpose of this targeted session was to call together researchers at the forefront of stem cell transplantation technologies aimed at the restoration of vision in order to evaluate the current progress of stem cell–based therapeutic strategies to improve vision. The session opened with discussion of the wide range of potential applications for stem cell research. These included using iPSCs to model developmental and degenerative disorders of the eye, for drug testing and gene correction, and engineering transplanted cells to secrete neuronal survival factors (“ex vivo” gene therapy). The participants agreed that the session would then focus on prospects for restoration of vision that has been lost to retinal degenerative disease, particularly by the replacement of RPE and photoreceptor cells, as rapid progress is being made in these areas. In this session a brief review of the recent successes in RPE and photoreceptor transplantation was discussed, as well as their limitations and deficiencies. Many detailed reviews of these topics are available for those interested in a more thorough exploration of these fields (Croze and Clegg, 2014; Fernandez-Robredo et al., 2013; Pearson, 2014; Ramsden et al., 2013; Reynolds and Lamba, 2014; Rowland, Buchholz, & Clegg, 2012; Westenskow, Kurihara, & Friedlander, 2014; Wright, Phillips, Pinilla, Hei, & Gamm, 2014). Finally, recommendations were provided to direct and stimulate future research and ultimately, to make these therapies available to patients suffering from vision loss.

Figure 4.1. RPE monolayers derived from human embryonic stem cells can be grown on a synthetic parylene substrate to provide a biocompatible replacement for Bruch's membrane (*scale bar* = 200 μm) (Image used with permission of Britney Pennington and Dennis Clegg.).

Transplantation of RPE

The RPE is a monolayer of pigmented hexagonal cells underlying the photoreceptor cells of the retina. This layer of polarized cells performs many functions that are critical to the homeostasis and proper functioning of the retina (Strauss, 2005). Age-related macular degeneration (AMD) and Stargardt macular dystrophy (SMD) are characterized by the degeneration or dysfunction of the RPE, which can induce photoreceptor atrophy and/or death. Thus, cell replacement via stem cell transplantation therapies could be a promising treatment for these diseases. Human RPE transplantations have already been performed using autologous, fetal, and cadaver RPE in a small number of cases and have reported some limited visual recovery (da Cruz, Chen Ahmado, Greenwood, & Coffey, 2007).

Conditions necessary for the production of highly enriched, pigmented RPE suitable for transplantation have been developed using human ESCs (hESCs) (Klimanskaya et al., 2004) and human iPSCs (hiPSCs) (Buchholz et al., 2009; Hirami et al., 2009), and recently identified RPE stem cells resident in the adult RPE layer (Salero et al., 2012). Biochemical analyses of hESC-RPE and hiPSC-RPE suggest that they are highly homologous to prenatal RPE in cellular structure and in gene and protein expression profiles; furthermore, *in vitro* analyses suggest that hESC-RPE and hiPSC-RPE can recapitulate the primary functions of RPE (Kokkinaki, Sahibzada, & Golestaneh, 2011; Meyer et al., 2011; Osakada, 2009; Singh et al., 2013; Ukrohne et al., 2012). Most *in vivo* analyses of the efficacy of hESC-RPE and hiPSC-RPE have been performed using the RCS rat, which harbors a mutation in *Mertk*, a gene crucial for photoreceptor outer segment phagocytosis. This mutation results in RPE dysfunction and the progressive loss of photoreceptors, which is also a feature of AMD. Subretinal injection of hESC-RPE or hiPSC-RPE into RCS rats can promote photoreceptor survival and function and can result in improved visual function compared with sham treatment (Carr et al., 2009; Gamm et al., 2007; Idelson et al., 2009; Lu et al., 2009; Lund et al., 2006; Ukrohne et al., 2012; Vugler et al., 2008). The mechanism by which the transplanted RPE cells promote photoreceptor survival is incompletely understood. As transplantation of human fetal neural progenitors achieves a similar effect (da Cruz et al., 2007), noncell autonomous effects, such as secreted factors, or stimulation of endogenous macrophages, may play a role. Recent demonstration of the developmental plasticity of RPE stem cells and their potential to generate mesenchymal progeny, including osteocytes (Salero et al., 2012), highlights the importance of assessing phenotypic stability of stem cell-derived RPE cells used for therapeutic transplantation.

