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# Cell transplantation strategies for retinal repair

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## Abstract

Cell transplantation is a novel therapeutic strategy to restore visual responses to the degenerate adult neural retina and represents an exciting area of regenerative neurotherapy. So far, it has been shown that transplanted postmitotic photoreceptor precursors are able to functionally integrate into the adult mouse neural retina. In this review, we discuss the differentiation of photoreceptor cells from both adult and embryonic-derived stem cells and their potential for retinal cell transplantation. We also discuss the strategies used to overcome barriers present in the degenerate neural retina and improve retinal cell integration. Finally, we consider the future translation of retinal cell therapy as a therapeutic strategy to treat retinal degeneration.

### Keywords

stem cell; progenitor cell; photoreceptor; retina; transplantation; degeneration

## Therapeutic strategies to restore the neural retina

Retinal degenerations, either inherited or age related, remain the largest cause of untreatable blindness in the developed world. While encompassing a range of causes, most have in common the loss of the sensory cells of the retina, the photoreceptors. Many therapeutic strategies aim to slow down the progression of retinal disease, as once photoreceptors are lost they will not regenerate. Stem cell therapy may have great therapeutic potential as a treatment for degenerative retinal disease, by providing the opportunity to replace the lost cells.

The most relevant clinical studies currently being conducted in patients with retinal degeneration are fetal retinal sheet transplants. This transplantation strategy relies on the immature retinal sheet extending cell processes and forming synaptic connections with the degenerate host retina. The rationale behind this is that the inner retinal neurons of the host remain intact and therefore only require synaptic connections with photoreceptors for visual function to be restored. To date, studies investigating retinal sheet transplantation in patients have shown some subjective visual improvement (Humayun et al., 2000; Berger et al., 2003; Kaplan et al., 1997; Radtke et al., 1999). A recent clinical study of retinitis pigmentosa and age-related macular degeneration patients who received fetal retinal sheet transplants (neural retina and retinal pigment epithelium, RPE), reported improvements in vision for 7 out of 10



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patients, although the direct beneficial effects of the fetal retinal grafts are difficult to assess as all patients also received intraocular lens implants. Importantly, no overt immunological responses to the transplanted tissue were observed. However, the possibility of effector cell-mediated immune responses against the retinal grafts were not examined, and graft rejection cannot be completely discounted (Radtke et al., 2008).

Previous animal studies investigating retinal sheet transplantation have demonstrated increased visual responses localized to the region of the host neural retina overlaying the graft. Due to the lack of control animals with nonfunctional transplanted retinal sheets, it is difficult to determine whether this is the result of increased synaptic connectivity between the host and grafted retinal neurons, or a trophic response induced by the fetal retinal sheet on the remaining host photoreceptors (Mohand-Said et al., 2000; Arai et al., 2004; Liljekvist-Soltic et al., 2008; Seiler et al., 2005). In the former scenario, the intervening inner retinal layer of the graft forms a barrier to photoreceptor connectivity between the host inner retinal neurons and the graft photoreceptor layer. Therefore, synaptic connections made are unlikely to represent the normal principal retinal circuit, which comprises a single bipolar and ganglion cell, and may result in atypical visual responses (Fig. 1).

In summary, fetal retinal sheet transplants appear to offer limited potential for retinal repair but are currently one of few therapeutic options for most progressive retinal degenerations. Another therapeutic strategy currently under investigation is the transplantation of retinal cell suspensions. In theory, cell transplantation has the potential to not only maintain the diseased neural retina but also restore visual function and acuity. To date cell transplantation to restore the neural retina is still being investigated in animal models of photoreceptor degeneration, and clinical application is a distant prospect. However, the future therapeutic application of cell transplantation to human retinas must be considered and experimental strategies devised accordingly.

The brain and the neural retina are both derived from the neuroectoderm of the neural plate during embryonic development (Chow and Lang, 2001). Given that immature neurons and progenitor cells are intrinsically capable of migrating and differentiating during neural development, numerous studies have investigated the integration of brain-derived neural progenitors transplanted to the neural retina (Klassen et al., 2007b; Mellough et al., 2007; Mizumoto et al., 2003; Sakaguchi et al., 2003; Takahashi et al., 1998). However, cell transplantation to the adult retina has demonstrated limited cell integration of neural progenitor cells (Sakaguchi et al., 2005; Young et al., 2000). This was assumed to be due to the inhibitory environment present in the adult neural retina. Therefore, further studies have investigated the transplantation of neural precursor cells to the developing postnatal retina.

Promising results were observed after cell transplantation to the developing retina, and it was suggested that the age of the host tissue had a key role in determining the fate of transplanted precursor cells (Sakaguchi et al., 2003, 2004; Van Hoffelen et al., 2003; Chacko et al., 2000). Studies demonstrated well-integrated transplanted cells in all layers of the host retina. These cells exhibited retinal morphology for various cell types with extensive dendritic processes present in the plexiform layers, and all cells respecting the retinal architecture (Young et al., 2000; Takahashi et al., 1998). However, the integrated cells did not express any mature retinal cell markers, suggesting that their morphology was related to the retinal microenvironment in which they differentiated, rather than intrinsic signals (Marquardt and Gruss, 2002; Takahashi et al., 1998). Further studies using tissue-restricted reporter genes to demonstrate retinal cell fate determination also observed that integrated cells did not exhibit intrinsic features of mature retinal neurons (Sam et al., 2006).

