Research Article

PEDF and VEGF-A Output from Human Retinal Pigment Epithelial Cells Grown on Novel Microcarriers

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Human retinal pigment epithelial (hRPE) cells have been tested as a cell-based therapy for Parkinson's disease but will require additional study before further clinical trials can be planned. We now show that the long-term survival and neurotrophic potential of hRPE cells can be enhanced by the use of FDA-approved plastic-based microcarriers compared to a gelatin-based microcarrier as used in failed clinical trials. The hRPE cells grown on these plastic-based microcarriers display several important characteristics of hRPE found *in vivo*: (1) characteristic morphological features, (2) accumulation of melanin pigment, and (3) high levels of production of the neurotrophic factors pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor-A (VEGF-A). Growth of hRPE cells on plastic-based microcarriers led to sustained levels (>1 ng/ml) of PEDF and VEGF-A in conditioned media for two months. We also show that the expression of VEGF-A and PEDF is reciprocally regulated by activation of the GPR143 pathway. GPR143 is activated by L-DOPA ($1\,\mu$M) which decreased VEGF-A secretion as opposed to the previously reported increase in PEDF secretion. The hRPE microcarriers are therefore novel candidate delivery systems for achieving long-term delivery of the neuroprotective factors PEDF and VEGF-A, which could have a value in neurodegenerative conditions such as Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is the 2nd most common neurodegenerative disease. Risk for PD is primarily related to increased age, and the most vulnerable neurons are the dopaminergic neurons in the substantia nigra. Destruction of these neurons leads to a disruption of the pathway between the substantia nigra and striatum and produces a severe dopamine deficiency [1]. Dopamine plays an important role in the communication between the thalamus, striatum, and cortex. Reduction of dopamine levels leads to the cardinal motoric features of Parkinson's disease: tremor, generalized slowness of movement, and difficulty transitioning from one movement to the next [1]. The most effective form of treatment for PD is dopamine replacement therapy.

The two most pressing therapeutic challenges in PD are to (1) provide a stable level of dopamine replacement and (2) slow or halt disease progression as reviewed [2–4]. Pharmacological treatment of PD is satisfactory in the early stages of the disease but becomes problematic as the disease progresses. Treatment with the potent dopamine precursor L-DOPA is eventually required for almost all patients, and its erratic absorption and short half-life lead to the development of disabling fluctuations in the treatment response [5]. Providing a continuous intracerebral source of dopamine by using cell-based therapy has been at the front line of experimental efforts [6]. One strategy has been to use hRPE cells as a local source of L-DOPA [7–11]. Cell transplantation of hRPE using cross-linked gelatin microcarriers has been used in preclinical and clinical studies of PD and appears to be a

safe technique [8, 12, 13]. However, the therapeutic potential for this platform has been questioned due to the recently reported failure of the commercially sponsored Phase II clinical trial [13]. Among the several potential contributing factors to the lack of efficacy, we would like to point out three specifically. Firstly, there may have been a failure to achieve long-term survival of the hRPE monolayers on the gelatin-based microcarriers. Immune system responses to the graft may have been responsible for graft failure, and these could be ameliorated by the use of immunosuppression in future trials or avoided by the use of autologously produced hRPE cultured from a small biopsy. Secondly, long-term survival of the graft may also be hindered by digestion of the gelatin-based microcarrier, needed to keep hRPE as a differentiated functional monolayer. Inert plastic materials might provide for a more stable microcarrier since these will not be degraded. Finally, the failure to account for the role of neurotrophic factors in the positive results seen in preclinical and Phase 1 trials could be an important aspect. The clinical benefit found in the initial trial and previously described in both rat and nonhuman primate models of PD [8, 14] was presumed to be due to the production of L-DOPA. However, some of the observed benefit could be related to the release of neuroprotective factors [15] such as pigment epithelium-derived factor (PEDF) [16, 17] and vascular endothelial growth factor-A (VEGF-A) [17]. Indeed, primate studies of hRPE transplantation have indicated a persistent clinical benefit despite little or no long-term survival of the initial engraftment as assessed by both behavioral and neuroimaging measures [18]. These data suggest that a neurorestorative effect outlasting survival of the cell transplant was induced. The Phase II trial design lacked screening of the cells for normal levels of production of PEDF or VEGF-A prior to implantation. We believe it would be a mistake to allow the result of the single Phase II trial to prematurely halt further development of the hRPE platform. To reinvigorate this field, other properties and new manipulations of hRPE cells need to be put forth. It is in this regard that we emphasize and further extend the important findings that (1) PEDF and VEGF-A are neurotrophic and neuroprotective factors in culture models of PD [17, 19], (2) the PEDF level of hRPE cells is subject to variability depending on the source and also the pigmentation level [20], and (3) the PEDF level is regulated by L-DOPA via the G-protein-coupled receptor GPR143 (OA1) [21]. Further investigations of the neurotrophic potential of hRPE cells, due to secretion of PEDF and VEGF-A, have the potential to revive interest in the hRPE cell platform as cell transplantation therapy for PD. However, further understanding of the regulation of these factors in hRPE cells will be required, particularly since L-DOPA is central to the strategy of dopamine replacement using hRPE transplantation. A reciprocal regulation of PEDF and VEGF-A is known in many body tissues [16, 17, 22]; for example, when VEGF-A levels are high, PEDF levels are generally low and vice versa. Since activation of GPR143 up-regulates PEDF production and secretion, it would be logical to investigate if there is also a reciprocal effect on VEGF-A.

