

Embryonic stem cell trials for macular degeneration: a preliminary report



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Summary

Background It has been 13 years since the discovery of human embryonic stem cells (hESCs). Our report provides the first description of hESC-derived cells transplanted into human patients.

Methods We started two prospective clinical studies to establish the safety and tolerability of subretinal transplantation of hESC-derived retinal pigment epithelium (RPE) in patients with Stargardt's macular dystrophy and dry age-related macular degeneration—the leading cause of blindness in the developed world. Preoperative and postoperative ophthalmic examinations included visual acuity, fluorescein angiography, optical coherence tomography, and visual field testing. These studies are registered with ClinicalTrials.gov, numbers NCT01345006 and NCT01344993.

Findings Controlled hESC differentiation resulted in greater than 99% pure RPE. The cells displayed typical RPE behaviour and integrated into the host RPE layer forming mature quiescent monolayers after transplantation in animals. The stage of differentiation substantially affected attachment and survival of the cells in vitro after clinical formulation. Lightly pigmented cells attached and spread in a substantially greater proportion (>90%) than more darkly pigmented cells after culture. After surgery, structural evidence confirmed cells had attached and continued to persist during our study. We did not identify signs of hyperproliferation, abnormal growth, or immune mediated transplant rejection in either patient during the first 4 months. Although there is little agreement between investigators on visual endpoints in patients with low vision, it is encouraging that during the observation period neither patient lost vision. Best corrected visual acuity improved from hand motions to 20/800 (and improved from 0 to 5 letters on the Early Treatment Diabetic Retinopathy Study [ETDRS] visual acuity chart) in the study eye of the patient with Stargardt's macular dystrophy, and vision also seemed to improve in the patient with dry age-related macular degeneration (from 21 ETDRS letters to 28).

Interpretation The hESC-derived RPE cells showed no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after 4 months. The future therapeutic goal will be to treat patients earlier in the disease processes, potentially increasing the likelihood of photoreceptor and central visual rescue.

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Introduction

Since their discovery in 1998,¹ human embryonic stem cells (hESCs) have been thought a promising source of replacement cells for regenerative medicine. Despite great scientific progress, hESCs are among the most complex biological therapeutic entities proposed for clinical use.² In addition to the dynamic complexity of their biology, many regulatory concerns have hindered clinical translation, including the risk of teratoma formation and the challenges associated with histoincompatibility. Until reprogramming technologies, such as somatic-cell nuclear transfer³ or induced pluripotent stem cells,^{4,5} are further developed, diseases affecting the eye and other immunoprivileged sites will probably be the first pluripotent stem cell-based therapies in patients. It is well established that the subretinal space is protected by a blood–ocular barrier, and is characterised by antigen-specific inhibition of both the cellular and humoral immune responses.⁶

In the retina, degeneration of the retinal pigment epithelium (RPE) leads to photoreceptor loss in many sight-threatening diseases, including dry age-related

macular degeneration and Stargardt's macular dystrophy. Dry age-related macular degeneration is the leading cause of blindness in the developed world, and Stargardt's macular dystrophy is the most common paediatric macular degeneration. Although both are untreatable at present, there is evidence in models of macular degeneration in mice and rats that transplantation of hESC-derived RPE can rescue photoreceptors and prevent loss of vision.^{7,8} Among its functions, the RPE maintains the health of photoreceptors by recycling photopigments, metabolising vitamin A, and phagocytosing photoreceptor outer segments.^{9,10} In studies in the Royal College of Surgeons (RCS) rat, an animal model in which vision deteriorates because of RPE dysfunction, subretinal transplantation of hESC-derived RPE resulted in extensive photoreceptor rescue and improvement in vision without evidence of untoward pathological effects.⁷ These and other safety studies⁸ suggest that hESCs could serve as a potentially safe and inexhaustible source of RPE for the efficacious treatment of many retinal degenerative diseases.

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Transplantation of intact sheets and suspensions of primary RPE cells has been previously attempted in people, with mixed results; both in terms of graft survival and improvement in vision.^{11–18} However, there are advantages to the use of progeny obtained from hESCs as a source of replacement tissue for clinical studies. In addition to producing an unlimited number of healthy young cells with potentially reduced immunogenicity,^{19,20} the stage of in-vitro differentiation can be controlled to ensure optimum safety, identity, purity, and potency before transplantation into the targeted population of patients. The hESC derivatives must be free of pathogens, possess the appropriate characteristics of the differentiated cell, be of high purity, and free of undifferentiated cells. They must also be extensively tested in animals for absence of teratomas, migration of cells into other organs, and adverse effects. The goal of our studies was to assess the safety and tolerability of hESC-derived RPE cells, including teratoma formation, hyperproliferation of the cells, ectopic tissue formation, and immune rejection. We report our preliminary experience with two patients: one with dry age-related macular degeneration and one with Stargardt's disease.

