Article Title: Treatment of age-related macular degeneration with pluripotent stem cell-derived retinal pigment epithelium

Running Title: Age-related macular degeneration and RPE regeneration

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Abstract

Retinal pigment epithelium (RPE) degradation is central to the onset and progression of age-related macular degeneration (AMD), a growing and currently incurable form of blindness. Due to its key role in maintaining the retinal structure and homeostasis, cell replacement of the RPE monolayer has emerged as a promising therapy to rescue visual acuity in AMD patients.

Thanks to the tremendous progress of pluripotent stem cells technologies over the last decade, a potentially unlimited new source for RPE transplantation has reached clinical trials. This review summarizes the methods by which pluripotent stem cell-based RPE cells are produced for transplantation, the delivery methods currently being adopted and the latest clinical outcomes with regard to the treatment of AMD.

Keywords

Age-related macular degeneration, retinal pigment epithelium, pluripotent stem cells, regenerative medicine, cell therapy, stem cells, retina

Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in the developed world, and is expected to increase significantly as the ageing population grows. The global prevalence of AMD is currently thought to be approximately 170 million, and predicted to increase to 288 million by 2040.[1] In the UK, approximately 600,000 people are affected by AMD[2] and the cost of treatments per person per year ranges from £10-30K, with the lower range representing those in retirement.[3] A recent report estimates the total cost of

adult sight loss and blindness in the UK to be between £15.8 billion and £28.1 billion per year, depending on the year and disability weights used, with AMD accounting for 34% of total sight related healthcare costs.[4] Developing new and effective therapies for AMD would therefore be hugely valuable, not just for patient benefit but also to reduce financial burden.

AMD is a progressive disease of the macula, characterized by atrophy of the retina due to the degeneration of the light sensitive photoreceptor cells. The onset of AMD is caused by damage to the retinal pigment epithelium (RPE), which is a monolayer of highly specialized cells located posterior to retinal photoreceptor cells and anterior to Bruch's membrane (BrM) and the choroid. The RPE acts as an interface between the retina and choroid, and tight junctions connecting neighboring RPE cells contribute to its polarization and role in maintaining the blood-retinal barrier.[5] This prevents the diffusion and transport of non-specific material from the choroid whilst selectively transporting essential nutrients required by the photoreceptors for visual function.

A heathy RPE is essential for retinal homeostasis as it lends functional and metabolic support to the overlying photoreceptors. This is facilitated by numerous microvilli on the apical surface of the RPE, surrounding and interacting with each photoreceptor to allow maximum surface area contact.[6] Close interaction is essential for the RPE to carry out its multiple functions which include the removal of metabolic waste from the retina, protection of the retina from stray light, control of water and ion flow, replenishment of visual pigments for photoreceptors and phagocytosis of spent photoreceptor outer segments (POS).[6, 7] The latter of these functions is perhaps the most metabolically challenging as each RPE cell is estimated to phagocytose approximately 300 million POS discs in a lifetime.[8] All of these

functions however are essential for photoreceptor survival, and thus any damage to the RPE can have severe consequences for vision.

As we age, the RPE becomes fatigued due to oxidative damage and changes occur in the composition of BrM.[9, 10] These changes may be associated with an unbalanced regulation of ECM components by the ageing RPE,[11, 12] resulting in increased level of BrM proteins combined with overall remodelling of the extracellular matrix.[13-16] The most prominent change in composition of the BrM is the accumulation of lipoproteins, which have been secreted by the RPE.[17-20] This is thought to generate a lipid barrier within BrM,[21, 22] leading to a decrease in permeability and impaired metabolic exchange between the photoreceptors, RPE and choroid.[23, 24] Consequently, waste products, termed 'drusen', can accumulate within and around the RPE in the macular region.[25, 26] Drusen are considered the hallmark for AMD and can be relatively harmless when small in number and size. However, excessive drusen accumulation, and specifically the presence of large soft drusen in the macula, is considered a major risk factor of AMD[27, 28] and thought to promote inflammation.[29] Over time, drusen accumulation and chronic inflammation results in degeneration of the RPE and choroid, preventing essential maintenance of the photoreceptors and ultimately leading to photoreceptor cell death and irreversible blindness.[30, 31] This type of AMD is termed dry AMD. However, approximately 10-15% of patients with dry AMD can also develop neovascular or wet AMD, which occurs when an excessive amount of vascular endothelial growth factor (VEGF) is released by the RPE as a result of degeneration and inflammation,[29] triggering the uncontrolled growth of new blood vessels. Due to the existing age-related changes in the RPE/BrM, these new vessels can break through into the RPE and retina causing disruption and damage. In addition, these new vessels are fragile and leaky and have the potential to develop into retinal bleeds which can cause the physical displacement of the

RPE and retinal layers. This can lead to a rapid deterioration in vision due to resulting photoreceptor cell death (Figure 1).