Further investigation of the neurotrophic potential of hRPE cells should be coupled with research to aid in the development of microcarriers with increased long-term stability. Such studies would allow the development of more stringent criteria that could be used to screen hRPE cell lines and predict their likelihood of robust clinical efficacy. The experiments described here take a first step in this direction by (1) examining the levels of PEDF and VEGF-A secretion of hRPE on different types of plastic-based microcarriers and (2) investigating if there is an effect of L-DOPA on regulation of VEGF-A secretion as has been shown for PEDF secretion.

2. Materials and Methods

2.1. RPE Culture. RPE cells were isolated from human donor eyes obtained from Advanced Bioscience Resources as described [23, 24] and maintained in Chee's essential medium replacement media (CEM-RM). To seed the microcarriers, hRPE cells (1×10^6) were plated in a 12.5 cm^2 flask coated with 3% agar and gently rocked for 2 hours with $100\,\mu$L microcarriers delivered as a 1:1 ratio (v/v) microcarrier to media slurry, $200\,\mu$L total volume. The total number of microcarriers differed among the microcarrier types as the microcarrier size differed, but the packed volume of microcarriers was the same in each group. We used four types: the gelatin-based CultiSpher microcarriers (macroporous gelatin microcarriers, diameter: 130–380 μm; Percell Biolytica, Astorp, Sweden), as well as 3 plastic-based microcarriers from Solohill Engineering (Ann Arbor MI) that are free from animal products and FDA approved: Hillex II (modified polystyrene with cationic trimethyl ammonium, diameter: 160–180 μm), Plastic Plus (cross-linked polystyrene, cationic charged, diameter: 90–150 μm), and ProNectin F (trademark Solohill, polystyrene coated with recombinant RGD containing protein, diameter 90–150 μm) microcarriers.

2.2. PTU + L-DOPA Treatment. To determine whether GPR143 signaling activity controlled VEGF-A secretion, we used a strategy similar to that which illustrated the receptors control over PEDF [21]. hRPE cells were plated into 24-well plates in CEM and maintained at confluency for 3-4 months to facilitate differentiation of the monolayers [23]. Because L-DOPA is the endogenous ligand for GPR143, and all pigmented cells produce L-DOPA, we used 200 μM phenyl thiourea (PTU) to inhibit the endogenous production of L-DOPA by tyrosinase, which then downregulates the GPR143 signaling pathway, allowing us to control the signaling pathway and use a defined L-DOPA concentration. In the presence of PTU, we then added 1.0 μM L-DOPA to stimulate the GPR143 signaling pathway. Each treatment was on the cells for 72 hours, after which the media was collected and immediately frozen at $-80°$C.

2.3. Photomicrography. Images were acquired digitally on an Olympus IX70 inverted microscope and camera using Olympus MagnaFire software using a 20x objective (400x magnification).

2.4. ELISA Measurement of VEGF-A, PEDF, GDNF, and BDNF. Enzyme-linked immunosorbent assays (ELISAs) were used to determine the concentrations of VEGF-A (R&D Systems, Minneapolis, MN, USA), PEDF (Chemicon International, Temecula, CA, USA), glial-cell-line-derived neurotrophic factor (GDNF; Promega, Madison, WI, USA) and brain-derived neurotrophic factor (BDNF; Promega, Madison, WI, USA) in conditioned medium from hRPE cells grown on microcarriers or normal tissue culture flasks. The medium was harvested from the hRPE cultures every 3-4 days to produce the conditioned medium (CM) samples over long-term time courses. The CM was frozen at $-80°$C immediately after harvest. The ELISAs were performed in duplicate as directed by the manufacturers. ELISA results were obtained using an EMAX Microtiter Plate Reader (Molecular Devices, Downingtown PA, USA).

3. Results

3.1. Growth of hRPE on Microcarriers. The hRPE cells were seeded onto the microcarriers and followed for 2 months in culture. Figure 1 shows example photomicrographs of the different plastic-based microcarriers covered with monolayers of hRPE at 1 and 2 months after seeding. Figures 1A and 1B depict hRPE cells on Hillex II microcarriers, at 1 month and 2 months. The hRPE formed a clearly visible monolayer around the microcarrier with the characteristic melanin pigment and the polygonal morphology reminiscent of the normal histological features found *in vivo* [23]. Figures 1C and 1D depict hRPE cells on Plastic Plus microcarriers, and Figures 1E and 1F depict hRPE cells on ProNectin F microcarriers. From the visual inspection, the Hillex II microcarriers appear to have the most uniform monolayer, but all microcarriers had significant clumping. The hRPE cells remained as a monolayer on the Hillex II microcarrier most frequently. The hRPE cells on the Plastic Plus microcarriers generally appeared as an amorphous mass of cells and microcarriers as illustrated. The hRPE cells on the ProNectin F microcarriers were generally present as a monolayer but not as regular as on the Hillex II microcarriers.

3.2. PEDF Concentration Time Course. The PEDF present in hRPE media was measured in a series of ELISAs. In all, the ELISA results show a significant PEDF secretion into the medium by hRPE cells over 2 months.

