Replacement of the RPE monolayer

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Abstract

There are numerous scenarios in which replacing the diseased RPE monolayer is an attractive but as yet unrealised goal. The proof of concept that vision can be improved by placing a healthy neuroretina onto a different, healthy, underlying RPE layer is demonstrated in patch graft transplantations. The surgical procedure to relocate the neuroretina is both complex and is hampered by postoperative complications and as such newer replacement procedures are also being investigated including stem cell replacement therapies. Past studies have largely focused on using cell suspensions and have had disappointing outcomes largely due to the lack of control over cellular differentiation, incomplete attachment onto Bruch's membrane and subsequent integration into the existing RPE monolayer. The choice of which cells to transplant is still under investigation and is complicated by factors such as the ease of collection of an adequate sample, rejection following implantation, the age of the cells and ethical issues. In all these situations, however, understanding the mechanisms of cellular differentiation are likely to be prerequisite to future successes.

The current research into replacing the RPE monolayer is briefly discussed with reference to our experiences comparing IPE and RPE cells in an *in vitro* environment.

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degeneration. The recent introduction of anti-VEGF agents (eg, ranibizumab, and so on) have shown great benefit with more than 35% of patients with classic choroidal neovascularisation (CNV) experiencing a 15-letter visual improvement at 1 year.¹ The VEGF pathway, however, is only one component of the angiogenic response which is in turn only a part of the overall wound healing response occurring at the macula.² At the time of diagnosis, the subretinal neovascular membranes are already composed of established vascular networks and most of the benefit from anti-VEGF agents may be due to cessation of plasma leakage from incompetent new vessel walls.^{3,4} In addition, with the repeated injections and limited regression of the CNV scar observed, there are a large number of patients for whom no treatment is available. In addition the reality of the much greater problem of non-neovascular AMD has yet to be addressed in any meaningful therapeutic way.

So, while progress in the last decade or so has been outstanding, the scientific community can ill afford to standstill as an ageing epidemic is almost upon us.

There are therefore a number of reasons, which make the idea of replacing the RPE monolayer worthy of even more extensive research. For example, the RPE does not regenerate during adult life and the cells have been exposed to a continuous workload, which as we age, understandably can take its toll. Such examples of this are well documented in the literature including lipofuscin accumulation, altered gene and protein synthesis, oxidative stress, development of drusen and the accumulation of advanced glycation end-products.^{5,6}

The concept of replacing RPE cells has been around for several decades and came to the fore when submacular surgery demonstrated that CNV excision is invariably accompanied with loss of adjacent RPE along with disruption of the underlying Bruch's membrane.⁷ The subsequent loss of RPE is consistent with RPE atrophy and the poor visual outcome that is

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Introduction

A wide ranging variety of diseases at the level of the RPE monolayer make replacing the diseased cells with a healthy monolayer an idealistic goal. The main disease targeted for such therapy has been age-related macular

seen after such surgery.⁸ Studies therefore began to focus on whether replacing the lost RPE cells with either homologous (fetal and adult RPE cells) or autologous sources of RPE and IPE cells following post surgical CNV removal could improve the visual outcome. The advantages and disadvantages of each cell source are discussed further below. However, it was the macular relocation and patch graft transplant procedures, which clearly demonstrated a 'proof of principle' for replacing the RPE monolayer. Case selection procedures are being developed using algorithm to assess suitability for macular translocation, which may increase surgical successes.⁹ However, Van Meurs *et al* and Stanga *et al*^{10,11} have shown some functional results following patch graft transplant procedures at 1 year and over 2 years follow-up respectively with improvement in visual function, fixation of the graft using ocular coherence tomography and its vascularisation confirmed by fluorescein and indocyanine green angiography. These procedures used autologous peripheral full-thickness grafts, which were composed of neurosensory retina, RPE, Bruch's membrane, choriocapillaris and choroid and were usually harvested from the midperiphery prior to translocation to the macula following CNV removal.¹² More recent data suggests that longer than 1 year function may be preserved in some patients with dry AMD.^{13,14} However, although autologous RPE transplantation can in principle restore vision in neovascular AMD, surgical variability and complications still remain high.¹⁵

Transplantation of adult RPE cells

Adult RPE have been transplanted into the subretinal space of various animal models as well as human. Methods have included autologous cells, genetically modified RPE cell lines, homologous and heterologous RPE cells as cell suspensions or grown as a monolayer on substrates prior to implantation.