Methods

Participants

We selected patients on the basis of several inclusion and exclusion criteria (webappendix), including end-stage disease, central visual loss, the absence of other clinically significant ophthalmic pathological effects, a cancer-free medical history, present cancer screening, the absence of contraindications for systemic immunosuppression, the ability to undergo a vitreoretinal surgical procedure under monitored anaesthesia care, and psychological suitability to participate in a first-in-human clinical trial involving hESC-derived transplant tissue. Patients provided written informed consent and ethical approval was obtained from the University of California, Los Angeles, institutional review board.

Procedure

We used hESC line MA09 cells²¹ to generate a master cell bank with Good Manufacturing Practices; this cell line has ex-vivo exposure with mouse embryo cells and is thus classified as a xenotransplantation product. The hESC master cell bank was thawed and expanded on mitomycin-C-treated mouse embryonic fibroblasts for three passages. After embryoid body formation and cellular outgrowth, we isolated pigmented RPE patches⁸ with collagenase. After purification and trypsinisation, the cells were expanded and cryopreserved at passage 2 for clinical use. We characterised RPE in-process and after freezing and formulation, including karyotyping, pathogen and phagocytosis assay testing, and differentiation and purity evaluation by morphological assessment, quantitative PCR, and quantitative immunostaining for RPE and hESC markers (webappendix).

In preclinical studies we injected hESC-RPE subretinally into National Institutes of Health (NIH) III immunodeficient mice (tumorigenicity and biodistribution studies), and dystrophic RCS rats and ELOVL4 mice (efficacy studies) as described elsewhere.⁸ To detect human cells in the injected eyes and other organs, we used DNA quantitative PCR, designed to amplify human Alu Y DNA sequences, and immunostaining of paraffin sections for human mitochondria and human bestrophin (webappendix).

For clinical studies we thawed, washed, and resuspended vials of cryopreserved MA09-RPE at a density of 2×10^3 viable cells per μL of BSS Plus (Alcon, Hünenberg, Switzerland). A vial containing the appropriate volume of formulated RPE and a paired vial containing the appropriate volume of BSS Plus at $2-8^\circ\text{C}$ were delivered to the operating room. Immediately before injection, the two vials were reconstituted in a 1 mL syringe to obtain the targeted injection density of 333 viable RPE cells per μL . 150 μL of reconstituted RPE was injected through a MedOne PolyTip cannula 25/38 delivering the targeted dose of 50 000 viable RPE cells into the subretinal space of each patient's eye.

We did pars plana vitrectomy including surgical induction of posterior vitreous separation from the optic nerve anteriorly to the posterior border of the vitreous base. Submacular injection of 5×10^4 hESC-RPE cells in 150 μL was delivered into a preselected region of the pericentral macula that was not completely lost to disease. We carefully chose transplantation sites on the basis of optical coherence tomographic data suggesting the presence of native, albeit compromised, RPE and similarly compromised overlying photoreceptors, to optimise the chances of transplant integration and potential for photoreceptor-cell rescue. We thought transplant engraftment within a completely atrophic central macula was unlikely in view of the loss of Bruch's membrane in advanced atrophic disease.²² Further, complete macular atrophy does not mimic central macular status in earlier stages of degeneration, which might be the ultimate therapeutic target of a stem-cell-based regenerative transplant strategy.

The immunosuppression regimen included low-dose tacrolimus (target blood concentrations 3–7 ng/mL) and mycophenolate mofetil (ranging from 0.25 to 2.00 g orally per day) a week before the surgical procedure and continued for 6 weeks. At week 6, the regimen calls for discontinuation of tacrolimus and a continuation of mycophenolate mofetil for an additional 6 weeks. These studies are registered with ClinicalTrials.gov, numbers NCT01345006 and NCT01344993.

Role of the funding source

The sponsor of the study participated in study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding authors had full

See Online for webappendix

access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Controlled hESC differentiation resulted in greater than 99% pure RPE (figure 1). A single six-well plate of pigmented patches produced about 1.5×10^8 RPE cells (sufficient to treat >50 patients). The cells displayed typical RPE behaviour, losing their pigmented cobblestone morphology during proliferation and redifferentiating into a monolayer of polygonal cuboidal pigmented epithelium once confluence was established. Quantitative PCR showed that markers of pluripotency (OCT4, NANOG, and SOX2) were substantially downregulated, whereas the transient marker of neuroectoderm differentiation, PAX6, and RPE markers, RPE65, bestrophin, and

MITF, were expressed at high levels (webappendix). In mature cultures greater than 99% of the cells were positive for ZO-1 and bestrophin, PAX6, or both (PAX6 disappearing in more mature cells). After cryopreservation, cells were thawed and formulated for transplantation. Staining for PAX6, MITF, or both (figure 1) was done on formulated RPE cultured overnight, confirming greater than 99% RPE purity. After further culture PAX6/bestrophin and ZO-1 immunostaining was similar to preharvest cultures, and a potency assay showed greater than 85% of the cells phagocytosed bioparticles (figure 1).