There is currently no curative treatment for AMD, and only patients with the wet form of AMD can receive treatment to stabilize the disease. This involves regular injections with anti-VEGF drugs to reduce neovascularization and prevent further fluid accumulation, which has been shown to stabilize and indeed improve vision in the majority of patients.[32, 33] However, anti-VEGF treatment does not prevent disease progression already in motion, and RPE cells that have already been damaged or lost will inevitably result in neighboring photoreceptor cell death. Furthermore, patients receiving anti-VEGF treatment may be susceptible to developing mechanisms of resistance, rendering the therapy ineffective.[34] New treatments are therefore sought after and recent advances in stem cell research and cell therapies offers the opportunity and potential to treat AMD by replacing the damaged layers of the macula.

RPE dysfunction and degradation is central to AMD pathogenesis and therefore an obvious target when considering cellular therapies. It is also the most straightforward layer of cells to generate and replace given its monolayer structure in comparison to the retinal network or underlying vasculature. Generating stem cell-based RPE cells suitable for transplantation has therefore been the focus of a growing number of research groups over the past decade.

Stem cell-derived RPE production in clinical trials

Since the first RPE transplantations three decades ago,[35, 36] tremendous progress has led to the point where we are now pioneering stem cell-based RPE grafts in humans.

Autologous harvesting of RPE from adjacent layers in the patient's retina has proven technically difficult and of poor clinical efficacy. Indeed, proof of principle studies have long-looked for alternative sources of RPE.[37]

Traditionally, alternative RPE sources used for transplantation were of primary and fetal origin,[35, 36] followed by transformed RPE lines[38] and later by tissues from non-RPE origin as diverse as iris or bone marrow.[39-42] However, none of these have proven to meet the clinical demands of a growing AMD patient population, which would require abundant and readily available RPE cells for transplantation.

A promising cell source that lifts the constrains of primary tissues by potentially providing unlimited and accessible RPE cells has been found in human pluripotent stem cells (hPSCs). Pluripotent stem cells have the potential to grow indefinitely *in vitro* as undifferentiated stem cells, while retaining the ability to specialize into any cell type.[43] They are either derived from the inner cell mass of the human blastocyst (Embryonic stem cells, hESCs) or by genetic reprogramming of somatic cells (induced pluripotent stem cells, hiPSCs) (Figure 2).[43, 44]

Since their initial isolation in 1998, hPSCs have been successfully differentiated *in vitro* into almost any cell type, from hepatocytes to neurons, often after complex protocols. However, thanks to the intrinsic nature and default developmental pathways of hPSCs, the differentiation into RPE cells has been an almost serendipitous process. Indeed, hPSCs spontaneously differentiate into pigmented RPE *in vitro* upon removal of basic fibroblast growth factor (bFGF) from the self-renewing growth media.[45-52]

It is perhaps not surprising that RPE cells were one of the first cell type to be derived from hPSCs and are now leading the way in clinical trials with variations of the spontaneous-based differentiation protocol. Spontaneous differentiation into functional RPE is initiated by bFGF withdrawal from the media of confluent 2D culture of hESCs or hiPSCs. A variation to this step is the formation of hESCs-derived 3D embryoid bodies that then set down in the dish prior the differentiation.[53] Pigmented foci of RPE cells emerge amongst other differentiated cells after 3 to 5 weeks (Figure 2).[52] These RPE foci are visible with the naked eye and can be isolated via manual dissection and seeded into a smaller vessel on top of an extracellular matrix substrate. Over the next several weeks in the protocol, such purified cells reach a confluent monolayer with typical RPE phenotype: presence of cobblestone epithelial morphology, tight junctions, apical polarity, microvilli, pigmentation, metabolic maturity with secretion of PEDF/VEGF and positivity to RPE-specific markers.[52] At this stage, depending on the specific transplantation strategy, the matured RPE cells are further grown on a selected scaffold or substrate prior isolation for injection (see also chapter 3 for injection methods).