Photoreceptor rescue has been documented by injecting healthy RPE cell suspensions prior to the onset of photoreceptor degeneration in the RCS rat model. Unfortunately, atrophy of photoreceptors was not prevented by transplantation of RPE cells at a later stage of the disease, which may indicate a limited time frame of opportunity for successful transplant procedures. In addition, human RPE cell lines also have been successfully transplanted into the subretinal space of the RCS rats and shown preservation of photoreceptors, visual responses and cortically mediated vision.^{16,17}

The environment into which cells are transplanted remains a valid concern. In AMD patients the problems to surmount immediately following CNV excision include the remnants of Bruch's and disrupted choroid

as this will govern the microenvironment and thus the fate and success of the cell transplant.^{12,18} Thus, it is important that cells are transplanted with an underlying substrate at the outset. The choice of substrate is covered elsewhere in more detail (see Sheridan *et al*¹²) but in particular it must have excellent biocompatibility, be porous and sustain an intact monolayer of cells. Modifying the patient's own Bruch's membrane may represent an option for some patients but as Bruch's can present itself as either aged, diseased, fragmented or even missing this will be a difficult issue to resolve. Peripheral RPE cells have been harvested and then transplanted, as a cell suspension, into the same eye post CNV removal.^{19,20} Although visual improvement and preservation of retinal thickness have been shown in some patients at follow-up, many other cases have been complicated by extensive PVR, retinal detachment, cell clumping with failure to form an intact monolayer or cell migration.

More recently, 12 patients have received a homologous transplant of RPE cells on a gelatine substrate following surgical removal of a subfoveal CNV membrane, who subsequently underwent immunosuppressive treatment post transplant.²¹ Unfortunately, although the transplanted cells appeared not to be rejected, there was no significant difference between 1 year and preoperative values of best-corrected visual acuity, contrast sensitivity or reading speed. Histological analysis of one patient revealed the RPE transplant did not establish a monolayer and RPE survival was poor. This type of failure may be circumvented if an intact differentiated monolayer on a suitable underlying substrate could be transplanted initially rather than relying on the cells to attach to an aged Bruch's membrane, proliferate, differentiate and integrate with the neural retina before any further photoreceptor loss occurs. Indeed, preventing dedifferentiation of the monolayer will no doubt play a key role in future transplant procedures. Agents that specifically target dedifferentiated cells rather than their differentiated counterparts should be of value to help maintain an intact functioning monolayer.^{22,23} Many studies have shown growth of healthy cells (RPE and IPE) on biodegradable and non-biodegradable artificial substrates and are discussed outside of this review.¹² We believe that non-degradable artificial substrates hold the greatest promise of achieving such monolayers and satisfying regulatory body approval.

Fetal RPE cells

The Alverre study was the first to demonstrate some initial success in humans with subretinal transplantation of small monolayer patches of human fetal RPE in AMD

patients following removal of neovascular membranes. Unfortunately at 3 months post transplantation, retinal function was lost and fixation on the graft was no longer observed. In addition, leakage around the graft was also evident indicating the transplanted cells were being rejected.²⁴ Crucial confounding factors such as rejection have also been well documented in most animal models studied and additional complications such as cells clumping or multilayering, rosette formation and photoreceptor loss with resultant drop in visual function have been reported. However, sheets and fetal cell suspensions have been shown to integrate within the monolayer and preserve the normal retinal lamination as well as form some connections with photoreceptors and subsequent visual responsiveness.^{25–27}

In addition to transplanting fetal RPE, transplantation of a fetal neuroretina/RPE complex in a 64-year-old woman with retinitis pigmentosa resulted in improvement in visual acuity at 5-year follow-up.²⁸ However, this appears to be an isolated case because no such change was observed in any other patient studied. In addition, the patients who could tolerate the triple immunosuppression for 6 months showed no apparent immune rejection. Although earlier studies on patients without immunosuppressive drugs which showed no apparent signs of graft rejection at 6 months post transplant demonstrated features associated with rejection that became evident at 1 year post transplant.²⁹ This form of operation is unlikely to play a major role in future treatment modalities even if the results had given spectacular improvements in vision as there are serious ethical and logistical issues involved. The ethical issues of using fetal tissue have been widely reported and the logistics of using one fetus per transplant also makes it unfeasible.

Transplantation of IPE cells

Obtaining an autologous supply of cells, which are relatively easy to harvest, has resulted in numerous studies providing compelling evidence for the use of IPE cells as an alternative cell type for transplantation in AMD. IPE cells can be harvested from a simple surgical procedure, such as an iridectomy³⁰ and provide an autologous source of cells thus overcoming the problems associated with immune rejection and long-term immunosuppression. In addition, the IPE cells may afford the advantage of being relatively healthier than peripheral RPE, which may to some extent be affected by the underlying disease.¹² IPE and RPE have been shown to possess a number of key characteristics in common which make successful use of these cells plausible. IPE cells share the same embryonic origin as the RPE (the neuroepithelium), are heavily pigmented, exhibit similar

cellular morphological features, express cytokeratin markers (Figure 1) and exhibit a similar mRNA expression profile for cytokines and their receptors relative to RPE.³¹

In addition, we and others³¹ have been able to demonstrate that human IPE have the ability to phagocytose photoreceptor outer segments (POS) *in vitro*³¹ whereas bovine IPE have been reported to express mRNA for proteins involved in retinol metabolism, such as RPE65, CRALBP and 11-cis-retinol dehydrogenase.