Since the hESCs were exposed to animal cells, the master cell bank and RPE were extensively tested for animal and human pathogens. We confirmed the cells were free of microbial contaminants, including animal

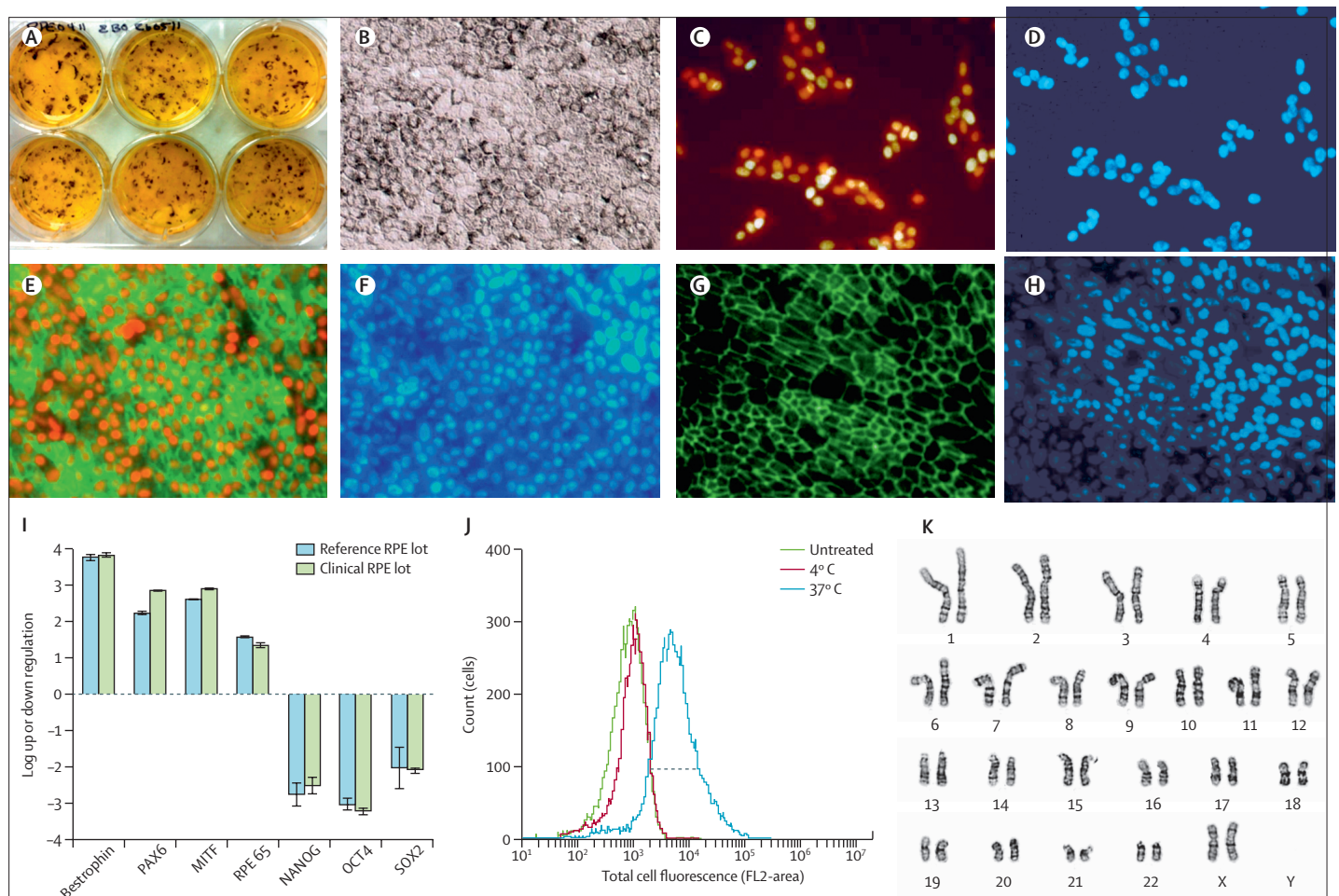


Figure 1: Characterisation of RPE generated from hESC-MA09 cells

A six-well plate with pigmented patches of RPE formed in differentiating culture of embryoid bodies (A) and assessment of molecular markers in thawed and formulated RPE (B–H). (B) Hoffman modulation contrast microphotography of 3-week-old RPE after formulation showing that the confluent cobblestone monolayer with medium pigmentation has been established. (C) MITF/PAX6 merged (MITF=red, PAX6=green). (D) DAPI corresponding to MITF/PAX6. (E) Bestrophin (red)/PAX6 (green) merged. (F) Corresponding DAPI. (G) ZO-1. (H) Corresponding DAPI. Note that near 100% of cells in C–H are positive for the marker(s) assessed. Magnification $\times 400$ (B–H). (I) Quantitative PCR showing up-regulation of RPE markers and down-regulation of hESC markers in the thawed clinical RPE compared with a reference RPE lot. (J) Flow cytometry histogram showing phagocytosis of PhRodo bioparticles by hES-RPE at 37°C and at 4°C (control). (K) normal female (46 XX) karyotype of the clinical RPE lot. MITF and PAX6 (C, D) were assessed in overnight cultures of freshly formulated cells and bestrophin/PAX6 and ZO-1 immunostaining was done on 3-week-old cultures. Quantitative immunohistochemical staining was done with standard methods with the percentage of positive stained cells normalised to the number of DAPI stained nuclei inspected. Assessment of RPE purity and the extent of differentiation were based on the percentage of bestrophin, PAX6, ZO-1, and MITF stained cells. RPE=retinal pigment epithelium. hESC=human embryonic stem cells.