Although the original spontaneous method remains the gold standard and the one approved for clinical-grade RPE production, the issues of low efficiency and extended culture have driven research to more directed methods that could be beneficial in clinical manufacturing. Indeed, there is now a plethora of research protocols for step-wise and developmentally guided hPSCs-derived RPE differentiation. RPE differentiation has been improved via the combinatorial manipulation of the TGF, BMP, FGF/MAPK and Wnt signaling pathways.[54-60] Furthermore, inhibition of Rho-associated kinase (which plays crucial roles in anoikis, cell-cell contact and other cellular functions), has been shown to improve RPE efficiency via increased survival and maintenance of the RPE epithelial phenotype.[58, 61] Finally, several

groups are generating RPE within a 3D retinal organoid, although problems with protocol robustness, cellular maturity and sheer stress are currently limiting this method to research or high-throughput screenings rather than the clinic. [62]

Cellular identity, maturity, functionality and safety are all key components in the development of an efficacious cell replacement therapy.[63, 64] Part of the clinical appeal of hPSCs-derived RPE is that they appear to resemble naïve RPE in terms of molecular signature and functionality, which is crucial to structurally and metabolically support the photoreceptors and ultimately treat AMD. [51, 52, 65, 66]

Another important issue when dealing with any allogenic cell source remains that of immunocompatibility, which is especially critical for PSC-based cell therapies, where the benefits of long-term engraftment would be out-balanced by the risks posed by an indefinite immunosuppression in the context of chronic rejection.[67, 68] HESC and their progeny are highly immunogenic, and studies in genetically identical mice showed that even a single Mayor Histocompatibility Complex class I gene difference induced some type of rejection. [69] In view of the immunogenicity of hESCs, huge efforts have been placed on the development of banks of HLA-typed hESCs required to achieve partial or full matching between donor and recipient for a large percentage of the population.[70] However this goal remains elusive, as current clinical-grade hESCs banks are insufficient to cover even national populations, due to the scarcity and preciousness of the sourcing embryonic material. Until this is achieved, an immunosuppression regime will likely have to be in place in hESCs-derived RPE replacement therapy, despite the eye being an immune-privileged site.

On the other hand, using hiPSCs as pluripotent source could overcome this challenge. In essence, hiPSCs are generated from the patient's own cells, therefore the RPE derivatives possess the exact genetic background of the patient, and complete immunocompatibility should be expected.[67] Although deriving an hiPSCs line for each patient requires longer and expensive methods compared to readily available allogenic hESCs lines, carefully selected super-donors, or HLA engineering[71], could make the generation of a global HLA-typed hiPSC bank a possibility.[70, 72] However, the use of hiPSCs is not without hurdles. Notably, albeit still controversial, it has been proposed that reprogramming might lead to re-expression of early developmental antigens for which self-tolerance is not established, posing issues of autoimmune response for autologous hiPSC transplantation.[68]

Even more pressing are the concerns around the genetic stability of the cell lines, since the reprogramming itself can pose risks on the genetic and epigenetic integrity of the hiPSCs, which are not all fully understood.[73, 74] As clear example of this concern, a recent clinical trial with hiPS-derived RPE was halted in one patient due to the detection of DNA copy number aberrations in chromosome X of the transplantable cells.[75] Indeed, the initial protocols for the generation of iPSCs involved retro-viral induction of key transcription factors including the oncogene c-Myc, which raises concerns associated with genome integration and tumorigenicity in terms of clinical use.[76] Research in the field of iPSC production has therefore been heavily focused on developing non-integrating and oncogene-free reprogramming platforms.[77] The genomic integrity of hiPSCs is surely one of their biggest clinical challenges, but one also shared by hESCs. Indeed, it is known that a proportion of hESCs acquire genetic abnormalities in culture, some involving known oncogenes.[78, 79] While the scientific community is working on the evaluation of the effects and risks associated with the genetic instability of pluripotent stem cells, the safety of hPSCs-derived therapies could

be enhanced by the use of established cancer-associated genetic tests on the hPSCs and their differentiated progeny.[79]

Methods of RPE transplantation

The choice of cell source and methods to generate bona fide RPE is but one challenge facing cell therapeutics for AMD. Equally critical is the decision regarding the delivery of the RPE into the eye.