For each of the four types of microcarrier with monolayers of hRPE cells, the PEDF concentration was measured for samples collected every few days for 2 months, as shown in Figure 2. The cells on the CultiSpher microcarrier secreted insignificant amounts of PEDF. This was explained by the inability of the CultiSpher to create a sufficient base for hRPE cells to form a healthy monolayer. The other three microcarrier types, Hillex II, Plastic Plus, and ProNectin F all cultured with hRPE from the same donor eye, provided comparable PEDF levels. After a short rising phase, maximal expression levels were reached at about 3-4 ng/mL from the Hillex II microcarriers and about 2 ng/mL from Plastic Plus and ProNectin F microcarriers, and the PEDF concentration

remained stable for the duration of the experiment (2 months) as shown in Figure 2.

3.3. VEGF-A Concentration Time Course. The VEGF-A present in hRPE media was measured in a series of ELISAs. In all, the ELISA results show a significant VEGF-A secretion into the medium by hRPE cells over time.

For each of the four types of microcarrier with monolayers of hRPE cells, the VEGF-A concentration was measured for samples collected every few days for 2 months, as shown in Figure 3. The cells on the CultiSpher microcarrier secreted insignificant amounts of VEGF-A, which again was attributed to largely unsuccessful growth of cultures of hRPE cells on CultiSpher over time. The other three microcarrier types Hillex II, Plastic Plus, and ProNectin F, provided comparable VEGF-A levels. After a rising phase of about 20 days, stable expression levels were reached at about 15 ng/mL, and the VEGF-A concentration remained stable for the duration of the experiment (2 months) as shown in Figure 3. The higher variability within the PEDF versus the VEGF-A time course is explained by the fact that the PEDF concentration measured is much closer to the detection threshold than the VEGF-A concentration.

3.4. GDNF and BDNF Concentration Time Course. The GDNF present in hRPE media was measured in a series of ELISAs. In all, the ELISA results showed a significant GDNF secretion into the medium by hRPE cells grown on Hillex II, Plastic Plus, and ProNectin F microcarriers only over the first 3 weeks as depicted in Figure 4. After day 22 the GDNF level had dropped below the detection threshold. Even at the start, the GDNF concentration was only in the pg/mL range, compared to VEGF-A and PEDF for which ng/mL concentrations were measured.

We also examined if BDNF was present in hRPE media as measured in a series of ELISAs. In all, the ELISA results showed that the hRPE cells did not secrete any measurable BDNF into the medium on all microcarriers tested at any time point (data not shown).

3.5. Regulation of Neurotrophic Output of hRPE. We have previously shown that GPR143 signaling regulates PEDF production in hRPE cells [21]. To extend this finding, we investigated regulation of VEGF-A output in hRPE cells, using the tissue-type, stable, pigmented monolayer for a minimum of 3 months prior to experiment initiation. We used the same paradigm used by Lopez et al. 2009 [21] to define PEDF regulation in hRPE cells. The tyrosinase inhibitor PTU was used to block endogenous L-DOPA production and allow control of OA1 signaling without complicated endogenous activity. We added 200 μM PTU + 1 μM L-DOPA which caused a significant decrease in VEGF-A production that was restored to baseline when L-DOPA was removed. This decrease of VEGF-A production after addition of L-DOPA compared to the untreated cells ($P <$ 0.0005, paired t-test with Bonferroni Correction, $n = 9$) is shown in Figure 5. After the L-DOPA was removed, there was
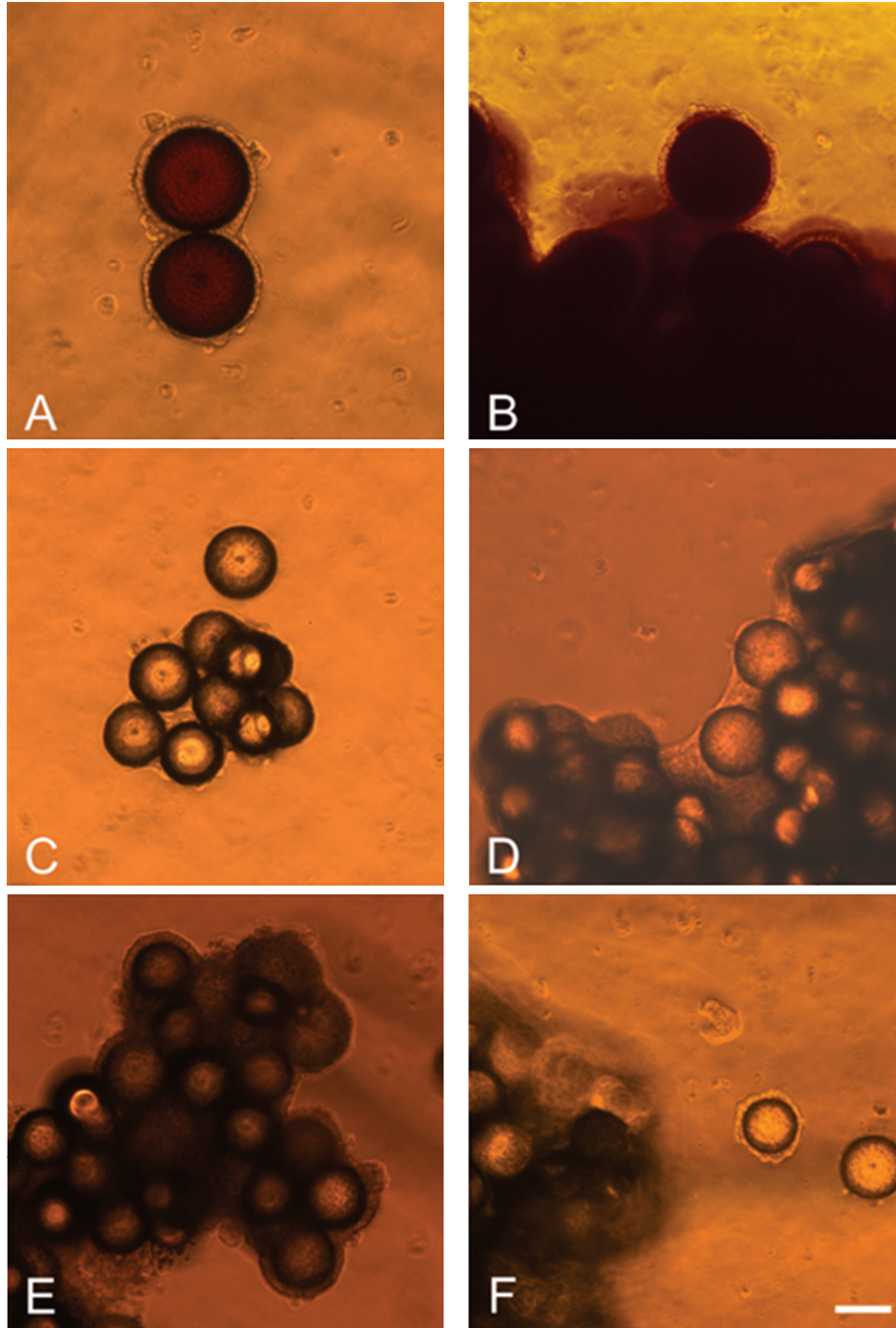
FIGURE 1: Example photomicrographs for hRPE cells grown on different plastic-based microcarrier types at 1 month (A, C, E) and two months (B, D, F). Hillex II microcarriers are shown in A and B, Plastic Plus microcarriers are shown in C and D, and ProNectin F microcarriers are illustrated in E and F. Note the characteristic melanin pigment and the polygonal morphology reminiscent of the normal histological features found *in vivo*. Scale bar = 100 μm.

