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Advancing a stem cell therapy for age-related macular degeneration

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Abstract

The retinal pigment epithelium [RPE] is a multifunctional monolayer located at the back of the eye required for the survival and function of the light-sensing photoreceptors. In Age-related Macular Degeneration [AMD], the loss of RPE cells leads to photoreceptor death and permanent blindness. RPE cell transplantation aims to halt or reverse vision loss by preventing the death of photoreceptor cells and is considered one of the most viable applications of stem cell therapy in the field of regenerative medicine. Proof-of-concept of RPE cell transplantation for treating retinal degenerative disease, such as AMD, has long been established in animal models and humans using primary RPE cells, while recent research has focused on the transplantation of RPE cells derived from human pluripotent stem cells [hPSC]. Early results from clinical trials indicate that transplantation of hPSC-derived RPE cells is safe and can improve vision in AMD patients. Current hPSC-RPE cell production protocols used in clinical trials are nevertheless inefficient. Treatment of large numbers of AMD patients using stem cell-derived products may be dependent on the ability to generate functional cells from multiple hPSC lines using robust and clinically-compliant methods. Transplantation outcomes may be improved by delivering RPE cells on a thin porous membrane for better integration into the retina, and by manipulation of the outcome through control of immune rejection and inflammatory responses.

Keywords: Pluripotent stem cells, retinal pigment epithelium, age-related macular degeneration, cell transplantation, stem cell therapy

Abbreviations: AMD: Age-related Macular Degeneration; AMD-GA, dry AMD identified by geographic atrophy; AMD-CNV, wet AMD identified by choroidal neovascularization; cGMP: current Good Manufacturing Practice; hESC: human embryonic stem cells; hiPSC: human induced pluripotent stem cells; PLLA, poly-L-lactic acid; PLGA, poly lactic-co-glycolic acid; PSC: pluripotent stem cell; RPE: retinal pigment epithelial.

INTRODUCTION

The retinal pigment epithelium is a multifunctional monolayer required for the health and function of neighbouring photoreceptor cells in the retina. Age-related macular degeneration [AMD] is associated with loss of retinal pigment epithelial [RPE] cells which cover the Bruch's membrane at the back of the eye and support the survival and function of the photoreceptor cells which detect light. Dysfunction or death of RPE cells in the macula region causes loss of photoreceptor cells and permanent central blindness.

AMD is a highly prevalent irreversible visual impairment. The onset of dry AMD is characterized by formation of drusen deposits leading to complement activation and chronic inflammation of the RPE with subsequent loss of photoreceptor function. Damaged RPE cells cannot clear debris efficiently and so condition a toxic environment for photoreceptors. In wet or neovascular AMD, choroidal vessels proliferate and invade the retina and leak blood and fluid under the retina, leading to rapid loss of RPE cells and ultimately loss of vision (Figure 1). While anti-angiogenic factors like anti-VEGF inhibitors have been used as drugs to suppress vascularization in wet AMD, this is not always effective, is not curative and involves multiple injections into the eye. Currently there is no available treatment available for dry AMD that improves vision.