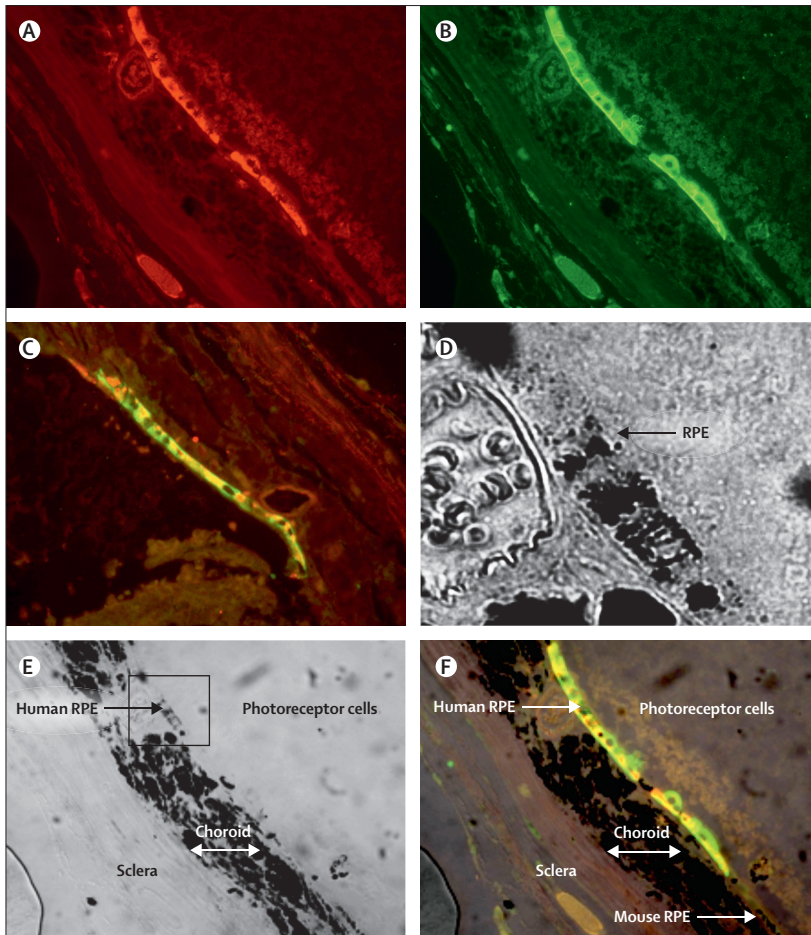


Figure 2: Survival and integration of RPE generated from hESC-MA09 into an NIH III mouse eye after 9 months Section stained with anti-human mitochondria (A) and anti-human bestrophin (B). Note the precise colocalisation of human mitochondria and bestrophin staining in the same cells (C; A and B merged) and absence of staining in mouse RPE (F; A, B, C, and E merged). Frame on the bright field image (E) is enlarged in D to show morphology of human RPE. Magnification $\times 200$ (A–C, E, and F), D is additionally magnified $\times 4.5$. RPE=retinal pigment epithelium. hESC=human embryonic stem cells. NIH=National Institutes of Health.

and human viral pathogens (webappendix). The final RPE product had normal female (46 XX) karyotype (figure 1); a high sensitivity assay ruled out the presence of contaminating hESCs: examination of 2 million/9 million cell RPE samples (at P1/P2) stained for OCT4 and alkaline phosphatase showed no presence of pluripotent cells. Tumorigenicity, biodistribution, and spiking studies done in NIH III mice showed no adverse or safety issues in any animals. Additionally, we did not identify tumours in animals injected with $5\text{--}10 \times 10^4$ RPE cells spiked with either 0.01%, 0.1%, or 1% undifferentiated hESCs, whereas undifferentiated hESCs developed teratomas by 2 months in all animals. Survival of hRPE was confirmed in the eyes of all the animals up to 3 months after injection, and in 48 (92%) of the 52 animals at 9 months (webappendix). hRPE survived for the lifetime of the animals and integrated into the mouse RPE layer; although morphologically indiscernible from the host RPE (figure 2), they could be identified by

immunostaining and expressed bestrophin in a typical basolateral fashion. Ki-67 staining showed a low level of proliferation 1–3 months after transplantation, but we did not identify Ki-67-positive cells at 9 months, suggesting that the hESC-RPE had formed quiescent monolayers.