The inability of neural progenitor cells to differentiate into photoreceptors, when transplanted into the developing eye, suggests the lineage restriction of these cells to brain-related cell types (Klassen et al., 2004a). Therefore, a more appropriate cell source for transplantation studies to the retina might be neural retinal progenitor cells. These cells develop in the retinal microenvironment and may therefore have fewer inhibitory intrinsic signals enabling retinal-specific cell differentiation, compared with neural brain-derived progenitor cells. Retinal progenitors isolated from embryonic retinas have been transplanted into young (P17) dystrophic S334ter rats. The integration of these cells was observed in the form of neurite extensions into the host retina (Qiu et al., 2005). Similar to studies using brain-derived neural progenitor cells, limited integration of retinal progenitor cells was observed after transplantation to adult retinas. Greater neurite extensions were observed following transplantation to young or developing postnatal retinas. However, the use of degenerate models with no remaining outer nuclear layer (ONL) makes it difficult to determine the extent of cell integration and mature retinal cell morphology of transplanted photoreceptors.

It was therefore assumed that the adult retina constituted an environment that inhibited retinal progenitor cell integration and differentiation possibly due to a lack of extrinsic cues that are present during development. However, recent studies have demonstrated morphological integration of early postnatal retinal precursor cells into the normal adult retina (MacLaren et al., 2006; Bartsch et al., 2008). The study by MacLaren et al. (2006) demonstrated that the integration of fully differentiated and functional photoreceptors can be achieved after transplantation into the adult retina, but only if the donor cells are postmitotic photoreceptor precursors. This was a surprising finding as it had been assumed that multipotent progenitor cells would be the best source of donor cells. Instead, these results suggest that the intrinsic nature of transplanted cells, rather than the extrinsic environment, is of greater importance for cell integration (Fig. 2). Unlike integrated neural progenitor cells, as well as mature photoreceptor morphology, the integrated photoreceptor precursor cells also demonstrated correctly localized mature retinal markers, such as rhodopsin (Rho) and peripherin-2 in the outer segments, and ribbon synapse proteins in the integrated spherules. They also demonstrated functional synaptic connectivity by increased light-induced pupil constriction following subretinal transplantation of functional compared with nonfunctional precursor cells, when transplanted into the *rho*$^{-/-}$ mouse (MacLaren et al., 2006). These results show that the ontogenetic stage of transplanted cells is crucial for the successful integration of retinal cells into the adult host ONL.

## Cell sources for retinal transplantation

One fundamental problem for the application of photoreceptor cell transplantation for human retinal disease is that an appropriate source of the precursor cells is required. Postmitotic photoreceptor precursor cells can be derived from the P1-5 postnatal mouse retina. However, equivalent human retinal cells would have to be derived from second-trimester fetuses. Ethical considerations aside, such tissue is in very limited supply and may not provide a consistent source of cells for retinal cell transplantation. An expandable source of cells that could be cultured in vitro to the correct ontogenetic stage for transplantation may, therefore, be a more appropriate and reproducible source of photoreceptor precursor cells. Several potential such sources are discussed in the following text, including adult retinal stem-like (RS) cells, Müller stem-like (MS) cells, and embryonic stem (ES) cells.

Lower vertebrates such as fish and amphibians retain greater regenerative abilities than mammals. With regard to the eye, they continuously add new retinal neurons to the adult retina as they grow (Straznicky and Gaze, 1971; Johns, 1977; Johns and Easter, 1977). These new cells are added at the peripheral edge, at the ciliary margin zone (CMZ), in a

manner that is thought to recapitulate embryonic retinal cell development (Harris and Perron, 1998). A similar zone of proliferating cells has been found in the chick that contributes to the postnatal growth of the retina (Fischer and Reh, 2000). However, the presence of a CMZ in the retina of the mouse has not been detected (Kubota et al., 2002). It has been speculated that the existence of a population of adult stem-like cells isolated from the ciliary body of the retina in mammals is the evolutionary equivalent of cells from the CMZ (Tropepe et al., 2000).

A population of quiescent cells from the ciliary body of the mammalian retina were discovered to proliferate in vitro, express immature retinal markers, and upon differentiation express markers of mature retinal cell types (Tropepe et al., 2000; Ahmad et al., 2000). These adult-derived RS cells can be grown as neurospheres with epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF2) and differentiated by culture on substrate-coated plates in a growth factor–free serum-containing medium, similar to adult neural stem (NS) cells (MacNeil et al., 2007; Ahmad et al., 2000). The addition of Wnt3a and FGF2 to adult RS cell neurosphere cultures has been shown to have an additive effect on cell proliferation, resulting in greater numbers of secondary neurospheres (Inoue et al., 2006). RS cell neurospheres have been derived from the iris, ciliary body, and pars plana, but not the anterior neural retina (Gu et al., 2007; Haruta et al., 2001; MacNeil et al., 2007). Further to the studies in rodent and porcine eyes, RS cells have also been isolated from adult human retinal tissue and shown to form neurospheres in vitro (Carter et al., 2007; Mayer et al., 2005).