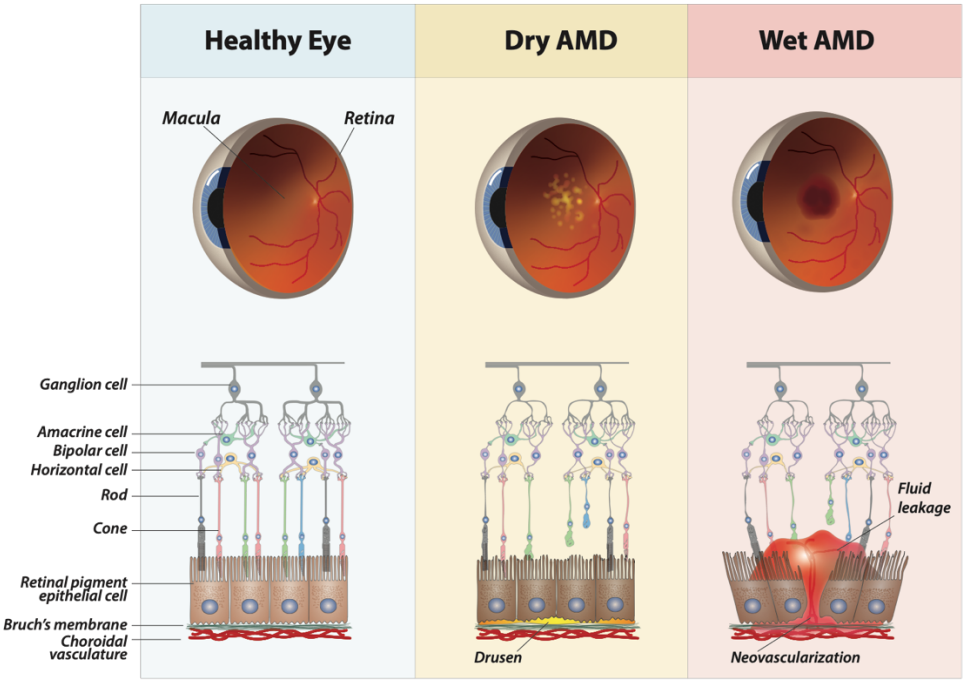

Figure 1. Age-related Macular Degeneration (AMD) can manifest as Dry and Wet (or neovascular) forms, each having a different disease aetiology. Dry AMD is characterized by the formation of drusen deposits behind the retina, while neovascularization and blood leakage into the eye characterize Wet AMD.

It has been estimated that the number of people living with AMD will reach 196 million by 2020 and that this will increase to 288 million by 2040 [1]. That study showed that 17% of 45- to 85-year old adults would suffer some loss of vision or blindness due to AMD. The incidence of AMD is predicted to accelerate due to an ageing population, diet, smoking and overexposure to light. Treatment options are limited and only partially effective, and there is urgent need to develop new strategies for cellular therapy particularly in the case of dry AMD.

RPE cell transplantation aims to halt or reverse vision loss by preventing the death of photoreceptor cells and is considered one of the most viable applications of stem cell therapy within the field of regenerative medicine. Early studies of RPE cell transplantation to treat retinopathies identify rescue of photoreceptor function in animal studies [1]. However, the challenge with RPE cell transplantation is in the source of sufficient numbers of cells to effect therapy. Clinical trials involving human adult or foetal RPE cells have not proven successful

due to the limited number and proliferative potential of available cells [2].While mesenchymal stem cells have been reported as promising for therapy of retinal diseases , clinical trials have not been reported. Accordingly, the use of RPE cells derived from pluripotent stem cells has been readily adopted.[3]

Several overseas groups have reported that the transplantation of stem cell-derived RPE cells in AMD patients is safe and can improve vision. Recent studies include The London Project to Cure Blindness [2], The California Project to Cure Blindness [3] and the Riken Laboratory for Retinal Regeneration [4]. The most recent paper from the London group [2] reported significant improvement in vision in two [of two] patients with neovascular or wet AMD using a cell-on-membrane construct with some similarity to that developed in this Centre by Surrao et al. [5, 6]. These published clinical trials demonstrate proof-of-concept for a stem cell therapy to treat AMD and provide details of implant size and choice of patient group.

Despite significant clinical progress, published methods used for RPE cell production from stem cells remain labour-intensive and not readily amenable to commercial scale clinical application. Although several groups have reported high efficiencies in RPE cell production [7-9], their methods require the use of undefined animal products that carry risk of patient infection. Most clinical trials have generated hPSC-RPE cells using spontaneous differentiation under xeno-free/defined conditions [2, 3, 10]. Conversely, published studies showing more efficient differentiation of hPSCs to RPE cells use undefined reagents [such as Matrigel] and have not been adapted to current Good Manufacturing Protocols [cGMP] for integration into human clinical trials. Routine treatment of large numbers of AMD patients will require the ability to rapidly and efficiently generate clinical-grade RPE cells. It will be necessary to develop methodology for rapidly producing hPSC-RPE cells at high yield and high homogeneity from possibly multiple PSC lines under xeno-free/defined clinical grade conditions in order to provision cells for a large clinical cohort.

IMPORTANT CONSIDERATIONS IN STEM CELL THERAPY FOR AMD

Making sufficient RPE cells under clinical-grade conditions

In terms of a suitable source of cells for transplantation, an important consideration is the number of cells which can be generated. While there have been reports of transplantation of