In subretinal transplantation studies in the RCS rat model, discussed above, RPE cells were delivered in the form of a bolus. These researchers reported numerous challenges associated with this form of delivery: (1) the aged and damaged basement membrane is not an adequate substrate for transplanted RPE, (2) generating a polarized monolayer of transplanted RPE cells is difficult, (3) survival of transplanted cells is low, and (4) disruption of the blood–brain barrier increases the chances of detrimental immune responses (Carr et al., 2009; Idelson et al., 2009; Lu et al., 2009; Sugino et

Table. List of Approved and Ongoing Clinical Trials Using Stem Cell Technologies for the Treatment of AMD

Therapy	Developer	Status of Product
Suspension hESC-RPE cells	Advanced Cell Technologies, Inc. (Marlborough, MA, USA)	Phase 1 clinical trials for dry AMD and Stargardt
Suspension hESC-RPE cells	Cell Cure, Inc. (Alameda, CA, USA)	Preclinical Studies; dry AMD
Suspension neural progenitors	Stem Cells, Inc. (Newark, CA, USA)	Phase 1 clinical trial; dry AMD
Suspension cord blood mesenchymal stem cells	Janssen Pharma, Inc. (Titusville, NJ, USA)	Phase 1 clinical trial; dry AMD
iPSC-RPE monolayer	Riken CDB (Kobe, Japan)	Approved for clinical research for wet AMD
Autologous adult RPE	NY Neural Stem Cell Institute (Rensselaer, NY, USA)	Preclinical studies; dry AMD
iPSC-RPE monolayer on a biodegradable scaffold	NEI (Bethesda, MA, USA)	Preclinical studies; dry AMD
hESC-RPE monolayer on a polyester scaffold	London Project to Cure Blindness / Pfizer (London, UK)	Approved for phase 1/2 clinical trial for wet AMD and RPE tears
hESC-RPE monolayer on a parylene scaffold	The California Project to Cure Blindness	Preclinical studies, IND enabling studies underway; dry AMD

al., 2012). To circumvent some of these challenges, alternative delivery methods are being developed which employ semipermeable or porous scaffolds (Fig. 4.1) (Lu et al., 2012; Mathieson et al., 2012; Sheridan, Williams, & Grierson, 2004; Stanzel et al., 2014; Williams et al., 2005).

While delivery of a cell population on a structural support will likely prove superior to bolus injections, this method will present other challenges and will require additional optimization; larger areas of cell coverage may be more difficult to address with scaffolds compared with dissociated cell injections. Conditions will need to be identified that enable the transplanted RPE cells to align correctly, between the neural retina and the vascular choriocapillaris, and to function properly (e.g., efficient phagocytosis, growth factor secretion, and transport functions) so that further photoreceptor damage and progression of the disease is blocked or limited.

Because of the promising results seen in efficacy studies in the RCS rat model, numerous clinical trials have recently been approved for stem cell-based therapies for AMD (Table).

A preliminary report from one trial (Schwartz et al., 2012) found no evidence of tumors or adverse events in two patients. The results of these trials are highly anticipated because they will be very informative as to the viability of this approach.

Transplantation of Photoreceptors

Photoreceptors are specialized afferent retinal neurons responsible for the detection of light entering into the eye. Rod photoreceptors are extremely sensitive, able to detect a single photon of light. Because of this sensitivity, low-light vision relies exclusively on rod photoreceptors. Cone photoreceptors, on the other hand, are much less sensitive, but are necessary for color vision and high-acuity vision. A variety of inherited retinal degenerations including retinitis pigmentosa (RP) result in the loss of photoreceptors, and these diseases are good candidates for stem cell-based therapies. Because in most inherited retinal degenerative diseases the inner retinal circuitry remains largely intact, at least initially, newly transplanted photoreceptors need only make short synaptic connections to contribute to restoration of visual function.

The identification of an ideal donor cell for efficient photoreceptor transplantation has proven challenging. Transplantation of intact sheets of fetal human retina in two RP patients reported subjective visual improvements

Figure 4.2. Newly integrated GFP+ photoreceptor cells within adult mouse retina, 3 weeks after subretinal transplantation of photoreceptor precursor cells isolated from postnatal day 44 retina. (Reprinted with permission. Lakowski et al. (2011). Effective transplantation of photoreceptor precursor cells selected via cell surface antigen expression. *Stem Cells*, 29, 1391–1404.)