Differentiated neurosphere cultures give rise to both neuronal and glial cell types, suggesting multipotentiality. However, the expression of a small number of mature retinal markers may not indicate completely differentiated and functionally mature retinal cell types (MacNeil et al., 2007; Kokkinopoulos et al., 2008). Several studies have investigated the induction of mature retinal phenotypes in RS cell cultures from both the adult ciliary body and iris by retroviral transduction of photoreceptor relevant transcription factors. The expression of *Crx* or *Otx2* in both cell types demonstrated the directed differentiation of cells positive for Rho, recoverin, and transducin protein expression (Akagi et al., 2004). In contrast, transduced mesencephalon-derived NS cells displayed little Rho immunoreactivity, suggesting that NS cells require greater manipulation to differentiate toward retinal cell lineages (Akagi et al., 2004; Haruta et al., 2001). In further studies, primate iris-derived cells were induced to differentiate into Rho-positive cells after transduction with a combination of both *Crx* and *NeuroD* retroviral vectors. Both rat and primate differentiated cells were shown to hyperpolarize after light stimulation, suggesting the generation of functional photoreceptor cell types (Akagi et al., 2005). Similar to this study, genetically modified mouse RS cells electroporated to express *Crx* have also been shown to induce differentiated cells that exhibit some functional properties of mature retinal photoreceptors (Jomary and Jones, 2008). This was in contrast to RS cells electroporated with a control plasmid, which differentiated to express mature photoreceptor cell markers but did not demonstrate light-sensitive properties (Jomary and Jones, 2008). These studies suggest that RS cells could be induced to differentiate into light-sensitive rod photoreceptor phenotypes; however, the expression of mature retinal markers by differentiated cells does not necessarily equate to functional photoreceptors (Bradford et al., 2005). Therefore, extensive in vitro characterization of differentiated cells is required prior to retinal cell transplantation. The potential of these cells to function in vivo and improve visual responses following transplantation into the degenerate neural retina has yet to be established (Akagi et al., 2005). RS cells isolated from the ciliary body or iris tissue have, to date, shown limited potential for cell integration after transplantation into adult wild-type or degenerate retinas (Akagi et al., 2003; Chacko et al., 2000; Klassen et al., 2007a; Canola et al., 2007). This is most likely due to reduced numbers of RS cell-derived photoreceptor precursors at the correct ontogenetic stage (MacLaren et

al., 2006). Further investigation of homogeneous populations of transplanted cells at characterized stages of differentiation may enable RS cell-derived transplants to integrate with the host retina (Canola and Arsenijevic, 2007; Akagi et al., 2003).

To confirm that cultured cells can integrate into the neural retina, the transplantation of cultured retinal progenitor cells isolated from the embryonic retina has been investigated by a number of groups. The majority of studies have demonstrated the differentiation of transplanted progenitor cells into mature retinal phenotypes in the subretinal space. However, little integration into the host retina was observed following the subretinal transplantation of these cells (Akagi et al., 2003; Chacko et al., 2000). Other studies have observed very little differentiation and mature retinal cell marker expression, and concluded that progenitor cells required further differentiation in vitro prior to cell transplantation (Yang et al., 2002). Klassen et al. have examined the integration of cultured P1 retinal cells in the *rho*$^{-/-}$ mouse and demonstrated *gfp* (green fluorescent protein)-positive transplanted cells within the host neural retina. These integrated cells expressed mature retinal photoreceptor markers but lacked mature retinal cell morphology (Klassen et al., 2004b). A possible explanation for the differences observed in vivo following cultured retinal progenitor cell transplantation is the different culture conditions used. However, these investigations suggest that the culturing of cells in vitro prior to transplantation does not inhibit their migratory potential.

There has been some debate as to whether RS cells constitute a neural adult stem cell population like those found in the subventricular and subgranular zones of the brain, or whether these cells have limited self-renewal suggesting a progenitor-like phenotype (Xu et al., 2007; Inoue et al., 2005; Liu et al., 2005; Engelhardt et al., 2004; Kokkinopoulos et al., 2008; ). A growing number of investigations have found that RS cells can only be sustained in vitro for a limited period (Liu et al., 2005; Inoue et al., 2005; MacNeil et al., 2007). Due to the difficulty of propagating retinal cells individually, it is impossible to perform clonogenic analysis to establish if these cells divide asymmetrically. A study comparing the growth characteristics of adult rat-derived RS cells with those of NS cells demonstrated a lack of cell proliferation and self-renewal after 8 weeks in vitro for the former, while NS cells continued to proliferate in neurosphere cultures (Liu et al., 2005). It therefore remains to be determined whether RS cells can be sufficiently expanded in vitro for therapeutic purposes.

In the brain, the radial glial cells of the adult hippocampus proliferate and differentiate into neurons throughout life (Seri et al., 2001, 2004). A similar phenomenon has been observed in the adult neural retina of fish, with the generation of new neurons from the equivalent glial cell type in the retina, the Müller cells (Raymond et al., 2006; Bernardos et al., 2007). Several studies have demonstrated that Müller cells from the adult mammalian central retina also have some stem-like characteristics in vitro. This includes the formation of neurospheres and the expression of NS cell markers such as *Sox2, Pax6*, and *Chx10* (Lawrence et al., 2007; Das et al., 2006a; Nickerson et al., 2008). A spontaneously immortalized cell line of MS cells has been established from human retinal tissue, and their expansion did not appear to be limited like that of RS cells (Limb et al., 2002). Following differentiation, MS cells have been shown to express mature retinal cell markers, including peripherin, recoverin, and S-opsin (Lawrence et al., 2007; Das et al., 2006b). Of note, recent investigations in the *Chx10*$^{orJ/orJ}$ mouse have demonstrated a population of cells present in the central neural retina that exhibit properties similar to those of ciliary epithelium-derived RS cells (Dhomen et al., 2006; Kokkinopoulos et al., 2008). As the mutation of *Chx10* results in reduced retinal progenitor cell proliferation and microphthalmia, it has been suggested that these cells represent a dormant progenitor cell population that is maintained in the mutant central neural retina but not in wild-type retinas (Dhomen et al., 2006). When

cultured in vitro, these cells express glial cell markers and may represent a similar Müller progenitor cell population. Similar to the differentiation of RS cells into mature retinal cell types, differentiated cells derived from MS cell cultures have yet to be functionally characterized to confirm that they represent fully differentiated retinal neurons.