isolated mature and fetal RPE cells into animal models [11-13], it is difficult to obtain enough of these cells for effective transplantation to multiple patients. The field has therefore adopted the transplantation of RPE cells differentiated from pluripotent stem cells [PSCs], and procedures for cell differentiation involving both spontaneous and directed differentiation [14, 15]. Most reported culture systems producing RPE cells for clinical trial use hESCs as a starting cell source, with hiPSCs used more recently. There is variability amongst the procedures used in clinical trials [Table 1]. Derivation of hESC-RPE cells through long-term spontaneous differentiation followed by cell isolation and maturation has been commonly used [16-19]. Although this approach is sufficient for small trials of 10 to 20 patients, it would be insufficient and cost prohibitive for the production of the cell numbers required to treat the estimated 200 million AMD patients worldwide. Methods have been published that can generate RPE cells at high efficiency and speed using chemically defined signals that mimic stages of eye development, but these have been applied to research and not to clinical testing [7, 9, 20]. The efficiency and robustness of clinical grade cell production lags behind research-grade methods. For clinical grade cell preparation, all culture components need to be approved by the Therapeutics Goods Administration [TGA] and Federal Drug Administration [FDA]. No published procedure produces RPE cells at high efficiency without the use of cell culture components that are not xeno-free and carry risk of infection to patients. The application of directed differentiation methods to AMD therapy will require rapid, efficient manufacturing procedures to produce pure populations of RPE cells at scale, sufficient for testing and validation of batches ahead of use.

Table 1. Clinical trials using pluripotent stem cell derived RPE cells to treat AMD.

Clinical trial ID	Phase	Disease	No.	Cell Type	Cell line	Differentiation Protocol	Delivery Method	Sponsor[s]	Ref.
NCT01344993 NCT02563782 NCT02463344	I/II	AMD-GA	9	hESC-RPE	MA-09	Spontaneous	Sub-retinal suspension	Ocata Therapeutics [Astellas]	[24, 25]
UMIN000011929	I	AMD-CNV	2	hiPSC-RPE	Autologous	Directed [42]	Monolayer	Riken Institute for Developmental Biology	[4]
On hold	I/II	AMD-CNV	2	hiPSC-RPE	Non-autologous	Directed [42]	Monolayer	Riken Institute for Developmental Biology	
UMIN000026003	I	AMD-CNV	-	hiPSC-RPE	Allogeneic	Directed [42]	Monolayer	Kobe City Medical Centre General Hospital	
NCT02590692	I/IIa	AMD-GA	5	hESC-RPE	CPCB	Spontaneous	Monolayer on substrate Paralyene C	Regenerative Patch Technologies	[3]
NCT01691261	I	AMD-CNV	2	hESC-RPE	SHEF-1.3	Spontaneous	Monolayer on substrate Polyethylene terephthalate	Pfizer	[2]
NCT01674829	I/II	AMD-GA	12	hESC-RPE	MA09	Spontaneous	Sub-retinal suspension	CHA Bio Biotech	[26]
NCT03305029	I	AMD-GA	3	hESC-RPE	SCNT-HESC	Spontaneous	Sub-retinal suspension	CHA University	
NCT02286089	I/II	AMD-GA	24	hESC-RPE	HAD-C 102	Directed [9]	Sub-retinal suspension	BioTime CellCure Neurosciences	
NCT02903576	I/II	AMD-GA AMD-CNV	18	hESC-RPE	MA09	Spontaneous	Sub-retinal suspension Monolayer on substrate	Federal University of Sao Paulo	
NCT03046407	I/II	AMD-GA	10	hESC-RPE	Q-CTS-HESC-2	Spontaneous	Sub-retinal suspension	Chinese Academy of Sciences	
NCT02755428	I/II	AMD-GA	10	hESC-RPE	Q-CTS-HESC-2	Spontaneous	Sub-retinal suspension	Chinese Academy of Sciences	
NCT02749734	I/II	AMD-CNV	15	hESC-RPE	Q-CTS-HESC-2	Spontaneous	Sub-retinal suspension	Chinese Academy of Sciences	

AMD, age-related macular degeneration; AMD-GA, dry AMD identified by geographic atrophy; AMD-CNV, wet AMD identified by choroidal neovascularization.

RPE cells have limited proliferative potential *in vitro*, and so large-scale production will necessarily involve rapid, efficient expansion at an early stage of passage. In this laboratory, we have developed a protocol that uses chemical cocktails to instruct cell differentiation to RPE cells [21]. The entire process utilizes synthetic, human or clinical grade components, and involves no components that carry risk to human patients, and is therefore highly likely to obtain FDA and TGA approval [21]. The process has also been adapted to an adherent format which makes it easier to produce cells at scale, and potentially in an automated, closed system. It appears to be a robust procedure and has been used effectively on several hESC lines to give the same cell output despite natural variation between stem cells of different origin.