We harvested two lots of RPE at different levels of pigmentation (melanin content was 4.8 pg per cell [SD 0.3] for the lighter pigmented lots and 10.4 [SD 0.9] for the more heavily pigmented lots). We processed cells from both lots with the protocol for clinical transplantation described in the Methods section. After extrusion through the injection cannula, we seeded the cells onto gelatin-coated plates and monitored for attachment and subsequent growth. RPE from the lighter pigmented lot showed a minimal number of floating cells in overnight cultures; most of the cells had attached and spread, displaying typical RPE morphology for this stage of growth (figure 3). After 3 days in culture, the number of lighter pigmented cells had increased from 4.0×10^4 to 10.6×10^4 cells (figure 3). By contrast, the heavily pigmented RPE had large numbers of floating cells; only a small proportion of the cells attached and survived, with a substantially decreased number of cells ($<10\%$ [9.0×10^3] noted in the lighter lot) after 3 days in culture (figure 3). These results suggest a strong correlation between the stage of RPE differentiation and the ability to adhere and thrive in vitro. The RPE lot we used had a melanin content of 4.1 pg per cell with similar attachment and growth to the lighter lot. Stresses associated with the freeze–thaw cycle, washings, centrifugation, and extrusion through the injection cannula might account in part for the recorded differences between lightly and heavily pigmented cells.

We did not identify any hyperproliferation or abnormal growth in either patient at any point during the follow-up period when assessed by detailed biomicroscopic and indirect ophthalmoscopic clinical examinations at multiple postoperative assessments. Absence of teratoma formation was confirmed with laboratory studies including spectral domain optical coherence tomography, high resolution digital fundus photography, autofluorescence imaging, and fluorescein angiography studies done serially throughout the follow-up period.

Neither patient studied had hESC-RPE proliferation outside the subretinal space on clinical examination or laboratory testing. After extensive scanning of the preretinal space of both patients with spectral domain ocular coherence tomography, we detected a single pigmented cell on the retinal surface of the patient with Stargardt's macular dystrophy. This cell is near the retinotomy site and has not shown any sign of proliferation or contraction.

At no point after transplantation did we detect any signs of intraocular inflammation or hyperproliferation in either patient. Absence of clinically detectable inflammation was corroborated with slit lamp

biomicroscopic photography, fundus photography, intravenous fluorescein angiography, and spectral domain ocular coherence tomography (webappendix). Both operated eyes were free of inflammation throughout the postoperative period. Indeed, we did not detect any signs of clinically significant intraocular inflammation related safety signals such as uveitis, cataract, macular oedema, secondary glaucoma, rises in intraocular pressure, serous retinal detachment, pain, or photophobia. Both eyes studied had an uneventful postoperative surgical course with mild conjunctival haemorrhage and hyperaemia resolving within the first postoperative week. Both patients were comfortable during the postoperative period and tolerated the procedures well. Neither patient needed postoperative analgesia.

A secondary concern is whether the subretinal bleb created by the transplant injection might lead to retinal detachment. We noted 100% flattening of the surgical bleb in both patients at 4 h after transplantation. At no point did we detect retinal detachment or any lesion predisposing to retinal detachment in either eye studied.

Without immunohistological analysis, anatomic evidence of hESC-RPE has been difficult to confirm in the patient with dry age-related macular degeneration. On postoperative day 1, the bed of the transplant bleb seemed to be hyperpigmented on biomicroscopic examination. However, on subsequent serial assessments we could not confirm clinically detectable hyperpigmentation. During the first postoperative week, the patient with age-related macular degeneration did not comply with the immunosuppression regimen and blood concentrations of the agents dropped below the therapeutic range.

We noted anatomical evidence of hESC-RPE survival and engraftment in the patient with Stargardt's macular dystrophy. We detected clinically increased pigmentation at the level of the RPE within the bed of the transplant beginning at postoperative week 1 to month 3 (figure 4). The transplanted cells seem to have involved regions of complete pretransplant RPE loss as well as regions of pretransplant RPE compromise (figure 4). Spectral domain ocular coherence tomograph images collected at postoperative month 3 show survival and engraftment of hESC-RPE. Our findings confirm the recorded morphological RPE features of the pigmentation, and localise the transplanted cells to precisely the desired anatomical location.

We recorded functional visual improvements in both patients. Despite the lack of anatomical evidence, the patient with macular degeneration had functional improvements that included best corrected visual acuity changes from 21 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (20/500) at baseline to 33 ETDRS letters (20/200) at week 2. By week 6 she was reading 28 ETDRS letters (20/320) and has remained stable at this level through postoperative month 3. Goldman visual fields done by an experienced certified examiner (from