So far, cultured MS cells have shown limited integration into host retinas following transplantation, similar to RS cells (Singhal et al., 2008; Lawrence et al., 2007; Bull et al., 2008). Increased integration was observed in degenerate retinas following chondroitinase ABC treatment at the time of cell transplantation, suggesting that chondroitin sulfate proteoglycans (CSPGs) form a significant barrier to cell migration and integration (Singhal et al., 2008). MS cell migration was enhanced further by substantial immune suppression, demonstrating a combinational effect (Singhal et al., 2008). As microglia can be activated by CSPGs and their breakdown products have been shown to exert anti-inflammatory effects, it is likely that an innate immune response against the transplanted MS cells is at least partially inhibiting successful cell integration (Jones and Tuszynski, 2002; Jones et al., 2002; Rolls et al., 2006). Further characterization of the developmental stage of the transplanted population may enable the functional integration of photoreceptor cells derived from this source. However, immune suppression would be required for the long-term integration of human-derived MS cells in models of retinal degeneration.

In contrast to adult-derived RS cells, which exhibit limited self-renewal, ES cells isolated from the inner cell mass of the blastocyst can be grown in culture for indefinite periods of time, after which they can be induced to differentiate into cell lineages of all three germ layers (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1995, 1998; Suemori et al., 2001; Pera et al., 2000; Reubinoff et al., 2000). Therefore, established ES cell lines could provide an expandable source from which to derive photoreceptor precursor cells for retinal transplantation. Of concern for future clinical application is the culturing of ES cells with animal-derived reagents such as animal serum and animal-derived feeder layers, or by the use of animal cell culture conditioned medium. This is because the approval of therapeutic agents for use in humans requires them to be free of pathogens and animal contamination. Several studies have successfully cultured human ES cells in serum-free conditions and without feeder layers (Amit et al., 2004; Xu et al., 2001). A human ES cell line was recently established without the use of animal contaminated reagents, demonstrating that this should not be an issue for future clinical therapies (Ludwig et al., 2006a, b).

The differentiation of ES cells into neural progenitors that can produce the three main neural cell lineages of neurons, astrocytes, and oligodendrocytes has been well established (Joannides et al., 2007). Further to this, human ES cells have been shown to differentiate into various types of neurons, including dopaminergic neurons and oligodendrocytes (Yan et al., 2005; Perrier et al., 2004; Zhang et al., 2001; Nistor et al., 2005). Transplantation studies involving these differentiated cell types have demonstrated the potential of ES cells to produce differentiated neural cell populations that can be used for cell transplantation strategies (Nistor et al., 2005; Keirstead et al., 2005; Rodriguez-Gomez et al., 2007). The differentiation of ES cells into retinal cell lineages has not achieved the same progress as that seen for other neural cell types of the brain. However, recent advances in cell culture techniques have demonstrated the possibility of producing mature retinal cells from mouse, primate, and human ES cells (Osakada et al., 2008; Lamba et al., 2006). Previous studies have shown the differentiation of mouse ES cell-derived neural progenitors into photoreceptor-like cells after coculture with P1 or E6 retinal tissue. The differentiation of retinal cells was determined by immunohistochemistry and RT-PCR for photoreceptor-specific markers, including *Crx, Nrl, Rho kinase, arrestin*, and *interphotoreceptor retinoid-binding protein* (Zhao et al., 2002; Sugie et al., 2005). Despite the apparent generation of mature retinal phenotypes, the specific factors required to promote the differentiation of

these cells were not established. Further studies using more defined culture conditions demonstrated the differentiation of mouse and human ES cells into immature retinal cells. However, coculture with retinal explants or cell suspensions was still required for the expression of mature photoreceptor markers such as *recoverin* (Ikeda et al., 2005; Lamba et al., 2006). Recently, Osakada et al. demonstrated the generation of mature rod and cone photoreceptors from ES cells, with the use of defined culture conditions. They found 17.2±1.8% and 8.5±2.9% of cells were Rho and recoverin positive, respectively, after the stepwise differentiation of mouse and human ES cells (Osakada et al., 2008). Despite the demonstration of gene expression for phototransduction components in these cells, further evidence of their function is still required. It will be of great interest to determine whether these cells, if differentiated to the correct ontogenetic stage, could functionally integrate into the adult neural retina.

## Optimization of transplanted cell integration

Retinal disease has many different genetic and environmental causes, which result in a wide range of pathological conditions. A consistent outcome of these disorders is the degeneration and eventual loss of photoreceptors from the ONL. In order to replace these lost cells, transplanted photoreceptor precursors are required to migrate and integrate into the degenerated ONL. While the number of integrated photoreceptor precursor cells demonstrated in the adult neural retina is sufficient to restore the pupillary light reflex, only a relatively small number of transplanted cells integrate. Greater numbers of integrated cells would be required in order to improve visual acuity in degenerate models. As photoreceptor precursor cells are intrinsically capable of migrating and differentiating into the adult neural retina, it follows that other barriers must be present that limit extensive cell integration (MacLaren et al., 2006). The ability of transplanted cells to integrate within the host opossum retina has been shown to decline with host maturation (Sakaguchi et al., 2003, 2004). This coincides with the maturation of glial elements, such as Müller cells, which form anatomical barriers within the host retina, including the outer limiting membrane (OLM).