Successful photoreceptor rescue and survival have been demonstrated in a number of animal models following subretinal transplant of a suspension of IPE harvested from peripheral iridectomies. Despite the appearance of interaction with host POS,^{32–34} and phagosomes containing phagocytosed POS fragments being observed in the cytoplasm of transplanted IPE, complications were noted. In particular, areas of multilayering and degeneration were evident. Pilot studies of autologous IPE transplantation in humans

Figure 1 Photomicrographs of preconfluent cultures of primary BIPE cells demonstrating immunoreactivity to the cytokeratin antibody K8.13 (a) whereas IgG control is negative (b; mag. $\times 100$).

(again using cell suspensions) have shown some promise but results are variable between groups. Central fixation with stabilisation of vision has been documented in neovascular AMD and in some patients with traumatic RPE detachments,^{30,32,35,36} but complications such as those seen in RPE transplantation, including, retinal detachment, PVR, macular pucker and CNV recurrence have been reported.^{30,32,35} It is encouraging that recent follow up studies by Aisenbrey *et al*³⁷ have reported a stabilisation in visual acuity in 65% of patients who underwent autologous IPE transplantation after CNV removal and showed no signs of rejection or CNV recurrence.

As with RPE, IPE cells also have the ability to transdifferentiate and thus the rationale is that IPE implanted into the subretinal space may transdifferentiate or dedifferentiate into RPE cells or RPE-like phenotypes. However, if the correct environmental cues are not in place then the formation of an intact functioning monolayer is unlikely to occur.

Stem cells

Human embryonic stem cells have been known to differentiate into RPE cells for a number of years and their differentiation pathway is beginning to be elucidated.^{38,39} The use of ES-RPE cells holds promise in that a large number of cell monolayers could be generated to potentially supply the necessary graft volume required for large-scale therapy, while avoiding any genetic defects inherent in autologous RPE.^{38,40} However, the problem of rejection still needs to be fully solved. Other autologous stem cell sources may circumvent this problem, whether they are from induced pluripotent stem cells or existing adult stem niches. A number of stem cell populations have been identified in adult tissue in both ocular and non-ocular tissue. Their ability to self-renew and differentiate into one or more cell types, has lead to significant interest and efforts into developing new treatments for degenerative diseases.⁴¹ Several sources of cells have been transplanted into animal models of retinal degenerations with variable levels of success. Many studies have focused on cell integration with the neural retina but in the context of this review we will only discuss those tailored towards RPE integration or replacement. Although migration and integration of the stem cells into the host retina or RPE have been demonstrated with enhanced photoreceptor survival and function, complete integration still as yet, has not been achieved.^{42–46} A greater understanding of the molecular signalling cues and control pathways for specific differentiation into RPE and/or retinal neurones and precise stem cell delivery is needed to appreciate the full potential of stem cell-based treatments.⁴⁷ The recent

discovery of retinal stem cell niches that survive into adulthood (at the region of the Ora Serrata) which have the ability to differentiate into neural retinal cells as well as RPE cells may yield a new source of cells which can be differentiated into the relevant cell types prior to transplantation into patients.^{12,48,49} Retinal stem cells (RSCs) have been harvested from the pigmented and non-pigmented epithelium of the ciliary body of rats and humans.⁴⁸ These quiescent cells can be expanded *in vitro*^{12,49} and are known to express neural progenitor markers such as nestin.^{12,49} Although markers of mature inner and outer retinal cell types have been detected, very low levels of expression of RPE cell markers have been observed (<1%) even when the cells were plated onto a laminin substrate. Clearly RSCs by definition must have the potential to differentiate into RPE cells, but this process is only beginning to be elucidated *in vitro*^{12,49} and must continue to do so if it is to represent a therapeutic option. Further *in vivo* studies have demonstrated human RSCs transplanted into mice to survive up to 28 days and to integrate into the RPE monolayer as well as other layers of the neuroretina indicating there are a number of environmental queues involved. The eye also contains additional regions (eg, the human iris) that also have been shown to contain cells with stem cell like properties⁴⁸ but unlike the ciliary body-derived RSCs they have yet to be demonstrated to differentiate into RPE cells.⁵⁰

Summary of cell transplantation

Although the promise of replacing a damaged or diseased RPE monolayer with a new monolayer of cells shows great promise, clinically this potential has yet to be realised. Although stem cell-based therapies have shown some encouraging results, these studies are still at the pre-clinical stage of development. Studies which have made it through to the clinic (such as transplantation of autologous RPE or IPE cells) have shown some promising results but have also demonstrated a number of complications and hurdles that need to be cleared if such a treatment strategy is to have any realistic chances of prolonged success. Consequently, placing the cells as an intact differentiated monolayer on a non-degrading underlying porous substrate may overcome many of the current problems reported for cell transplant procedures such as, cell clustering, dysfunctional multilayering, cell migration, dedifferentiation and intractable PVR.

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