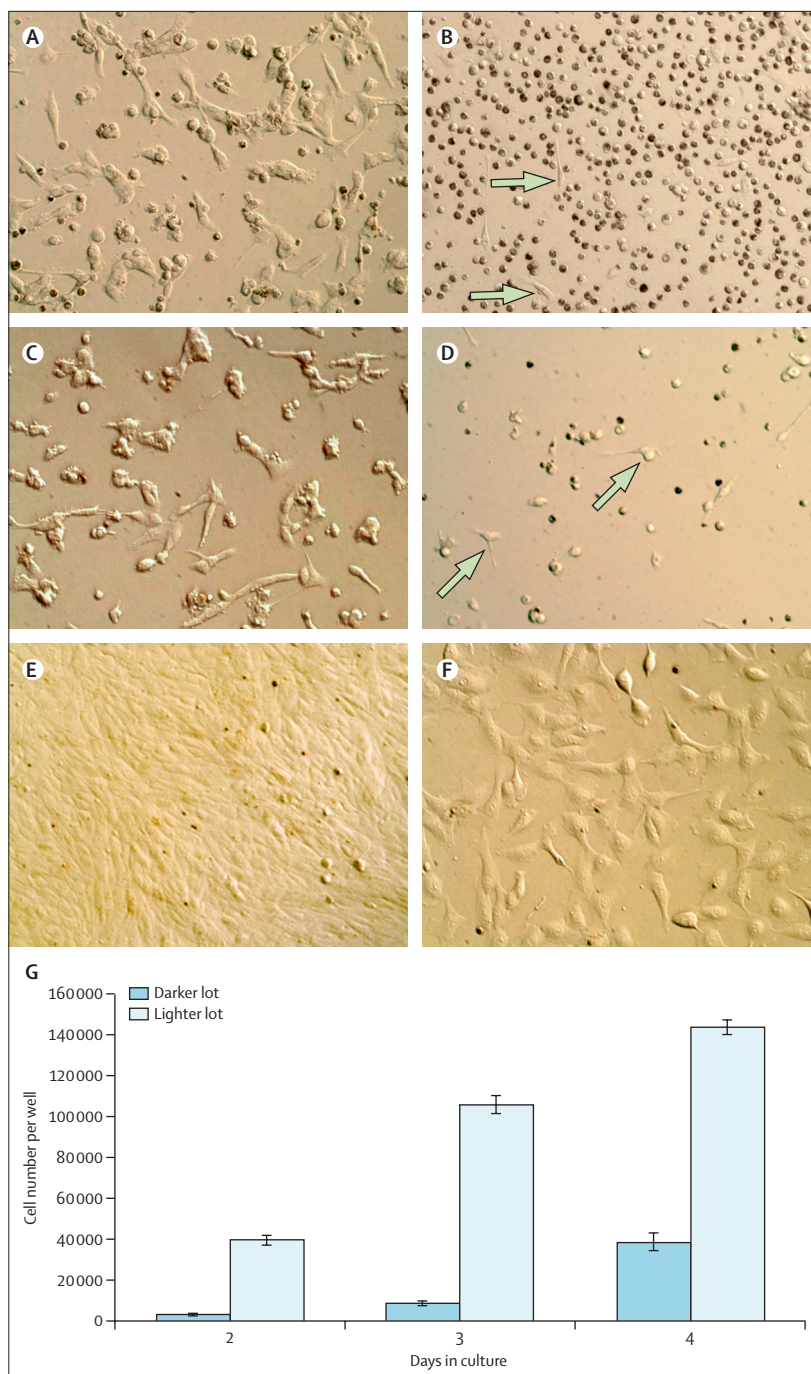


Figure 3: Difference in attachment and growth of RPE with different degrees of pigmentation
RPE from two lots were thawed, formulated, extruded through the injection cannula, and subsequently plated in cell culture medium on gelatin-coated plates. Micrographs show attachment and behaviour of cells from a lighter (A, C, E) and a darker (B, D, F) pigmented lot. A and B show the total cell population at 21 h after plating. The number of flat and adhering cells is an indication of relative plating efficiency. Note that most cells in the lighter pigmented lot (A) have attached, whereas most cells in the darker lot (B) remain rounded and unattached. C and D show the same cultures as A and B after removal of floating cells (arrows show flat adherent cells). E and F show the same cultures 3 days after plating. The lighter pigmented lot (E) has a greater number of cells and a confluent monolayer is established, whereas the darker lot (F) is still under confluent. Magnification $\times 200$. G illustrates the growth of RPE cells from both lots showing the total cell number per well on 3 consecutive days after plating. On the indicated day, cells were trypsinised and counted in a haemocytometer. Data are the mean cell number \pm SD of cell counts done on triplicate wells. RPE=retinal pigment epithelium.