The OLM has been shown to be a significant physical barrier to the migration and integration of photoreceptor precursor cells into the adult host ONL. OLM disruption, by the administration of the glial toxin alpha-aminoadipic acid (AAA), at the time of cell transplantation was shown to correspond with increased photoreceptor precursor cell integration (West et al., 2008). In mice with retinal dystrophy caused by defects in Crumbs homologue-1 (Crb1), a protein associated with adherens junction formation and stabilization, increased photoreceptor precursor cell integration has also been observed (Pearson et al., manuscript in preparation). However, OLM disruption has not been observed after retinal degeneration caused by other gene defects (Gouras and Tanabe, 2003; Sanyal and Hawkins, 1989). This suggests that the OLM would remain a significant barrier to transplanted photoreceptor cell integration in the majority of retinal degenerations (Fig. 3). The pharmacological induction of OLM disruption by AAA would not be suitable in degenerate retinas due to toxic effects on the supportive Müller glia (Pedersen and Karlsen, 1979; Ishikawa and Mine, 1983; Rich et al., 1995). An alternative method to induce transient OLM disruption is the use of small interfering ribonucleic acid (siRNA) to promote transcriptional gene silencing of relevant OLM-related proteins. Further investigation in degenerate models is required to establish the effect of OLM disruption on photoreceptor precursor cell integration in degenerate retinas.

A crucial difference between normal and degenerate retinas that may limit photoreceptor precursor cell integration is the presence of Müller cell activation in the latter. Following injury or degeneration of the neural retina, a process known as reactive gliosis occurs. This

can vary in severity depending on the initiating insult and is indicated by the expression of glial fibrillary acidic protein (GFAP) by Müller cell processes (Lewis and Fisher, 2003). In contrast, in uninjured retinas, GFAP is only expressed by astrocytes present at the inner edge of the neural retina. In addition to the upregulation of GFAP and vimentin by Müller cells during reactive gliosis, Müller cell processes have also been shown to form glial barriers along the outer edge of the retina after retinal detachment (Fisher et al., 2005; Lewis and Fisher, 2000, 2003). This glial scarring constitutes a barrier to integrating transplanted cells and is a characteristic of many late-stage retinal disease models (Zhang et al., 2004; Ekstrom et al., 1988; Sheedlo et al., 1995; Iandiev et al., 2006; Fan et al., 1996;).

Similar barriers to cell transplantation, such as the OLM and glial scarring, have been reported to limit the “integration” of retinal sheets with the host retina, as neurite extension does not occur in these regions (Zhang et al., 2003, 2004). Further to this, activated Müller cells and microglia are thought to produce increased extracellular matrix (ECM) components such as CSPGs, which have been shown to limit axon extension in the brain (Fawcett and Asher, 1999). Several studies have investigated the use of enzymes, such as chondroitinase ABC, neuraminidase X, and matrix metalloproteinase-2 (MMP-2), to break down these extracellular barriers in combination with cell transplantation and demonstrated encouraging results (Singhal et al., 2008; Suzuki et al., 2006, 2007; Zhang et al., 2007). It therefore seems that the investigation of techniques to reduce reactive gliosis and the subsequent glial scarring and ECM deposition will be important for successful photoreceptor precursor cell integration in late-stage retinal degeneration (Fig. 3).

One common feature of all retinal degenerations is cell death and the subsequent activation of the resident macrophage population, the microglia (Hughes et al., 2003; Hose et al., 2005; Zhang et al., 2005; Roque et al., 1996). This has also been demonstrated for injury-induced models of retinal degeneration (Harada et al., 2002). In our own investigations, we have noted that increased macrophage presence shortly after cell transplantation resulted in fewer integrated photoreceptors (unpublished results). It is not clear whether macrophages prevent precursor cell integration or cause the destruction of the integrated photoreceptors. However, the difference in inflammatory status between normal and degenerate retinas may be the cause of reduced photoreceptor cell integration observed in the latter. A recent study demonstrated increased numbers of sialoadhesin-expressing macrophages present in *rd1* and *rds* mouse models following precursor cell transplantation, and suggested that this may affect the survival of transplanted cells (Sancho-Pelluz et al., 2008). Previous studies have detected the presence of sialoadhesin-positive macrophages in untreated *rds* mice and a model of experimental autoimmune uveoretinitis (Jiang et al., 1999, 2006; Hughes et al., 2003). Sialoadhesin expression has been shown to contribute to the inflammatory response by promoting T cell and macrophage adhesion (Crocker et al., 1995; Jiang et al., 1999, 2006). Therefore, the increased inflammatory status of degenerate retinas may prompt the early rejection of transplanted cells, and initial innate immune suppression may be required to successfully transplant cells in these models of retinal degeneration (Fig. 3).

Immune rejection is a major problem in many transplantation paradigms. However, the brain and the eye are frequently described as immune-privileged sites, defined as sites that allow foreign grafts to survive for extended to indefinite periods of time. The eye contains several immune-privileged sites, namely, the anterior chamber, vitreous cavity, and subretinal space. Streilein et al. (2002) have performed extensive experiments examining the survival of neonatal retinal allografts in the eye. In combination with the eye maintaining an immune-privileged site, neonatal retinal tissue itself has been shown to be partially immune privileged when placed beneath the kidney capsule, a non-immune-privileged site. This is in contrast to skin grafts, a non-immune-privileged tissue type, which have been shown to be rejected by 12 days, and fully immune-privileged tissues, including the cornea and the RPE,

which survived for indefinite periods of time (Ng et al., 2002; Hori et al., 2000; Wenkel and Streilein, 2000).