From a surgical perspective, the subretinal injection of RPE is a delicate technique that can potentially lead to traumatic injuries to the eye and the retina. Serious complications may arise such as the induction of retinal detachment, large choroidal hemorrhages or RPE reflux which could lead to visual loss.[80] However, on the back of years of autologous and fetal RPE transplantations in both pre-clinical as well as human trials,[37] two major delivery system are leading the way in clinical trials for allogenic RPE transplantation: a "bolus" or a "patch" injection system (Figure 3).

Bolus injection

With the bolus injection method, matured RPE grown *in vitro* are detached and collected as a cell suspension at the end of the differentiation protocol and stored in frozen vials to be thawed prior transplantation. The surgeon then performs a pars plana vitrectomy followed by a retinotomy in the small affected area over the patient's macula.[53] The RPE cell suspension is then injected as a bolus of cells with a surgical cannula in the sub-retinal space of the macula (Figure. 3).

This technique is relatively simple compared to other types of transplantations, especially autologous, where a large retinal detachment is required in order to collect the RPE graft.[37]

However, there are several draw-backs to this delivery system. Some are associated with the vitreoretinal surgery itself, such as endophthalmitis infections or in more severe cases loss of vision with a rate of 2.5-3.3%.[81] More specific concerns with the bolus injection are the noted risks of reflux of RPE cells into the pre-retinal space[53] and the stress damage caused to cells when released by the cannula.[82]

Finally, a bolus injection raises biological issues linked with the RPE cells being in the form of a dissociated cell suspension.[83, 84] Physiologically, RPE cells are a polarized epithelial monolayer sitting above the Bruch's membrane. When grown *in vitro*, RPE also form a polarized monolayer but upon dissociation undergo de-differentiation and epithelial-to-mesenchymal transition (EMT) followed by a gradual re-epithelialization (MET).[85] Therefore, a bolus injection at transplantation does not replicate the physiologically polarized RPE monolayer and has been associated with poorer graft survival due to lack of proper monolayer morphology.[83, 86] Nevertheless, injection of an RPE suspension has shown to be a straightforward surgery, be well tolerated and lead to a pigmented graft in humans.[53]

Patch injection

This system is based on the delivery of an hESC-derived RPE monolayer, which can be sitting on a biocompatible scaffold or as a stand-alone sheet (Figure 3). The RPE patch allows the transplantation of a polarized, oriented and matured RPE monolayer, ready to replace the damaged RPE/Brunch's membrane without disruption of the epithelial morphology. With a patch implantation, the RPE are not only in the appropriate orientation, but there is less risk of them migrating away from the graft. Therefore, the use of a patch as alternative to dissociated RPE is surgically but also conceptually different. Indeed, a patch transplant aims to replace both the lost RPE monolayer as well as the damaged Bruch's

membrane by facilitating the graft integration within an already compromised disease microenvironment.[84]

Scaffolds

Although in essence similar, there are currently several biocompatible scaffold systems in clinical trials for growing and transplanting an RPE patch into the macula.

Nonbiodegradable substrates work on the principle that the RPE graft will permanently consist of the polarized RPE monolayer on top of a scaffold that replaces the diseased Brunch's membrane. Mimicking the Brunch's membrane properties, the scaffold must be permeable to soluble factors from the underlying choroid vessels and overall supports the RPE adhesion, polarity and metabolism.

Currently, two clinical trials are testing nonbiodegradable scaffolds for AMD. The London Project to Cure Blindness has piloted an hESC-derived RPE sheet grown on a biostable polyethylene terephthalate (PET) membrane, creating a 6mm x 3mm patch.[87] On the other hand, the University of Southern California Eye Institute is testing a hESCs-derived RPE monolayer grown on an ultrathin parylene substrate, creating a 6.25mm x 3.5mm patch.[88] Both types of patch are delivered after a pars plana vitrectomy and a small retinotomy over the macula region.[87, 88] Thanks to the sturdiness of those synthetic membranes, the patches are robust enough to be handled by the surgeon at the end of a custom-made delivery tool or insertion forceps. (Figure 3).