(Radtke, Aramant, Seiler, & Petry, 1999). However, transplanted partial or whole retinal sheets derived from rat or rabbit embryonic, or neonatal pig retina, showed very limited integration into the recipient retinal circuitry of animal models (Ghosh, Juliusson, Arner, & Ehinger, 1999; Ghosh, Wong, Johansson, Bruun, & Petters, 2004; Seiler et al., 2008; Turner, Seiler, Aramant, & Blair, 1988). Other research in rodent models has employed transplantation of dissociated cells: brain-derived neural stem cells, progenitors isolated from immature retinas, and ESC- and iPSC-derived retinal donor cells, each with varying degrees of success. Details of these studies are available in recent reviews (Pearson, 2014; Reynolds & Lamba, 2014; Wright, Phillips, Pinilla, Hei, & Gamm, 2014). Currently, subretinal transplantations using suspensions of post-mitotic, photoreceptor precursor cells (cells that are already specified to differentiate into rod photoreceptors) have been the most successful (Lakowski et al., 2010; MacLaren et al., 2006; Pearson et al., 2012; Warre-Cornish, Barber, Sowden, Ali, & Pearson, 2014). Some of these transplanted rod precursor cells are able to migrate into the adult retina, differentiate, and acquire morphological features comparable with mature photoreceptor cells (Fig. 4.2) (Bartsch et al., 2008; Eberle et al., 2012; MacLaren et al., 2006; Warre-Cornish et al., 2014).

Transplantation of rod precursors (isolated from postnatal mouse retina) into a genetic model of rod dysfunction has been shown to improve rod-mediated vision following integration of more than 25,000 new rod photoreceptors (Pearson et al., 2012). These recent studies in animal models are resolving the type and developmental stage of differentiated hESC/iPSC-derived cells that will be optimal for clinical photoreceptor transplantation therapy.

Conditions necessary for the *in vitro* production of progeny resembling photoreceptor cells from ESCs and iPSCs have been developed over a number of years (Lamba, Karl, Ware, & Reh, 2006; Meyer et al., 2009; Osakada et al., 2008). Human ESC- and mouse iPSC-derived retinal cells have been transplanted into mouse models, and in some cases differentiate to express photoreceptor markers within the mouse retina (Lamba, Gust, & Reh, 2009; Tucker et al., 2011). More recently three-dimensional (3D) culture systems have been developed that are remarkably able to generate optic vesicles and laminated retinal tissue *in vitro* (Fig. 4.3) (Eiraku et al., 2011; Meyer et al., 2011; Nakano et al., 2012).

Since 3D systems appear to closely recapitulate normal embryonic development of the retina, they provide a good renewable source of developing photoreceptor cells for transplantation. Transplantation of rod precursors isolated from 3D mouse ESC-derived retinal cultures has proven more effective than conventional 2D systems but is currently less

Figure 4.3. Human iPSC derived multilayered retina-like structures. RECOVERIN+ photoreceptor precursor cells (*red*) observed in the thickened ONL. (Reprinted with permission. Phillips, M.J., Wallace, K.A., Dickerson, S.J., Miller, M.J., Verhoeven, A.D., Martin, J.M., . . . Gamm, D.M. (2012). Blood-derived human iPS cells generate optic vesicle-like structures with the capacity to form retinal laminae and develop synapses. *Investigative Ophthalmology & Visual Science*, 53(4), 2007–2019.)

efficient than transplantation of rod precursor cells isolated directly from the neonatal retina (Gonzalez-Cordero et al., 2013; West et al., 2012). Notably, ESC-derived rod precursors isolated at a developmental stage similar to postnatal days 4 to 8 were shown to integrate more efficiently compared with cells at other stages (Gonzalez-Cordero et al., 2013). Given the extensive proliferative capacity and differentiation potential of pluripotent stem cells, for effective and safe transplantation, optimal differentiated progeny need to be purified and proliferative cells excluded (Gonzalez-Cordero et al., 2013; Lakowski et al., 2011; West et al., 2012). Further development of these therapies will require refinement of methods to generate sufficiently high numbers of transplantable rod and cone cells and to increase their long-term survival, integration, and function post transplantation. In addition, the success of these transplantations is limited by the integrity of the outer limiting membrane (OLM) and the extent of gliosis in the recipient tissue, both of which remain to be explored in greater detail (Barber et al., 2013; Pearson et al., 2010). Even with these challenges, stem cell-based cell replacement therapies for treatment of retinal diseases resulting in the loss of photoreceptors look very promising.