Of greatest relevance to photoreceptor precursor cell transplantation is that the subretinal space has been shown to elicit immune deviation after cell-associated or soluble antigen administration. The immune deviation of eye-derived antigens is a form of immune tolerance, a state of specific immunological unresponsiveness, mediated by antigen-specific T regulatory cells, also referred to as suppressor T cells (Streilein and Niederkorn, 1985; Wilbanks and Streilein, 1990). These cells are produced in the spleen and suppress delayed-type hypersensitivity immune reactions to alloantigens present in the eye. However, the immune deviation of alloantigens present in the subretinal space is lost if RPE cell viability is compromised or the outer blood-retinal barrier is disrupted (Wenkel and Streilein, 1998). Transplantation of neonatal retinal allografts to the subretinal space and vitreous cavity have been shown to induce immune deviation by 12 days, whereas transplantation to the subconjunctival space promoted antigen-specific delayed hypersensitivity (Jiang et al., 1993). However, neonatal retinal allografts eventually deteriorate in both the anterior chamber and the subretinal space by 35 days (5 weeks) post implantation. This appears to coincide with the loss of immune deviation and the onset of donor-specific delayed hypersensitivity (Jiang et al., 1995; Streilein et al., 2002).

The eye therefore represents a partially immune-privileged site and appears to eventually reject allogeneic cells transplanted to the subretinal space. This may be of concern for long-term retinal repair by cell transplantation. It remains to be seen whether a homogenous population of cultured photoreceptor precursor cells would elicit immune rejection following transplantation to the neural retina. Cultured neural progenitors have been shown to be less immunogeneic compared with freshly dissociated neural progenitors, the most likely explanation for this is the lack of donor-derived microglia in the cultured cell population (Hori et al., 2003; Ma and Streilein, 1998). Further investigation of cultured retinal progenitor cells transplanted to the subretinal space is required to establish the relevant issues of immune rejection for photoreceptor precursor cell transplantation.

## Future considerations for retinal cell therapy

Studies have shown that precursor cells at the correct ontogenetic stage can migrate and integrate into the adult host ONL and form functional synaptic connections (MacLaren et al., 2006). Several studies have since demonstrated mature photoreceptor morphology of integrated precursor cells in adult retinas (MacLaren et al., 2006; West et al., 2008; Bartsch et al., 2008). Recent studies of ES cells have established defined culture conditions to differentiate ES cells into photoreceptors (Osakada et al., 2008). Despite the recent advances in the production of ES cell-derived retinal cells, these may not translate into successful cell transplantation strategies, namely, due to the foreign nature of these cells with regard to the host immune system. Classic immunosuppressive drug therapy could be used, or alternatively, a human ES cell bank of cell lines characterized by human leukocyte antigens (HLA) could be created to provide closely matched differentiated cells (Taylor et al., 2005). Several studies have investigated novel ways to promote prolonged immunological tolerance to transplanted alloantigens in the eye, such as the transplantation of retinal progenitors combined with immature dendritic cells or alpha-melanocyte-stimulating hormone–induced T regulatory cells to develop or transfer immune tolerance, respectively, against the alloantigens present. Such strategies appear to lead to enhanced transplanted cell survival (Ng et al., 2007; Oishi et al., 2007). It may therefore be possible to exploit the eye’s natural immune deviation response to enable prolonged transplanted cell survival.

Barriers to photoreceptor precursor cell transplantation, such as the OLM and glial scarring, would still be present in the adult human retina. Intriguingly, however, cystoid macular edema (CME) is a condition seen in the end stages of many diseases of the outer retina, such as retinitis pigmentosa and diabetic maculopathy; microscopic examination of pathological specimens have shown that CME represents an intracytoplasmic swelling (edema) of Müller cells in the foveal region (Yanoff et al., 1984) which is similar to the effects of AAA described in previous studies (West et al., 2008; Ishikawa and Mine, 1983; Pedersen and Karlsen, 1979). Therefore, the diseased human fovea may have reduced OLM integrity and, as a result, constitute a particularly favorable site for future retinal cell transplantation strategies. This would be especially important if cone photoreceptor precursor cells are also able to integrate into the adult ONL, as observed for rod photoreceptor precursors (MacLaren et al., 2006). Other conditions that might be particularly suitable for cell replacement strategies include inherited retinal degenerations due to defects in Crb1, which have also been shown to result in reduced OLM integrity (Mehalow et al., 2003; van de Pavert et al., 2007).

A recent advance in stem cell biology has been the reprogramming of adult human fibroblasts by retroviral transduction to generate induced pluripotent stem (iPS) cells. Three independent studies used various combinations of four transcription factors, known to be required for pluripotency in ES cells (Friel et al., 2005), to induce adult cells to acquire pluripotent characteristics (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008). However, the use of retroviral transduction of transcription factors results in multiple random insertions of the transgene, which can also lead to oncogenesis in certain circumstances (Cattoglio et al., 2007). At present, very small numbers of human ES cell-like iPS cell colonies are produced (around 1 in 1000 cells). Therefore, further investigation of this cell population is required to improve the efficiency of the methods used and establish virus-free protocols of induction that would be less oncogeneic and have greater viability for therapeutic applications (Nakagawa et al., 2008; Kim et al., 2008; Okita et al., 2007). It will, however, be of significant interest to determine whether the current differentiation protocols for human ES cell-derived retinal cells also work for human iPS cells.