Next-generation scaffolds based on biodegradable polymers are currently been tested in pre-clinical studies by the National Eye Institute and the National Institutes of Health (Bethesda,

MD, USA). Here, the substrate is made of Poly-lactic-co-glycolic acid (PLGA), a polymer with shape-memory properties that will slowly dissolve after surgery, leaving the above RPE monolayer in contact with the original Brunch's membrane.[89, 90]

Another patch strategy, and the only one which used autologous iPSCs-derived RPE in a clinical trial, utilizes an RPE patch made without an artificial scaffold.[75, 91] Firstly, the RPE monolayer is grown on a type I collagen gel as temporary scaffold inside a Transwell insert. Later in the protocol, the collagen is enzymatically dissolved and the RPE sheet cut from the insert, resulting in an RPE sheet without a basement substrate. At the final stage, the RPE sheet is cut into strips of 1.3mm x 3mm in size ready for surgery.[91]

Although there isn't yet a consensus around the best delivery system for allogenic RPE grafts, both the bolus and patch transplantation methods described above are showing promising results in PhaseI/II clinical trials in terms of safety and feasibility. Future efficacy and long-term trial studies will inform us on the most effective and safe delivery method for RPE engraftment, one that ultimately will have to correlate with the best success on visual acuity restoration in AMD.

Clinical Outcomes

Initial safety studies have showed that a bolus injection of hESC-RPE into the subretinal space of patients is well tolerated.[92] In this study, two patients were treated, one with advanced dry AMD and another with Stargardts disease. Patients were immunosuppressed with tacrolimus and mycophenolate for one week prior to surgery and six weeks following surgery, followed by mycophenolate only for a further six weeks. For the procedure itself, a dissociated suspension of 50,000 viable RPE cells was delivered to the pericentral macular

region. This site was chosen by using optical coherence technology (OCT) to detect an area with compromised RPE and photoreceptors, which had not been completely lost, to increase the likelihood of integration. There was no signs of rejection or immune reactions detected in the four months following surgery, and no evidence of tumor formation. This was an important clinical trial as it provided the first evidence that hESC-RPE could be a safe source of cells for the treatment of AMD. A follow up study with a larger patient group, nine patients with advanced AMD and nine with Stargardts disease, tested a range in cell doses (50,000 – 150,000 cells/eye). Although there was no evidence of serious adverse events in terms of rejection or safety, there were systemic adverse events likely associated with immunosuppression and postoperative complications that were consistent with pars plana vitrectomy surgery for macular disorders. Despite this, increased pigmentation was observed in the treatment area consistent with transplanted RPE. Further to this, patients with AMD showed an 11-15 letter improvement in best corrected visual acuity (BCVA) in six out of the nine patients, with no change in the remaining three.[53]

More recently, clinical results have been published from the hPSC-RPE patch transplantation method. One such clinical trial has transplanted a hESC-RPE monolayer using an ultrathin parylene membrane as a scaffold into four patients with advanced dry AMD.[88] Patients were immunosuppressed with a treatment of tacrolimus for 8 days prior to surgery and 60 days after. There was no detection of graft rejection or worsening of vision following transplantation and hESC-RPE integration with host photoreceptors was evident. Further there was no unanticipated severe events in response to the surgery, patch or immunosuppression only an anticipated adverse event involving a subretinal hemorage possibly related to the surgery. Researchers also reported an improvement in BCVA of 17

letters in one patient and an improvement in fixation (the ability of a subject to fixate on a specific location) in two patients.

A similar and simultaneous clinical trial has taken place involving the transplantation of hESC-RPE using a vitronectin coated polyethylene terephthalate (PET) membrane.[87] Similar to the trials mentioned previously, subjects received perioperative immunosuppression with prednisone but also accompanied by intravitreal implants of fluocinoline acetonide allowing a sustained release local to the surgical site. The two patients treated in this trial presented with an acute form of wet AMD and experienced a subretinal bleed and recent rapid vision decline within six weeks of surgery. These criteria suggest the existence of viable photoreceptors and a 12 month follow up showed that both patients displayed a significant improvement in visual acuity (BCVA of 29 and 21 letters) as well as visual fixation at the center of the patch. Another important result from this trial was the observed increase in reading speeds for both patients (zero – 48 and 83 words per minute respectively), an improvement not reported in the other trials, which provides evidence of viable photoreceptors in the central macula. There were three serious adverse events observed in this study which were unrelate to the patch. One of which was a related to immunosuppression and the remaining two likely due to the surgery itself.