Cell transplantation on support membranes

Cell therapy approaches to AMD treatment target the subretinal space between the photoreceptor layer of the retina and the damaged RPE so that implanted cells interact directly with photoreceptors. Early studies tested the implantation of single cells and cell aggregates or sheets [20, 22-26]. Although partially successful, RPE cell injection is a sub-optimal technique that relies on loose individual cells to self-integrate into the existing diseased tissue. Recent attention has focused on the delivery of cells as a sheet growing on a support membrane and has yielded more success [5, 27-29]. This is likely due to the delivery of a layer of mature, functional cells directly into the region requiring treatment. Mature cells are polarized and as functional monolayers can better survive as a group. They are immediately ready to function by clearing waste, secreting survival signals to retinal cells and reducing the activity of inflammatory cells in the eye [30]. By comparison, cells in suspension are more likely to be stressed at injection and unlikely to seed for growth on to the damaged RPE layer in the eye. Successful monolayer implants have involved cells grown and matured on membranes of various type including a PET polyester [Transwell®] membrane [2] a thin Parylene C construct [3, 29], as well as electrospun nanofibre membranes like poly-L-lactic acid [PLLA] and poly lactic-co-glycolic acid [PLGA] developed in this lab (Figure 2) [5, 27]. Unsupported cell sheets of RPE cells grown on collagen have also been used as a successful implant [4, 31]. While several different membrane types have been used for RPE cell implantation with some success, these have not yet been directly compared for therapeutic potential.