For retinal dystrophies caused by photoreceptor-specific gene mutations, autologous adult-derived cells do not initially appear to be the best source of new retinal neurons, as the genetic mutation will remain. However, by ex vivo gene therapy, they have the potential to replace and restore visual function in degenerate retinas. Future treatment for retinal degeneration due to photoreceptor cell loss may require a combination of gene and cell therapeutic strategies (Bainbridge et al., 2008; Maguire et al., 2008). An alternative to this is the use of allogeneic, but closely matched, adult donor cells from which photoreceptor precursor cells for transplantation can be generated. Similar to conventional organ transplantation, these cells could be derived from a close family member or HLA-matched donor tissue to reduce the possibility of transplanted cell rejection. However, for some retinal dystrophies that progress slowly, the integration of recently derived autologous photoreceptors may limit further degeneration, especially in diseases such as retinitis pigmentosa where the loss of peripheral rod photoreceptors leads to the secondary loss of cone photoreceptors vital for central vision. Therefore, the successful rescue of retinal degeneration via cell therapy is most likely to involve a combination of different strategies and methodologies, depending on the pathology of the retinal disease being treated.

In summary, the restoration of visual responses by photoreceptor precursor cell transplantation to the human retina remains a promising strategy for retinal repair. Many studies have demonstrated both the potential structural barriers to precursor cell transplantation present in the adult and degenerate retina, as well as the need for autologous cell transplantation to promote long-term survival of transplanted cells. Strategies to

modulate these factors have highlighted some important considerations for future transplantation studies. The transplantation of photoreceptor precursor cells derived from the recently discovered iPS cells will be of great interest for future regenerative strategies of the neural retina. Since this review was written several papers of related interest have been published, these include Cicero et al. (2009) and Hirami et al. (2009).

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## Abbreviations

| | |
|---|---|
| **AAA** | alpha-aminoadipic acid |
| **CME** | cystoid macular edema |
| **CMZ** | ciliary margin zone |
| **Crb1** | Crumbs homologue-1 |
| **CSPG** | chondroitin sulfate proteoglycan |
| **ECM** | extracellular matrix |
| **EGF** | epidermal growth factor |
| **ES cell** | embryonic stem cell |
| **FGF2** | fibroblast growth factor-2 |
| **GFAP** | glial fibrillary acidic protein |
| **GFP** | green fluorescent protein |
| **HLA** | human leukocyte antigen |
| **iPS cell** | induced pluripotent stem cell |
| **ONL** | outer nuclear layer |
| **OLM** | outer limiting membrane |
| **MMP-2** | matrix metalloproteinase-2 |
| **MS cell** | Müller stem-like cell |
| **NS cell** | neural stem cell |
| **Rho** | rhodopsin |
| **RPE** | retinal pigment epithelium |
| **RS cell** | retinal stem-like cell |
| **SiRNA** | small interfering ribonucleic acid |

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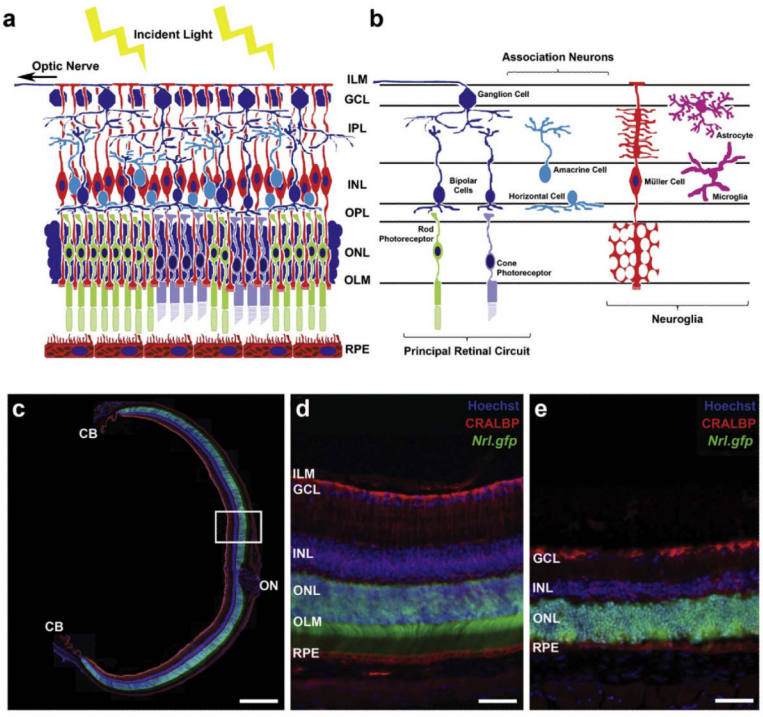


**Fig. 1.**

The mammalian retina. (a) A schematic diagram illustrating the layers of the mammalian retina (green rod and purple cone photoreceptors; red Müller cells and RPE; blue nuclei). (b) A schematic diagram illustrating the position of the various cell types present in the adult neural retina. These cells are subdivided into (i) the principal retinal circuit, (ii) the association neurons, and (iii) the neuroglia. (c) A sagittal retinal section from an *Nrl.gfp* (green; rod photoreceptors) mouse. Scale bar, 200 μm. (d) A single fluorescence image of an adult *Nrl.gfp* retinal section stained for CRALBP (red), a protein present in Müller cells and the RPE. Scale bar, 40 μm. (e) A single fluorescence image of a degenerating retinal section stained for CRALBP (red), demonstrating the disorganization and loss of photoreceptor cells (*Nrl.gfp*; green). Scale bar, 40 μm. Nuclei were counterstained with Hoechst 33342 (blue). CB, ciliary body; ON, optic nerve; ILM, inner limiting membrane; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OLM, outer limiting membrane; RPE, retinal pigment epithelium. (See Color Plate 1.1 in color plate section.)