Recommendations for Future Work

While the potential of stem cell therapies as a viable therapeutic strategy to improve vision has been clearly established in animal models, a considerable amount of ground work is still required in order to demonstrate the safety and benefit of these treatments as clinical therapies, to increase their effectiveness, and to expand the number of retinal diseases that can be treated through these technologies. Specifically, members of this target session enumerated the following concerns that merit additional exploration:

1. The potential for alternative, and possibly superior renewable sources of transplantable cells should be examined, such as restricted progenitors or partially reprogrammed autologous cells. Importantly, transplanted cell populations, regardless of their means of isolation and level of purity, require thorough characterization to determine their exact cellular composition and to ensure their safety with respect to toxicology and tumorigenicity. Target cell isolation should be enriched for optimal cell populations and exclude potentially hazardous or proliferative cells;
2. Cell culture systems (2D and 3D cultures with defined chemical stimulation) require further optimization to increase the health, homogeneity, and abundance of target cells prior to transplantation;
3. While current target cell delivery strategies include transplantation of cell suspensions, boluses, or combinations of

cells and support matrices (gels and scaffolds), these approaches require additional exploration and optimization (likely specific for each target disease);

4. Including additives at the time of transplantation to stimulate neuroprotection and/or the health and survival of transplanted cells (e.g., rod-derived cone viability factor or growth factors cotransplanted with photoreceptors), while at the same time promoting their stability (maintaining them in a desired, nonproliferative cell state) should be considered. Furthermore, efforts to increase the number of integrated cells, to stimulate their migration (when needed), and to enhance their proper localization post transplantation should be explored. Transplantation might be combined with additional treatments to bring about repair of damaged tissue, such as Bruch's membrane. Long-term studies of these metrics would prove useful;
5. Approaches to deliver RPE and photoreceptors (or other combinations of cells) simultaneously using support matrices should be developed, as this type of combinatorial treatment may augment the survival of transplanted cells and may be beneficial for retinal diseases that result in the loss of multiple cells types, such as Best's disease, AMD, and so on;
6. Additional strategies to measure the efficacy of the therapy and functionality of the cells following transplantation should be developed;
7. Because stem cell transplantation into the eye stimulates both a gliotic and an immune response, the events accompanying these responses, as well as any benefit that results from modulating these responses, should be documented. While it is common practice to immunosuppress following transplantation, systemic immunosuppression is costly and detrimental to the patient, and it is possible that aspects of the immune response are beneficial to the success of these therapies. It was also suggested that developing engineered cells that provide for localized immunosuppression in the eye could be beneficial; and
8. There is a great need for additional animal models, particularly to advance cone replacement therapies (since rodent retinas have relatively few cones and lack a fovea) and to develop treatments for more complex diseases such as AMD. Additional models to better gauge the potential and safety of the transplants and to enhance the development of these therapies prior to clinical trial would be desirable.
9. Although it is our hope and belief that stem cell transplantation technologies will prove beneficial for the treatment of multiple retinal diseases, a critical step in the development of these treatments is the design of carefully thought-out clinical trials. This targeted session stressed the importance of the following with regard to the design of current and future trials:
 - a. Clear rationale in the design of clinical trials is essential. While solely observing the gross physiological outcome of stem cell treatments can be informative, there is a growing need for conclusive clinical studies involving stepwise and standardized outcome measures;
 - b. The identification of target patient groups is essential for the successful interpretation of clinic trials. A better mechanism to classify patients with different manifestations of and stages of target retinal diseases is needed (in particular for AMD). Small, focused studies based on these classifications may be more informative;
 - c. Development of new imaging modalities to visualize and to track transplanted cells in patients and to measure the efficacy of these treatments for patients is needed; and
 - d. Importantly, patients who undergo clinical treatments should be given a clear explanation of expected outcomes (based on experimental evidence).
10. Finally, this targeted session spent a considerable amount of time discussing challenges that arise through the commercialization of these stem cell treatments in selective regions of the world, in particular when these treatments have not been sufficiently founded on rigorous experimental and clinical results. While it is our goal to provide these treatments to patients as quickly as possible, we realize that to do so in an undisciplined fashion could prove detrimental to the recipients of these treatments or be of no benefit to them, and could hinder the development of these technologies through the generation of negative public perceptions. The safety and success of these procedures requires a global effort. We call on scientists and clinicians from around the world to ensure that these treatments are done in a stepwise and careful fashion, while ensuring safety and with public reporting of trial results. It is essential to work together on this global goal of restoring vision to the blind.

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