The first and only hiPSC-RPE transplantation to date involved the treatment of chronic wet AMD using a substrate free sheet of RPE.[75] Although there was no change (neither improvement or deterioration) in visual acuity, a 12 month follow up revealed that the transplanted sheet remained intact and there was no sign of graft rejection, despite the lack of immunosuppression. A second patient was enrolled for this trial but was not treated due to concerns with the genetic changes in their iPS cells, and also due to this patient still showing

a moderate response to anti-VEGF treatment. Further enrolment in this trial was prevented in 2015 due to a change in Japanese Regenerative Medicine law. A recently published follow up shows four-year survival of the graft and evidence of functionality in the transplanted RPE.[93]

Conclusions

The combined results from these initial clinical trials (Figure 4) are encouraging in terms of safety and efficacy and indicate that pluripotent stem cell-derived RPE may be a safe and viable option for the treatment of AMD. However, as highlighted in this review, a number of scientific and clinical challenges still have to be addressed in this upcoming field. In order to successfully treat a growing AMD population with hPSC-derived RPE, focus will have to be placed on optimizing the most effective and safe protocol to generate RPE (iPSC vs hESC) with specific consideration of the genetic stability and HLA matching of the source and differentiated cells. Further to this, decisions will need to be made regarding the most efficient treatment system (bolus vs patch), bearing in mind the effects of the delivery method in terms of surgery complications and the survival of the transplant. Until the challenges associated with HLA-typed pluripotent stem cells are addressed, it is key to consider the type of immunosuppression regime applied and the whether there is a possibility of reducing the related systemic adverse events. Finally, identifying the best patient cohort for transplantation in order to achieve maximal visual recovery and consistency between results will become even more critical as AMD cell therapies move forward in clinic. Indeed, it is important to keep in mind when summarizing these treatments that we are currently targeting different stages of disease using different therapies. Given the nature of AMD and the loss of functional photoreceptors at the center of the macula at the late stages of the disease, it is crucial to know what level of photoreceptor cell rescue can be achieved when targeting advanced AMD (Figure 4). Ultimately, the extent to which central vision, paramount to

patient's quality of life, can be restored will be dictated by the level of photoreceptors survival at time of surgery.

The inception of hPSC-based therapies brings promise to diseases such as AMD. With current progress in stem cell research, it might be that we are looking at replacement of more complex retinal layers sooner than we think. For now, the successful transplantation of hPSC-derived RPE paves the way for scientists to explore cellular replacement therapies for AMD on a larger scale, with the knowledge that these treatments have the potential to be safe and well tolerated.

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Declaration of interest statement

The authors report no conflict of interest.

FIGURE LEGENDS

Figure 1.

Schematic representation of healthy and diseased retinas with respect to AMD. In the healthy (aged) retina (panel 1), communication between the RPE monolayer, the light sensitive photoreceptors and the choroid is essential for visual function. The apical side of the RPE contains numerous microvilli which engulf the outer segments of the photoreceptors allowing maximal surface area contact. This enables the RPE to efficiently maintain photoreceptor homeostasis. In early to intermediate stages of dry AMD (panel 2), accumulation of drusen causes disruption to the RPE resulting in inflammation, capillary loss and a breakdown in retinal homeostasis. In 10-15% of patients with AMD, an aged BrM together with inflammation can initiate neovascularization in and around the RPE and retina (wet AMD, panel 3). New fragile blood vessels can leak fluid and blood in the subretinal space, leading to a breakdown in retinal adhesion and degradation of the RPE/photoreceptor cell layers. Advanced stages of either dry or wet AMD (panel 4) will eventually result in the complete degradation of the RPE and photoreceptors and loss of central vision.

Figure 2.

Schematic overview of the original spontaneous differentiation protocol to generate functional RPE starting from either hESCs or hiPSCs. HESCs are isolated from the inner cell mass of the human blastocyst while hiPSCs are derived with genetic reprogramming (here illustrated the original Yamanaka's transcription factors) of somatic cells, such as fibroblasts. Pluripotent stem cells from both cell sources are able to spontaneously generate pigmented RPE foci after

bFGF withdrawal from the growth media. Pigmented foci are then isolated to obtain a pure monolayer that displays the characteristic phenotype of mature RPE.

Figure 3.

Diagram representation of the two main RPE delivery strategies currently in clinical trials: a bolus or patch system. The starting point is a population of hESCs or hiPSCs-derived RPE, produced with a spontaneous differentiation protocols and showing RPE characteristics and phenotype. With the bolus system, previously differentiated RPE are thawed as dissociated cell suspension before surgery. The RPE bolus is injected via a cannula in the subretinal space after a pars plana vitrectomy and retinotomy over the macula. With the patch system, a sheet of matured RPE monolayer (generally on a scaffold substrate) is cut in the final size just before being transplanted. Pars plana vitrectomy and macular retinal detachment is performed and the retinotomy enlarged and irrigated to remove all blood. The patch is then inserted with a delivery custom-made tool or forceps in the subretinal space.