a significant increase of VEGF-A production ($P < 0.0005$, paired t-test with Bonferroni Correction, $n = 9$) by the cells, returning toward baseline. The comparison of the cells at baseline and after the washout period (paired t-test with Bonferroni's correction, $n = 9$) shows no significant change in VEGF-A concentration.

4. Discussion

RPE cells have been studied as a transplantation platform for the treatment of PD based on their ability to produce L-DOPA as intermediate in the melanin synthesis pathway and thus provide a source of continuous intracerebral dopamine
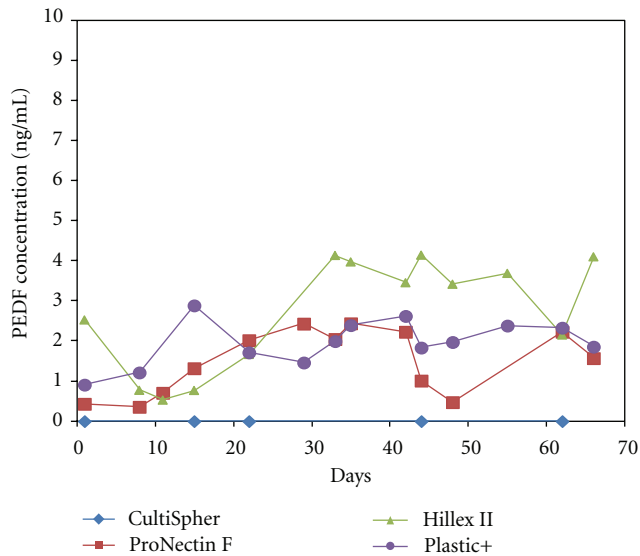
Figure 2: PEDF concentration time course. The PEDF concentration in the media from the hRPE cells on the different microcarriers was measured with an ELISA. After a short rising phase the PEDF concentration reached a plateau. The hRPE cells on Hillex II, Plastic Plus, and ProNectin F microcarriers secreted comparable amount of PEDF, whereas hRPE cells on the CultiSpher microcarriers did not secrete significant amounts of PEDF.
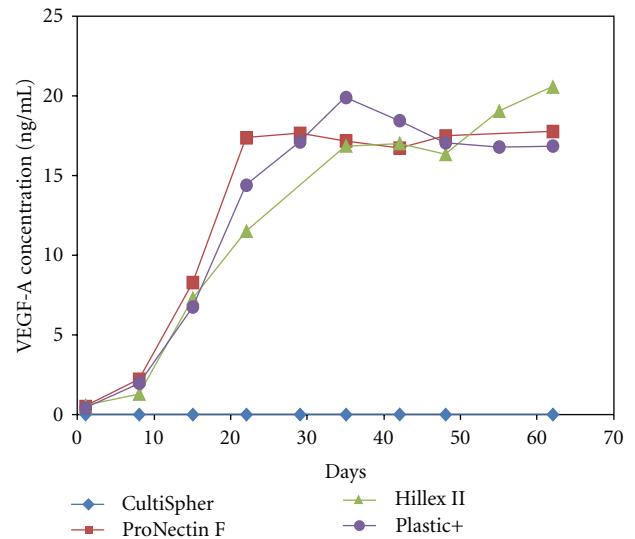