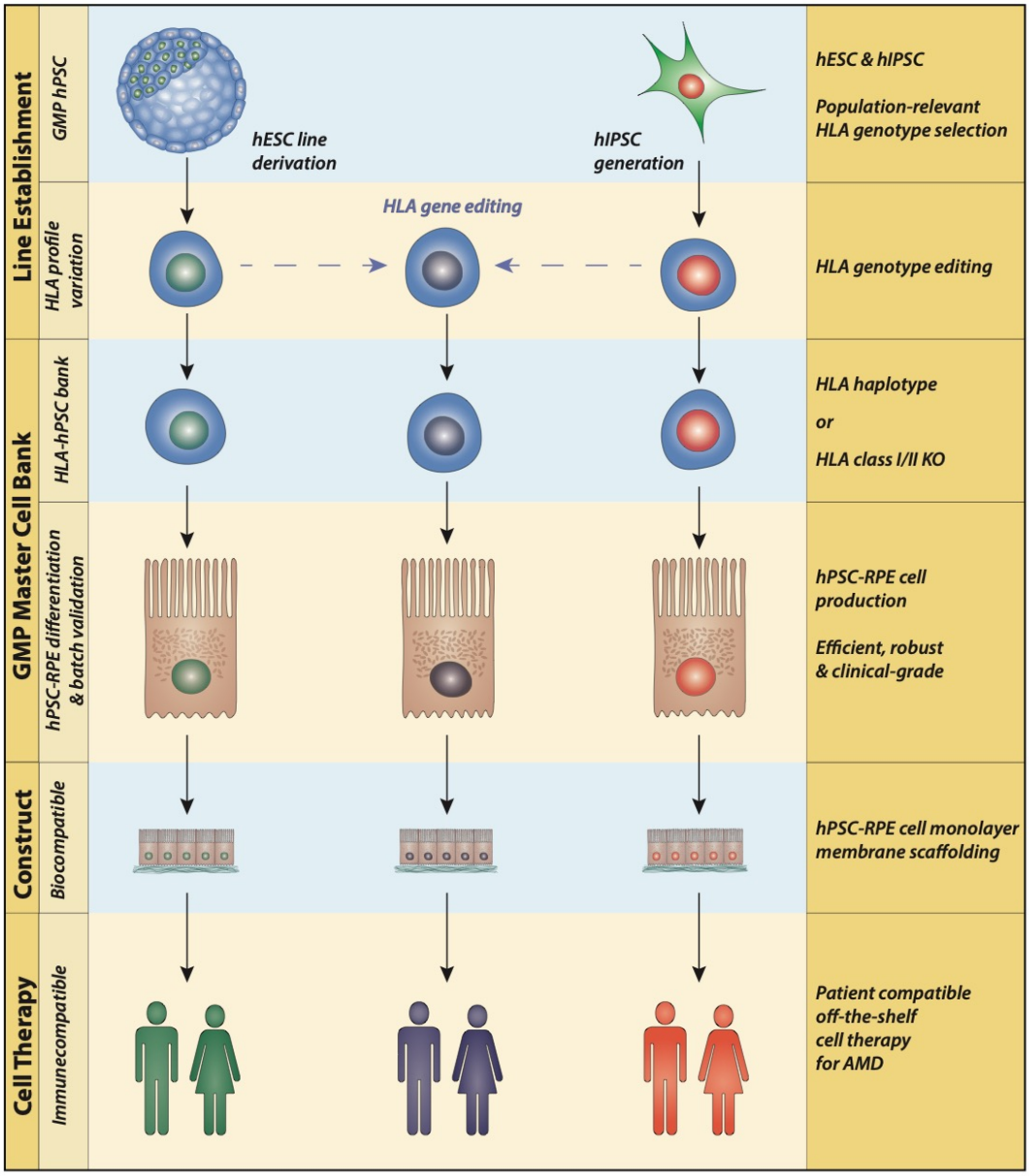

Figure 2. Provision of stem cell-derived retinal pigment epithelial (RPE) cell replacement therapy for Age-related Macular Degeneration (AMD). The path to the clinic involves the establishment of banks of human pluripotent stem cells (hPSCs) prepared under Good Manufacturing Practice (GMP) conditions to produce clinically acceptable cells. These could be derived from human embryonic stem cells (hESCs) or as human induced PSCs (hiPSCs) derived from fibroblasts or other somatic cell types. Banks of hPSC lines with broad immunocompatibility can be established by selection or by editing HLA genes to produce HLA 'super donor' haplotype li nes.hPSC-RPE cells are then generated under GMP conditions and frozen down to give a master cell bank of quality tested cells. For cell therapy in HLA-

matched AMD patients, hPSC-RPE cells are thawed and grown as a monolayer on biocompatible membranes. These constructs are implanted beneath the retina to support survival and function of photoreceptors and maintain vision.

In this lab, RPE cells grown on a thin PLLA membrane have shown optimized differentiation, polarization, viability and maturation [5, 6] [Surrao et al., unpublished data]. Thin, small fibre diameter electrospun membranes have a high level of porosity that allows exchange of water, waste and nutrients between RPE cells and the underlying blood supply. Another advantage is the increased surface area-to-volume ratio that enhances the coating of membranes with proteins that support cell attachment, monolayer formation and cell function [32]. We have tested four biodegradable polymers for their ability to ultimately form functional constructs for transplantation [5]. These are all polymers approved for clinical use by the USA Food and Drug Administration [FDA] and Australian Therapeutic Goods Administration [TGA]. The application of a protein coat enhances cell attachment, and xeno-free laminin and vitronectin have been used successfully in several studies [unpublished data].

In our Centre, a porous, thin, electrospun membrane coated with hPSC-derived RPE cells and having the modulus of the Bruch's membrane has been successfully transplanted into rat eyes and shown to support RPE cell survival and function at the interface with photoreceptors [Surrao et al., unpublished data]. Slowly biodegradable compositions like PLLA and PLGA would appear to be desirable, with the expectation that extracellular matrix production by transplanted RPE cells might replace the damaged Bruch's membrane over time. Coating the membrane with laminin [22], vitronectin [30] or other extracellular matrix protein, supports cell attachment, differentiation and monolayer formation, so providing the correct arrangement of cells needed for subretinal implantation into the eye.

LESSONS FROM IMPLANTATION STUDIES

Early clinical trials in patients with Stargardt's disease or dry AMD showed that hESC-RPE cells could be successfully transplanted across an HLA, or major histocompatibility barrier. Two separate clinical trials showed maintenance of a suspension of allogeneic hESC-RPE cells for at least one year following systemic immunosuppressive therapy with cyclosporin A and

tacrolimus for 3-4 months [24-26]. The ability to control inflammation and immune rejection of an allograft may be more challenging however if cells are transplanted as a monolayer on a foreign membrane, or if choroidal neovascularization is present as occurs in wet AMD. While it may be possible to control inflammation at least initially for a biodegradable membrane, if the membrane is non-biodegradable, inflammation and an immune rejection response may heighten with time, requiring prolonged administration of immunosuppressive therapy.

Recent clinical trials have involved the implantation of HLA-mismatched hESC-RPE cells grown on a porous, non-biodegradable membrane, combined with systemic and local immunosuppressive drug therapy for up to a year [2, 3]. These small Phase I trials provide evidence for the safety and efficacy of stem cell therapy for AMD and demonstrate survival of functioning RPE cells and visual improvement in some patients. However, general application of the therapy, and further clinical trials, may be precluded if long-term immunosuppressive drug therapy is needed to maintain an effective implant. For this reason, effective long-term cell replacement therapy may require the use of HLA-matched histocompatible implants delivered as a monolayer on membranes, and perhaps measures to control early inflammation. Furthermore, a biodegradable electrospun membrane of PLLA or PLGA [5, 6] might be more desirable as a support for a cell monolayer, particularly if immunosuppressive treatment is required only across the lifespan of the membrane.