Figure 4.

Schematic representation of the current PSC-derived RPE cell therapies for AMD in clinical trial. Schwartz et al[53, 92] (panel 1) have used a bolus injection of hESC-RPE cells in suspension in advanced dry AMD. Transplanted cells were shown to collect at the periphery of the atrophic region. In a different trial led by Mandai et al[75] (panel 2), hiPSC-RPE were grown on collagen gels allowing basement membrane deposition and the formation of a mature RPE monolayer. Before transplantation, collagenase was used to digest the underlying gel leaving only the hiPSC-RPE and its basement membrane. RPE sheets were cut into strips of 1.3mm by 3mm before injecting into the sub-retinal space of a patient with chronic wet AMD. The first treatment of patients with acute wet AMD was carried out by da Cruz et al[87]

(panel 3) and involved generating a patch of matured functioning hESC-RPE grown on a vitronectin coated polyethylene terephthalate (PET) membrane. The patch measured 6mm by 3mm which is almost large enough to cover the entire macula. Due to the rapid decline in vision and the 6-week timeframe in which the patients were treated, the majority of the photoreceptors are presumed viable. In a different trial again by Kashani et al,[88] patients with advanced dry AMD were treated with a patch of matured hESC-RPE grown on a synthetic parylene-C membrane. These cells have been polarized *in vitro* and are therefore transplanted as a fully functioning RPE monolayer. The size of the patch measured 3.5mm by 6.25mm, again large enough to cover the entire macula. In both advanced dry and chronic wet cases, photoreceptors are presumed to be dead or dying due to the timeframe (months rather than weeks) in which the patients were treated.