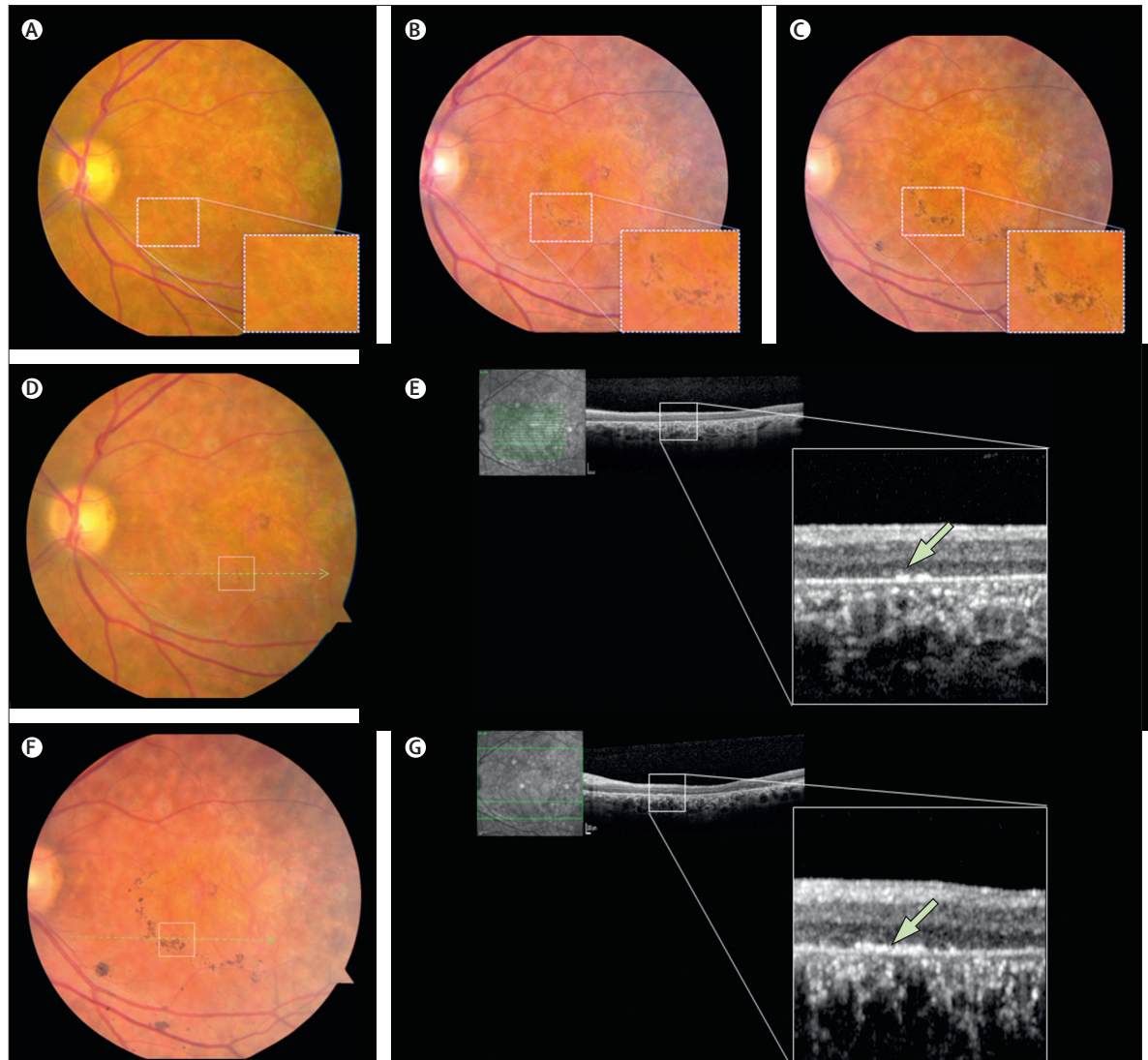


Figure 4: Images of the hESC-RPE transplantation site in the patient with Stargardt's macular dystrophy

Colour fundus photographs of the patient's left macula preoperatively and postoperatively (A–C). The region inside the rectangle bisects the border of the surgical transplantation site and corresponds to macular atrophy not included in the surgical injection. (A) Baseline macular colour image with widespread RPE and neurosensory macular atrophy. (B) Colour macular image 1 week after hESC-RPE transplantation. Note the mild pigmentation most evident in the region of baseline RPE atrophy. This pigmentation increased at week 6 (C). (D–G) Colour fundus photographs and SD-OCT images at baseline (D) and month 3 after transplant (F). Colour images show increasing pigmentation at the level of the RPE from baseline to month 3. Registered SD-OCT images (E, G) show increasing pigmentation is at the level of the RPE, normal monolayer RPE engraftment, and survival at month 3 (arrow) adjacent to region of bare Bruch's membrane devoid of native RPE. hESC=human embryonic stem cells. RPE=retinal pigment epithelium. SD-OCT=spectral domain ocular coherence tomography.

whom the treatment was not masked) showed no diminution of visual field; this could reasonably be interpreted as improved vision (preoperative and postoperative fields are shown in the webappendix). Confounding these apparent functional gains in the study eye, we also detected mild visual function increases in the fellow eye of the patient with age-related macular degeneration during the postoperative period.

We noted clear functional visual improvement in the study eye of the patient with Stargardt's macular dystrophy corresponding subjectively to the transplanted region of the posterior pole. At baseline the central

vision was hand motions. By week 2, best corrected visual acuity was improved to counting fingers (one ETDRS letter). We recorded continued improvement during the study period (five ETDRS letters [best corrected visual acuity 20/800] at 1, 2, and 3 months; table). The patient is very reliable and worked for years as a graphic artist. She reports subjectively improved colour vision and improved contrast and dark adaptation from the operated eye. Importantly, we have not detected functional visual change in the fellow eye, and no subjective improvement to the fellow eye has been reported. Similarly, Goldman visual fields done by an

	BCVA	ETDRS (number of letters)
Fellow eye		
Baseline	Hand motion	0
1 week	Hand motion	0
2 weeks	Hand motion	0
3 weeks	Hand motion	0
4 weeks	Hand motion	0
6 weeks	Hand motion	0
8 weeks	Hand motion	0
12 weeks	Hand motion	0
Operated eye		
Baseline	Hand motion	0
1 week	Counting fingers	0
2 weeks	Counting fingers	1
3 weeks	Counting fingers	3
4 weeks	20/800	5
6 weeks	20/800	5
8 weeks	20/800	5
12 weeks	20/800	5

hESC=human embryonic stem cells. RPE=retinal pigment epithelium. BCVA=best corrected visual acuity. ETDRS=Early Treatment Diabetic Retinopathy Study visual acuity chart.