Figure 3: VEGF-A concentration time course. The VEGF-A concentration in the media from the hRPE cells on the different microcarriers was measured with an ELISA. After a rising phase in the first 20 days after seeding the VEGF-A, concentration reached a plateau and remained stable until the end of the experiment. The hRPE cells on Hillex II, Plastic Plus, and ProNectin F microcarriers secreted comparable amount of VEGF-A, whereas hRPE cells on the CultiSpher microcarriers did not secrete significant amounts of VEGF-A.

replacement. In their normal anatomical location, RPE cells function to facilitate retinal survival and activity, in part by the secretion of the neurotrophic factors PEDF and VEGF-A [17, 25, 26]. These factors could potentially provide an additional benefit in PD by virtue of neuroprotective effects on dopaminergic neurons. Both of these molecules have been extensively studied with regard to their role in controlling tissue vascularity, where they have opposing effects to either, respectively, inhibit or promote angiogenesis [16, 17, 26]. In addition to their role in controlling angiogenesis, recent studies have supported an important neurotrophic function for both molecules in the CNS where they may work in concert [17].

Neuroprotective action of PEDF has also been described in a variety of neuronal cell types grown *in vitro* as well as in a postnatal organotypic culture model of motor neuron degeneration [27, 28]. PEDF prevented the death and atrophy of spinal motor neurons *in vivo* in the developing neonatal mouse after axotomy [29]. Of importance for the current study, PEDF has been shown to be both neurotrophic and neuroprotective in two *in vitro* models of PD [19].

It has been shown that VEGF-A has neurotrophic and neuroprotective properties in explants of the ventral mesencephalon [30]. VEGF-A has been shown to cause axonal outgrowth on cultured ganglia in a dose-responsive manner [31]. At VEGF-A concentrations (10–50 ng/mL) comparable to the level found in hRPE-conditioned media in this study, neuroprotective effects have been observed on cultured cells subjected to hypoxia and glucose deprivation [32]. Unfortunately, at higher concentrations, because of its angiogenic nature, VEGF-A may cause increased permeability of the

blood-brain barrier, which could be detrimental in causing cerebral edema or other untoward effects [33]. If hRPE cells are to be implanted in the brains of PD patients as a therapy, it will be important to establish that VEGF-A production is sufficient for neuroprotection but below the threshold for disruption of the blood-brain barrier.

The combined neurotrophic effects and the exact ratio of PEDF and VEGF-A could also be important considerations. A possible concerted neurotrophic effect by PEDF and VEGF-A in PD was suggested by Yasuda et al. [34] reporting a significantly positive correlation in the striatal levels of PEDF and VEGF-A in brains acquired at autopsy in PD patients. This study also monitored the striatal levels of PEDF relative to the VEGF-A levels in a rodent model following a toxic insult to the dopaminergic pathway. Acute damage to dopaminergic neurons induced a rise in PEDF levels in the CNS, supporting the hypotheses that PEDF acts as an endogenous natural neuroprotective response factor.

In this study we found that hRPE cells grown on plastic-based microcarriers retain their ability to produce both PEDF and VEGF-A, but we were unable to produce long-term cultures of hRPE cells on gelatin-based CultiSpher microcarriers. Thus, by virtue of the cells failing after initial attachment, we did not observe significant levels of either neurotrophic factor with those microcarriers. The hRPE cells transplanted in the Spheramine clinical trials utilized gelatin-based Spheramine microcarriers, and their potential to produce PEDF and VEGF-A was never investigated. The extent to which neuroprotection is operative in clinical trials
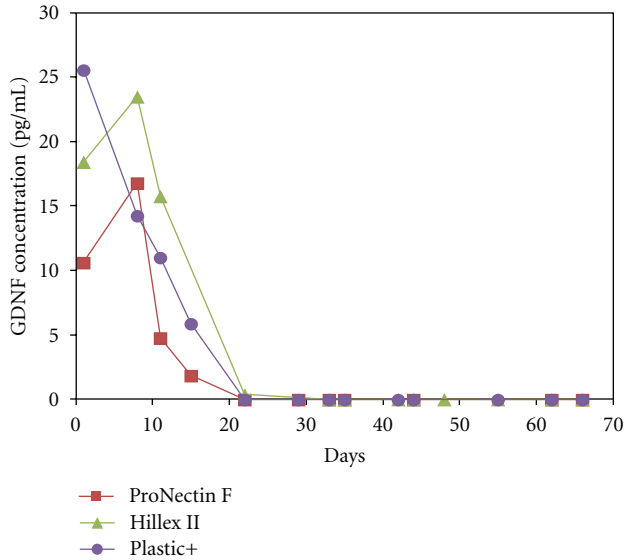
FIGURE 4: GDNF concentration time course. The GDNF concentration in the media from the hRPE cells on the different microcarriers was measured with an ELISA. The hRPE cells on Hillex II, Plastic Plus, and ProNectin F microcarriers secreted comparable amounts of GDNF at the start of the time course. The concentration was an order of magnitude less when compared to VEGF-A and PEDF. The GDNF concentration declined over the first 3 weeks, and there was no detectable amount of GDNF from day 22 on.
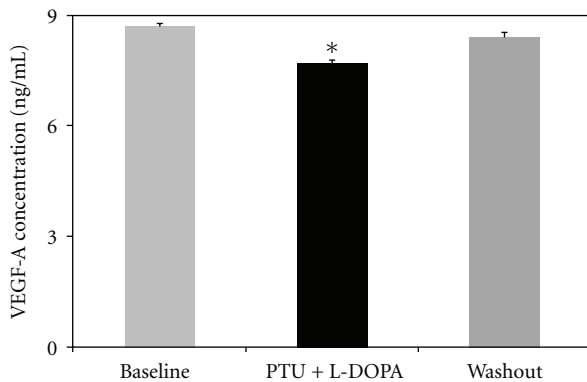