RPE cells in the normal eye are unique in their capacity to support an immune privileged environment. They produce a range of factors including transforming growth factor beta which supports maintenance of an immunosuppressed state within the eye [33]. A recent report now confirms that hESC-derived RPE cells also produce immunosuppressive cytokines and factors. These cells contribute to the maintenance of an immunosuppressed state in the damaged eye following transplantation in the absence of immunosuppressive drug therapy [20]. It has also been shown that both hESC-RPE cells and hiPSCs-RPE cells have unique potential above other PSC-derived differentiated cells like smooth muscle cells, to induce immunological tolerance [34]. For example, hiPSC-derived RPE cells are immune tolerated not only when implanted into the eye but also into other body sites [34]. In general, hESC-RPE cells and hiPSC-RPE cells have a characteristic immunosuppressive phenotype, and are weakly immunogenic, showing low expression of HLA Class I antigens, and expression of HLA Class II antigens only following interferon-γ treatment [31, 35].

In terms of their immune capability, hESC-RPE cells have clear capacity to inactivate T cells and induce T cell apoptosis, suggesting a direct inhibitory effect on T cell immunity [20]. Implantation of hESC-RPE cells subretinally into rats was shown to induce detectable blood levels of IL-10, a growth factor that supports development of regulatory T cells which mediate peripheral tolerance. The same hESC-RPE cells were also found to survive transplantation into vision-impaired RCS rats without administration of cyclosporin as an immunosuppressant [20]. Furthermore, RCS vision-impaired rats implanted with hESC-RPE cells showed greater improvement in photoreceptor function in the absence of immunosuppression than did animals given cyclosporin as an immunosuppressant [20]. A number of studies now indicate that both xenogeneic and allogeneic transplantation of hESC-RPE cells prepared as a cell suspension can occur successfully without long-term use of immunosuppression. It is likely therefore that immunosuppressive drug treatment negatively impacts the development of T cell-mediated tolerance, so that sustained immunosuppressive treatment following implantation of RPE cells into the eye may not be desirable.

Important considerations in the future design of a cell therapy for AMD will be histocompatibility between transplanted cells and the host, and the need for immunosuppressive drug therapy to achieve long-term transplantation without rejection. The two reported clinical trials involving hESC-derived RPE cells grown on a membrane did not use histocompatible cells [2, 3], and a third trial used hESC-RPE cells administered as cells in suspension [18]. Those grafts have been sustained for up to a year with no signs of rejection through administration of a combination of local and systemic immunosuppressants given over several months following implantation. Going forward, histocompatible transplantation could be achieved using patient-derived or MHC-matched hiPSCs for differentiation of RPE cells, or through the use of an allogeneic hESC cell line that has been engineered to no longer express HLA antigens [36].

Going forward, another option to allay graft rejection issues is to develop patient-specific hiPSC-derived RPE cells. The process of reprogramming, differentiation and validation of each line would make this a very costly and time-consuming activity, and this alone could preclude the patient-specific approach. More commercially feasible would be the provision of a bank of HLA-typed hESC-RPE and hiPSC-RPE cell lines, prepared, validated and banked for distribution to patients as needed. The development of super-donor cell banks of iPSCs for generation of differentiated cells of defined HLA haplotype has been considered in terms of

the number of lines required to treat a majority of a given regional population such as California [37] or Japan [38].

The need for a cell bank to provide MHC-matched iPSC-RPE cells for transplantation should however be weighed against the suitability of iPSCs as a starting cell population for differentiating RPE cells. In terms of RPE cell transplantation for AMD, there has been caution over the choice of hiPSCs as a starting cell population on the grounds that hiPSC-derived cells are prone to differentiative change and oncogenic transformation to give teratomas. In one study, the tumorigenic potential of iPSC-RPE cell lines was directly assessed through subretinal implantation into nude rats and no oncogenic transformation was detected after 12 months [39]. A clinical study however by the same group preparing similar hiPSCs-RPE cell sheets for implantation into two wet AMD patients reported oncogenic change in an hiPSC line derived from one patient which was then not implanted [4].