Table: Change in visual acuity after hESC-RPE transplantation in patient with Stargardt's macular dystrophy

experienced certified examiner from whom the treatment was not masked showed no diminution of visual field and could reasonably be interpreted as improved (preoperative and postoperative fields are shown in the webappendix).

Discussion

The therapeutic use of hESCs poses daunting translational challenges. We provide clinical evidence suggesting that hESC-derived cells might be safely transplanted into human patients (panel). In our study, we transplanted a low dose (5×10^4 cells) of RPE cells generated from hESCs into one eye of two patients with different forms of macular degeneration.

To optimise the chances the cells would attach to Bruch's membrane, we selected a submacular injection site where the macular anatomy (photoreceptors, Bruch's membrane, and RPE) was still present and potentially viable, thus maximising the potential that the transplanted cells would integrate with the native RPE and rescue compromised perimacular tissue. Both patients tolerated the transplant well without signs of postoperative inflammation, rejection, or tumorigenicity at the time of this report. Although the 4 month follow-up reported herein is short, ours and other studies confirm that teratoma formation usually happens within 8 weeks^{1,19,21,25,26} and is often detected within 4 weeks after transplantation.^{19,23} At 3 months, there was a nearly four-times increase in the weight of the eyes of animals

Panel: Research in context

Systematic review

We search PubMed for all publications, including clinical trials, meta-analyses, and reviews, with the terms "age-related macular degeneration", "dry-AMD", "Stargardt's macular dystrophy", "Stargardt's disease", and "stem cells" and identified no similar studies. Although several new drugs are available for the treatment of the exudative (wet) type of age-related macular degeneration, no proven treatments exist for patients with geographic atrophy (dry age-related macular degeneration).²³ Evidence from studies in animals suggests that stem cells could be useful as part of new treatment strategies for currently incurable degenerative retinal diseases.²⁴

Interpretation

Our study provides the first description of hESC-derived cells transplanted into the eyes of patients with two different forms of macular degeneration.

injected with undifferentiated hESCs because of teratoma growth. Similarly, immune rejection and clinical ocular safety signals relating to intraocular inflammation were expected during this period—we detected none. Although we did not detect any signs of transplant rejection or inflammation, it is possible the transplant was compromised.

Our findings suggest that transplanted hESC-RPE cells in the patient with Stargardt's macular dystrophy attached to Bruch's membrane and persisted for the duration of our observation period. At present, we do not know if the transplanted cells have reduced immunogenicity or whether they will undergo rejection without immunosuppression in the long term. Similarly, we are uncertain at this point whether any of the visual gains we have recorded were due to the transplanted cells, the use of immunosuppressive drugs, or a placebo effect.

Although the transplantation of intact sheets and suspensions of primary RPE cells has been previously attempted,^{11–18} RPE derived from adult and paediatric donors are restricted in both their capacity to proliferate²⁷ and their ability to differentiate in vitro.²⁸ Clinically, sheets of adult RPE engrafted into the subretinal space of patients with dry age-related macular degeneration have failed to improve visual function.²⁹ Although RPE derived from prenatal and postnatal tissue has been successfully dissociated and induced to grow in vitro,^{30–32} such sources are extremely limited and variable with regard to quality and expansion capacity. By contrast with adult and fetal tissue, a central feature of hESCs is that they have the capacity to proliferate indefinitely, providing a virtually unlimited source of youthful cells as starting material. Another crucial advantage is that the stage of in-vitro differentiation can be controlled to maximise survival and functionality. Attachment of the transplanted cells to

Bruch's membrane, and their subsequent survival and integration into the host RPE layer is crucial to the success of this therapeutic strategy. Our data show that the extent of RPE maturity and pigmentation might substantially affect subsequent attachment and growth of the cells in vitro.

Our study is designed to test the safety and tolerability of hESC-RPE in patients with advanced-stage Stargardt's macular dystrophy and dry age-related macular degeneration. So far, the cells seem to have transplanted into both patients without abnormal proliferation, teratoma formation, graft rejection, or other untoward pathological reactions or safety signals. Continued follow-up and further study is needed. The ultimate therapeutic goal will be to treat patients earlier in the disease processes, potentially increasing the likelihood of photoreceptor and central visual rescue.

Contributors

SDS and J-PH performed the transplant surgeries. SDS, J-PH, GH, VF-C, CKP, RMO, RG, IK, and RL contributed to data collection and analysis. SDS, EM, RG, IK, and RL contributed to study design. SDS, RG, IK, and RL contributed to literature search, data interpretation, and writing of the report.

Conflicts of interest

EM, RG, IK, and RL are employees of Advanced Cell Technology, a biotechnology company in the area of stem cells and regenerative medicine. The other authors declare that they have no conflicts of interest.

Acknowledgments

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