FIGURE 5: Regulation of VEGF-A expression in hRPE cells. Treatment of hRPE monolayers, grown in 24-well plates, with PTU (a tyrosinase blocker used to eliminate endogenous L-DOPA production) and 1μM L-DOPA leads to a decrease in VEGF-A concentration in the medium. After washout of PTU and L-DOPA, the VEGF-A concentration returns to the baseline levels. Data are presented as the mean of three experiments conducted in triplicate, error bars represent S.E.M., and an asterisk (*) denotes $P < 0.0005$ using paired t-tests with Bonferroni's correction between the PTU + L-DOPA group from both the baseline and the washout groups.

of hRPE transplantation remains speculative, but is in our opinion deserving of due consideration.

Additional reasons must also be considered to explain the failure of the Phase II clinical trial to show efficacy [13] despite promising preclinical [8–10, 14] and Phase I clinical trials [12]. Subsequent pathology data showed that most

cells did not survive implantation for more than 6 months [35], raising doubts about the long-term stability of the graft. Since one of the potential pitfalls of the clinical trial is that the gelatin-based Spheramine microcarriers might have been digested over time, we investigated nondegradable microcarriers in their potential to provide healthy hRPE growth, survival, and neurotrophic output [20, 36]. Culti-Spher microcarriers were ineffective in achieving healthy hRPE monolayers as evidenced by the lack of pigmented cells covering the microcarriers. Thus, it was not surprising that little PEDF or VEGF-A was produced using that substrate. Plastic Plus, ProNectin F, and Hillex II microcarriers on the other hand all have potential as microcarriers for hRPE growth and potential implantation. The hRPE cells appeared the most differentiated, pigmented, and regular in height and thickness on the Hillex II microcarriers. Interestingly, both the PEDF (2–4 ng/mL) and the VEGF-A (15 ng/mL) outputs of the hRPE cells on all three plastic-based microcarriers were very similar throughout the two-month time course, even though the appearance of the cultures was significantly different. We did not investigate the neurotrophic effect of the hRPE cells grown on microcarriers in this study, but based on our prior study evaluating the neurotrophic potential of conditioned media (RPE-CM) from 2-month-old fully differentiated hRPE cultures before and after PEDF depletion [20], we can conclude that over 50% of the neurotrophic effect on cultured neurons that was produced by hRPE cells is due to PEDF production. We can speculate that the remainder of that effect can be mostly attributed to VEGF-A, since we showed in the current study that VEGF-A is secreted throughout the study at significant levels whereas the hRPE cells do not produce measurable amount of BDNF at any time point (data not shown), and the GDNF level (Figure 4) drops below detection threshold by the 3rd week of culture.

In a parallel experiment, we investigated the regulation of the neurotrophic output in hRPE cells by L-DOPA. An interesting facet of the regulation of PEDF secretion is the linkage to an autocrine loop that regulates tyrosinase activity and pigment synthesis utilizing L-DOPA and GPR143. GPR143 is a G-Protein-coupled receptor with highest expression levels found in RPE cells. GPR143 signaling is critical for full expression of the L-DOPA synthetic enzyme tyrosinase. L-DOPA is not only a precursor for the formation of melanin, but also acts to increase melanogenesis by activating the GPR143 receptor creating a positive feedback loop. Loss of GPR143 signaling also reduces the output of PEDF from RPE cells. Genetic defects in GPR143 cause ocular albinism, a disorder of pigmentation that leads to vision loss due to changes in retinal neuron development and survival. The exact cause of the secondary neuronal loss observed in this disorder has been debated, but may reflect the decremental changes in PEDF production and subsequent loss of retinal neurotrophism.

A better understanding of the influence of L-DOPA on the neurotrophic output of RPE cells is also required for the potential use of RPE cell transplantation in PD. Since there is a reciprocal "yin and yang" like effect of PEDF and VEGF-A regulation [17], at work in many cell

types, including the eye, we sought to test whether GPR143 signaling, which upregulates PEDF secretion from hRPE cells, may downregulate VEGF-A secretion. To test this we used the same experimental method used previously to illustrate upregulation of PEDF by GPR143 signaling [21]. VEGF-A secretion was reduced significantly by induction of GPR143 signaling using the PTU/L-DOPA paradigm. Thus, we suggest that in hRPE cells, GPR143 up-regulates PEDF while simultaneously downregulating VEGF-A. In addition to the advance in the field of RPE cell transplantation, this observation has significant implications for our understanding of pigmentation-related eye diseases such as albinism and age-related macular degeneration.