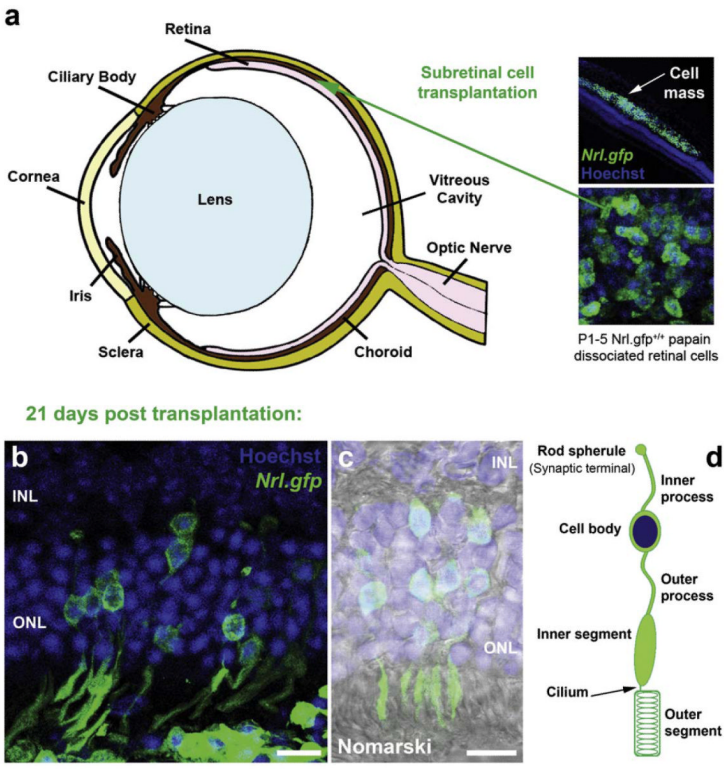


**Fig. 2.**

Photoreceptor precursor cell transplantation into the adult eye. (a) A schematic diagram of a mouse eye illustrating the subretinal transplantation of *Nrl.gfp* precursor cells (green) and the resulting cell mass (inserts). (b) A confocal image of integrated *Nrl.gfp* rod photoreceptors, 21 days after transplantation to an adult recipient. (c) A Nomarski confocal image of integrated *Nrl.gfp* rod photoreceptors. (d) A schematic representation of the structure of a rod photoreceptor. Nuclei were counterstained with Hoechst 33342 (blue). Scale bars, 20 μm. INL, inner nuclear layer; ONL, outer nuclear layer. (See Color Plate 1.2 in color plate section.)




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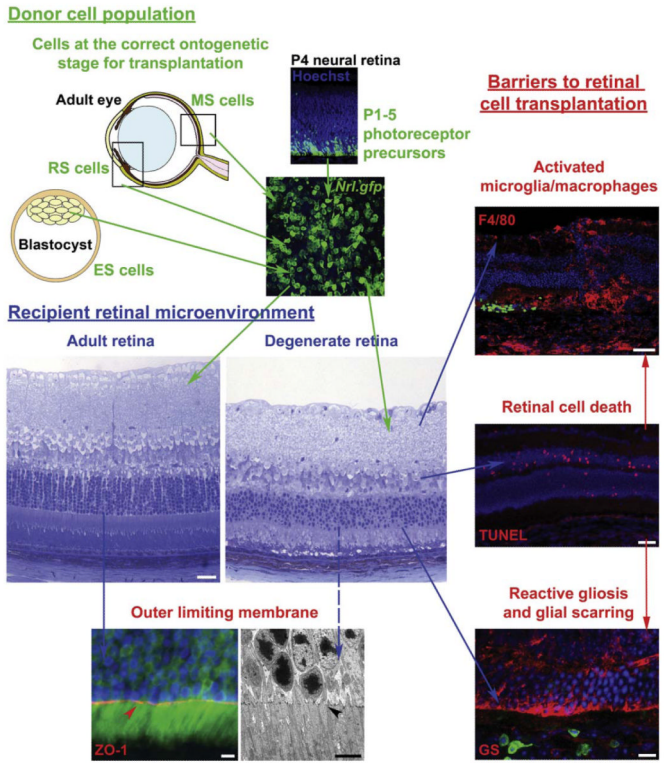


**Fig. 3.**

A summary of retinal cell transplantation strategies. A diagram to summarize the various retinal cell transplantation strategies and the related barriers that may limit transplanted photoreceptor cell integration in the adult and degenerate neural retina, as discussed in the main text. The donor cell population (top; green) can be derived from a variety of cell sources, but must be differentiated to the correct ontogenetic stage (postmitotic rod precursors, *Nrl.gfp*; green) prior to transplantation to enable photoreceptor cell integration into the host adult retina (MacLaren et al., 2006). The recipient retinal microenvironment (middle; blue) may also limit photoreceptor cell integration if the relevant barriers are not modulated at the time of transplantation. Scale bar, 50 μm. The relevant barriers to retinal cell transplantation and integration (right; red) are indicated. The outer limiting membrane (indicated by the red or black arrow head) forms a barrier to increased cell integration in the adult retina and in some models of retinal degeneration. Scale bars, 10 μm and 5 μm. Other barriers, present predominantly in the degenerate retina, include retinal cell death and the resulting activated microglia/macrophages and reactive gliosis/glial scarring. Scale bars, 50, 100, and 20 μm, respectively. Nuclei were counterstained with Hoechst 33342 (blue). ES cells, embryonic stem cells; GS, glutamine synthetase; MS cells, Müller stem-like cells; RS cells, retinal stem-like cells; ZO-1, zonula occludens-1. (See Color Plate 1.3 in color plate section.)