PROGRESS IN CLINICAL TRIALS

Macular degeneration is now recognized as a disease highly amenable to stem cell therapy. The introduction of hPSC-derived RPE cells into the subretinal space provides a means to replace the damaged retinal pigment epithelium. Already there have been multiple small clinical trials initiated involving hESC-derived RPE cells in several countries. Details of these trials are summarized in Table 1. These have involved both wet and dry forms of AMD characterized by geographic atrophy or choroid neovascularization. They have employed both hESC-derived and hiPSC-derived RPE cells. Most cells have been prepared through spontaneous differentiation which is a slow and prolonged procedure. Only one study has involved cells prepared through a protocol involving directed differentiation [9]. A Japanese trial represents the first implantation of autologous hiPSC-derived RPE cells into a patient with neovascular or wet AMD [4]. The combined studies have involved implantation of either cell suspensions, a monolayer sheet of cells grown on collagen, or cells grown as a monolayer on a substrate for implantation. Polymeric substrates tested to date include polyethylene terephthalate [PET] and Parylene C [2, 3].

The safety of hESC-RPE cell implantation into the subretinal space in humans was first demonstrated by Ocata Therapeutics [USA] [24, 25] and was confirmed by a Korean study

[26]. Those studies involved implantation of allogeneic cell suspensions into patients with either Stargardt's muscular dystrophy or dry AMD and followed for a year in the Korean study [10], and for 2 years in the American study [24], with no adverse proliferation of cells, no rejection or serious ocular effects, and no serious systemic effects. Both studies reported either improvement or no loss in visual acuity. However, when cells were transplanted as a suspension, it was more difficult for cells to localize and integrate into an existing retinal pigment epithelium [27]. A recent clinical trial involving 12 patients with Stargardt's disease given a suspension of hESC-RPE cells sub-retinally reported focal areas of hyperpigmentation in the subretinal region consistent with survival of transplanted cells, but no significant improvement in vision in those patients [18].

The efficacy of hiPSC-RPE cell implantation was reported for patients with neovascular or wet AMD receiving an autologous subretinal implant of cells grown as a sheet on collagen [4]. This was the first reported case of a successful hiPSC-derived stem cell therapy. A single patient maintained the graft without immunosuppressive drug treatment for up to a year, by which time there was no sign of rejection. Visual acuity was maintained but did not improve [4]. A second patient was withdrawn from the trial when mutations were observed in their differentiated autologous hiPSC-RPE cells.

Most relevant to the discussion here are the two clinical trials reported recently using cell-on-membrane implants. The *London Project to Cure Blindness* first initiated a clinical trial in 2015 to test the safety and efficacy of a 'patch' implant [2]. hESC-RPE were delivered subretinally on a polyester membrane coated with vitronectin into patients with advanced neovascular or wet AMD. This was the first successful trial delivering hESC-RPE cells grown on a non-degradable polyester [PET: Transwell®] membrane [2]. Subretinal implantation of the patch into 2 patients confirmed no serious retinal damage, with an improvement in visual acuity after a year. The *California Project to Cure Blindness* also reported results of a clinical trial in 2018 involving four patients with advanced dry AMD, who were implanted with hESC-RPE cells on a non-degradable Parylene C membrane [3]. Patients were followed for up to a year and showed no loss of vision, and with improved vision in one case.

In both clinical trials involving cell-on-membrane implants or 'patches', a monolayer of fully differentiated cells was delivered which was polarized and had tight junction barrier formation [2, 3]. The membrane allowed easy insertion and also provided cells in a fully functional form. Maintenance of cells on the membrane was monitored over time as dark

pigmented cells, and cells were seen to migrate from the patch to nearby areas devoid of RPE cells. Patients received perioperative systemic immunosuppressants, and in the case of the London Trial, local anti-inflammatories through use of a fluocinolone acetonide intravitreal implant. The controlled differentiation of cells to give RPE cells and their delivery as a membrane-supported functional monolayer, is thought to be an important element of this successful procedure.

No clinical trials have yet involved biodegradable nanofiber electrospun membranes as a support matrix for hESC-RPE cells. In this Centre, testing in RCS vision-impaired rats has begun and already shows successful implantation, survival and function of hESC-RPE cells grown on PLLA membranes, leading to a protective effect on photoreceptor survival [Surrao et al., unpublished data]. A recent study in pigs reported iPSC-RPE cells prepared as a 'patch' of cells grown on a biodegradable PLGA membrane [27]. Successful integration of cells into the retinal pigment epithelium was achieved using a membrane of the same composition but thicker fibre diameter as that developed in this Centre [5, 6]. Cells grown on membranes were implanted subretinally into pigs in which RPE had been damaged through laser injury as a model for dry or non-neovascular AMD. To date there have been no clinical trials to determine the effectiveness of long-term implantation of hESC-RPE cells on biodegradable membranes, or their impact on immunity in the eye.