Overall, it is apparent that hRPE cells grown on plastic-based microcarriers produce levels of both PEDF and VEGF-A that are in a range that would support a neuroprotective effect. The levels of PEDF (maximum around 4 ng/mL) were higher than the 1 ng/mL PEDF that was reported to be neuroprotective in both 6-OHDA and rotenone rat midbrain culture models [19]. In this paper we show that the secretion of VEGF-A is downregulated by L-DOPA. This effect was partial (less than 15%) and did not eliminate VEGF-A secretion under any conditions tested. The levels of VEGF-A (maximum around 15 ng/mL) are sufficient to expect a neuroprotective effect, since 1 ng/mL VEGF-A has been shown to provide a maximal protection in a 6-OHDA model of PD [33]. The VEGF-A levels also appear to be low enough that the negative effect of increased vessel formation may not be an issue. In rodent models, pathological vasculogenesis was observed after implantation of BHK cells expressing VEGF-A at approximately 40 ng/mL [33]. It is important to note that future manipulation of the GPR143 pathway could be used to optimize and balance the neurotrophic production of hRPE cells.

In future work the plastic-based hRPE microcarriers should be tested *in vivo* employing rodent models of neurodegeneration, to evaluate the long-term survival of the hRPE and the continued ability to release therapeutically relevant amounts of PEDF and VEGF-A after transplantation into the brain. The relative contribution of PEDF versus VEGF-A to the expected neurotrophic effects should also be further investigated *in vivo*.

5. Conclusion

Human RPE cells can be successfully grown and differentiated on 3 different types of FDA-approved plastic-based microcarriers over an extended period of time. The hRPE microcarriers produce significant amounts of two growth factors with neuroprotective potential, PEDF and VEGF-A. The regulation of both factors can be reciprocally regulated by modulation of the GPR143/OA1 pathway.

Acknowledgments

References

[1] C. W. Olanow, M. B. Stern, K. Sethi et al., "The scientific and clinical basis for treatment of Parkinson's disease," *Neurology*, vol. 72, no. 21, supplement 4, pp. S1–S136, 2009.

[2] F. Stocchi and C. W. Olanow, "Neuroprotection in Parkinson's disease: clinical trials," *Annals of Neurology*, vol. 53, no. 7, supplement 3, pp. S87–S97, 2003.

[3] J. M. Savitt, V. L. Dawson, and T. D. Dawson, "Diagnosis and treatment of Parkinson's disease: molecules to medicine," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 1744–1754, 2006.

[4] S. Fahn and D. Sulzer, "Neurodegeneration and neuroprotection in Parkinson disease," *NeuroRx*, vol. 1, no. 1, pp. 139–154, 2004.

[5] J. G. Nutt, "Motor fluctuations and dyskinesia," in *Parkinson's Disease: Diagnosis and Clinical Management*, S. A. Factor and W. J. Weiner, Eds., pp. 445–453, Demos Medical, New York, NY, USA, 2002.

[6] O. Lindvall and A. Björklund, "Cell therapy in Parkinson's disease," *NeuroRx*, vol. 1, no. 4, pp. 382–393, 2004.

[7] R. A. Bakay, C. D. Raiser, N. P. Stover et al., "Implantation of spheramine in advanced Parkinson's disease (PD)," *Frontiers in Bioscience*, vol. 9, pp. 592–602, 2004.

[8] D. J. Doudet, M. L. Cornfeldt, C. R. Honey, A. W. Schweikert, and R. C. Allen, "PET imaging of implanted human retinal pigment epithelial cells in the MPTP-induced primate model of Parkinson's disease," *Experimental Neurology*, vol. 189, no. 2, pp. 361–368, 2004.

[9] J. Flores, I. L. Cepeda, M. L. Cornfeldt, J. R. O'Kusky, and D. J. Doudet, "Characterization and survival of long-term implants of human retinal pigment epithelial cells attached to gelatin microcarriers in a model of parkinson disease," *Journal of Neuropathology and Experimental Neurology*, vol. 66, no. 7, pp. 585–596, 2007.

[10] T. Subramanian, D. Marchionini, E. M. Potter, and M. L. Cornfeldt, "Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemi-parkinsonian rats ameliorates behavioral deficits without provoking a host immune response," *Cell Transplantation*, vol. 11, no. 3, pp. 207–214, 2002.

[11] R. L. Watts, C. D. Raiser, N. P. Stover et al., "Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease," *Journal of Neural Transmission*, no. 65, pp. 215–227, 2003.

[12] N. P. Stover, R. A. E. Bakay, T. Subramanian et al., "Intrastriatal implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson disease," *Archives of Neurology*, vol. 62, no. 12, pp. 1833–1837, 2005.

[13] R. E. Gross, R. L. Watts, R. A. Hauser et al., "Intrastriatal transplantation of microcarrier-bound human retinal pigment epithelial cells versus sham surgery in patients with advanced Parkinson's disease: a double-blind, randomised, controlled trial," *The Lancet Neurology*, vol. 10, no. 6, pp. 509–519, 2011.

[14] I. L. Cepeda, J. Flores, M. L. Cornfeldt, J. R. O'Kusky, and D. J. Doudet, "Human retinal pigment epithelial cell implants ameliorate motor deficits in two rat models of parkinson disease,"

Journal of Neuropathology and Experimental Neurology, vol. 66, no. 7, pp. 576–584, 2007.

[15] C. Jomary, R. M. Darrow, P. Wong, D. T. Organisciak, and S. E. Jones, "Expression of neurturin, glial cell line-derived neurotrophic factor, and their receptor components in light-induced retinal degeneration," *Investigative Ophthalmology and Visual Science*, vol. 45, no. 4, pp. 1240–1246, 2004.

[16] J. Tombran-Tink and C. J. Barnstable, "PEDF: a multifaceted neurotrophic factor," *Nature Reviews Neuroscience*, vol. 4, no. 8, pp. 628–636, 2003.

[17] T. Falk, R. T. Gonzalez, and S. J. Sherman, "The Yin and Yang of VEGF and PEDF: multifaceted neurotrophic factors and their potential in the treatment of Parkinson's disease," *International Journal of Molecular Sciences*, vol. 11, no. 8, pp. 2875–2900, 2010.

[18] Y. Ma, S. Peng, J. Flores et al., "Abnormal metabolic brain network in parkinsonian macaques: modulation by retinal pigment epithelial (RPE) cell implantation," *Neurology*, vol. 71, pp. 154–155, 2008.

[19] T. Falk, S. L. Zhang, and S. J. Sherman, "PEDF is neurotrophic and neuroprotective in two in vitro models of Parkinson's disease," *Neuroscience Letters*, vol. 458, no. 2, pp. 49–52, 2009.

[20] B. S. McKay, B. Goodman, T. Falk, and S. J. Sherman, "Retinal pigment epithelial cell transplantation could provide trophic support in Parkinson's disease: results from an in vitro model system," *Experimental Neurology*, vol. 201, no. 1, pp. 234–243, 2006.

[21] V. M. Lopez, C. L. Decatur, W. D. Stamer, R. M. Lynch, and B. S. McKay, "L-DOPA is an endogenous ligand for OA1," *Plos Biology*, vol. 6, no. 9, article e236, 2008.

[22] J. I. Patel, J. Tombran-Tink, P. G. Hykin, Z. J. Gregor, and I. A. Cree, "Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: implications for structural differences in macular profiles," *Experimental Eye Research*, vol. 82, no. 5, pp. 798–806, 2006.

[23] J. Hu and D. Bok, "A cell culture medium that supports the differentiation of human retinal pigment epithelium into functionally polarized monolayers," *Molecular Vision*, vol. 7, pp. 14–19, 2001.

[24] D. J. Rak, K. M. Hardy, G. J. Jaffe, and B. S. McKay, "Ca^{++}-switch induction of RPE differentiation," *Experimental Eye Research*, vol. 82, no. 4, pp. 648–656, 2006.

[25] D. Bok, "The retinal pigment epithelium: a versatile partner in vision," *Journal of Cell Science*, vol. 106, no. 17, pp. 189–195, 1993.

[26] A. K. Olsson, A. Dimberg, J. Kreuger, and L. Claesson-Welsh, "VEGF receptor signalling—in control of vascular function," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 5, pp. 359–371, 2006.

[27] M. M. Bilak, A. M. Corse, S. R. Bilak, M. Lehar, J. Tombran-Tink, and R. W. Kuncl, "Pigment epithelium-derived factor (PEDF) protects motor neurons from chronic glutamate-mediated neurodegeneration," *Journal of Neuropathology and Experimental Neurology*, vol. 58, no. 7, pp. 719–728, 1999.

[28] M. M. Bilak, S. P. Becerra, A. M. Vincent, B. H. Moss, M. S. Aymerich, and R. W. Kuncl, "Identification of the neuroprotective molecular region of pigment epithelium-derived factor and its binding sites on motor neurons," *Journal of Neuroscience*, vol. 22, no. 21, pp. 9378–9386, 2002.

[29] L. J. Houenou, A. P. D'Costa, L. Li et al., "Pigment epithelium-derived factor promotes the survival and differentiation of developing spinal motor neurons," *Journal of Comparative Neurology*, vol. 412, no. 3, pp. 506–514, 1999.

[30] W. F. Silverman, J. M. Krum, N. Mani, and J. M. Rosenstein, "Vascular, glial and neuronal effects of vascular endothelial growth factor in mesencephalic explant cultures," *Neuroscience*, vol. 90, no. 4, pp. 1529–1541, 1999.

[31] M. Sondell, G. Lundborg, and M. Kanje, "Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system," *Journal of Neuroscience*, vol. 19, no. 14, pp. 5731–5740, 1999.

[32] K. L. Jin, X. O. Mao, and D. A. Greenberg, "Vascular endothelial growth factor: direct neuroprotective effect in in vitro ischemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 18, pp. 10242–10247, 2000.

[33] T. Yasuhara, T. Shingo, K. Muraoka et al., "The differences between high and low-dose administration of VEGF to dopaminergic neurons of in vitro and in vivo Parkinson's disease model," *Brain Research*, vol. 1038, no. 1, pp. 1–10, 2005.

[34] T. Yasuda, M. Fukuda-Tani, T. Nihira et al., "Correlation between levels of pigment epithelium-derived factor and vascular endothelial growth factor in the striatum of patients with Parkinson's disease," *Experimental Neurology*, vol. 206, no. 2, pp. 308–317, 2007.

[35] E. S. Farag, H. V. Vinters, and J. Bronstein, "Pathologic findings in retinal pigment epithelial cell implantation for Parkinson disease," *Neurology*, vol. 73, no. 14, pp. 1095–1102, 2009.

[36] M. Ming, X. Li, X. Fan et al., "Retinal pigment epithelial cells secrete neurotrophic factors and synthesize dopamine: possible contribution to therapeutic effects of RPE cell transplantation in Parkinson's disease," *Journal of Translational Medicine*, vol. 7, article 53, 2009.