ADVANCING A CELL THERAPY FOR AMD

Development of optimal cell-on-membrane constructs for implantation

Transplantation of mature RPE cells prepared as a monolayer on a synthetic, biomimetic Bruch's membrane is considered a promising therapy for AMD. However, the synthetic scaffolds used in clinical trials to date do not mimic the Bruch's membrane or the microenvironment of the retinal pigment epithelium. The native human Bruch's membrane is a 2-4 μm thick extracellular matrix comprised of mainly collagen and elastin, which supports RPE cell adhesion, migration, differentiation and maturation. One argument is that biodegradable, electrospun membranes will support cell growth for a prolonged period following implantation in the eye until cells integrate within the existing retinal pigment epithelium. Electrospun nanofiber PLLA and PLGA membranes have been developed with a

thickness, porosity, thin fibre diameter, and protein coating which resembles the Bruch's membrane [5, 6]. A thinner fibre diameter of ~70nm, and laminin coating of the membrane, represent advances over the first PLGA membrane developed and tested for RPE cell growth [40].

Production of hPSC-RPE cells under clinical grade conditions

In order to pursue human trials of hPSC-RPE cells, therapy-grade cell products must be generated according to cGMP regulations. The quest for an efficient cGMP compliant protocol to produce differentiated cells requires xeno-free hPSC lines, small molecule inducers to direct differentiation, and cGMP compatible media and surfaces for cell growth. Most of the hESC-RPE cell lines used in clinical trials were produced through spontaneous differentiation [2, 18] or through the use of compromised protocols of directed differentiation [7, 9] and are not readily adaptable to commercial scale cell production under clinical or cGMP conditions.

A differentiation protocol has been developed in this lab which is unique in that it is rapid, directed, efficient and completely xeno-free. By this procedure ~90% of hESCs can be directed to an RPE cell fate in two weeks using small molecules in serum-free/feeder-free cultures and under xeno-free/chemically-defined conditions [21]. Large scale production of differentiated cells from multiple hPSC lines will require protocols which allow scale up of cell production to meet clinical needs. Production of cells for clinical application will require robust, rapid, efficient and xeno-free methods for differentiation capable of producing large numbers of mature functional cells in a short time window. It will be important to optimise cell production through generation of higher yields of cells, so reducing the need for rederivation of differentiated cells and quality control associated with each batch preparation.

Maintenance of the immunoprivileged state of the eye

Transscleral incision and subretinal implantation could compromise the immune privileged environment of the eye. However, published reports, also confirmed by us, indicate that hESC-RPE cells as implants are tolerated for at least 4 weeks without immunosuppression in animal models [20, 27]. Inflammatory responses reflected by microglia activation are to be expected with incision and insertion of a foreign body, since microglia play an important role

as resident immune cells in maintaining immune homeostasis [41]. This type of response could be controlled through use of anti-inflammatory drugs at least perioperatively.

Implantation into a diseased or damaged eye also raises the issue of the environment in which the implanted cells must survive. Inflammation is a key factor in the pathogenesis of AMD and could prevent successful implantation. Strategies must be developed to control the inflammatory environment and to support the development of tolerance to the graft. Previous studies have shown that long-term use of systemic immunosuppression is not beneficial to successful acceptance of hESC-RPE cells transplanted into rats [20]. In fact, immunosuppressive drug therapy could inhibit induction of a regulatory T cell response needed to establish and maintain tolerance to the graft.

CONCLUSION

The success of stem cell therapies is entirely dependent on the quality of cells transplanted, their purity, functional competence and survival. The effectiveness of the therapy will then be dependent on a whole-of-problem approach involving the perfection of supporting membranes, the development of surgical techniques, and the management of the immune environment into which cells are transplanted. Despite a number of clinical trials reporting the preparation of hPSC-derived RPE cells and their placement as a subretinal implant, none is supported by a procedure for producing cells according to a protocol which will deliver stem cell-based therapy to the clinic. A protocol for cell production and differentiation is needed which can be applied to commercial scale cell production where cells are produced rapidly, under completely defined, small molecule and xeno-free conditions, which can then be quality tested and then banked for future clinical use. Future Clinical Trials will require production of homogenous, differentiated cells prepared under cGMP conditions, and in numbers reflecting the needs of AMD patients.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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