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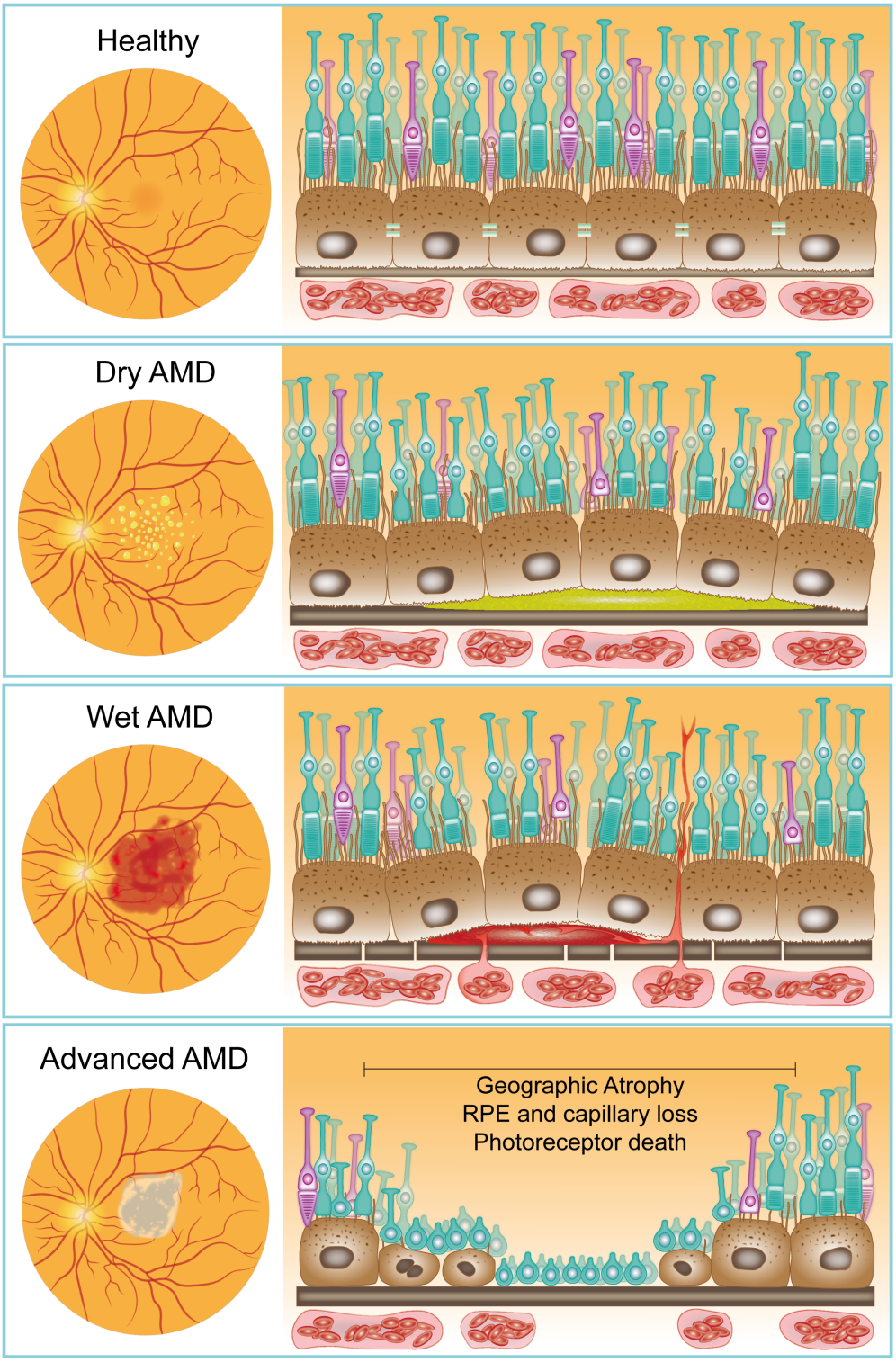
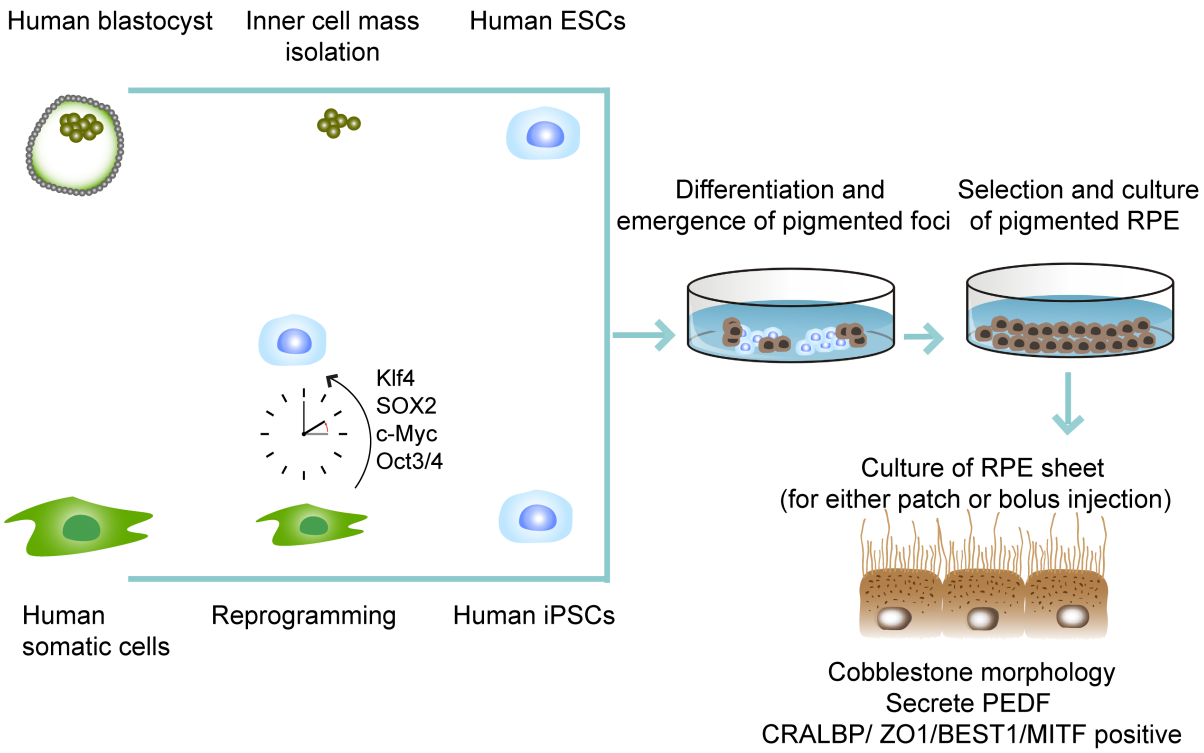
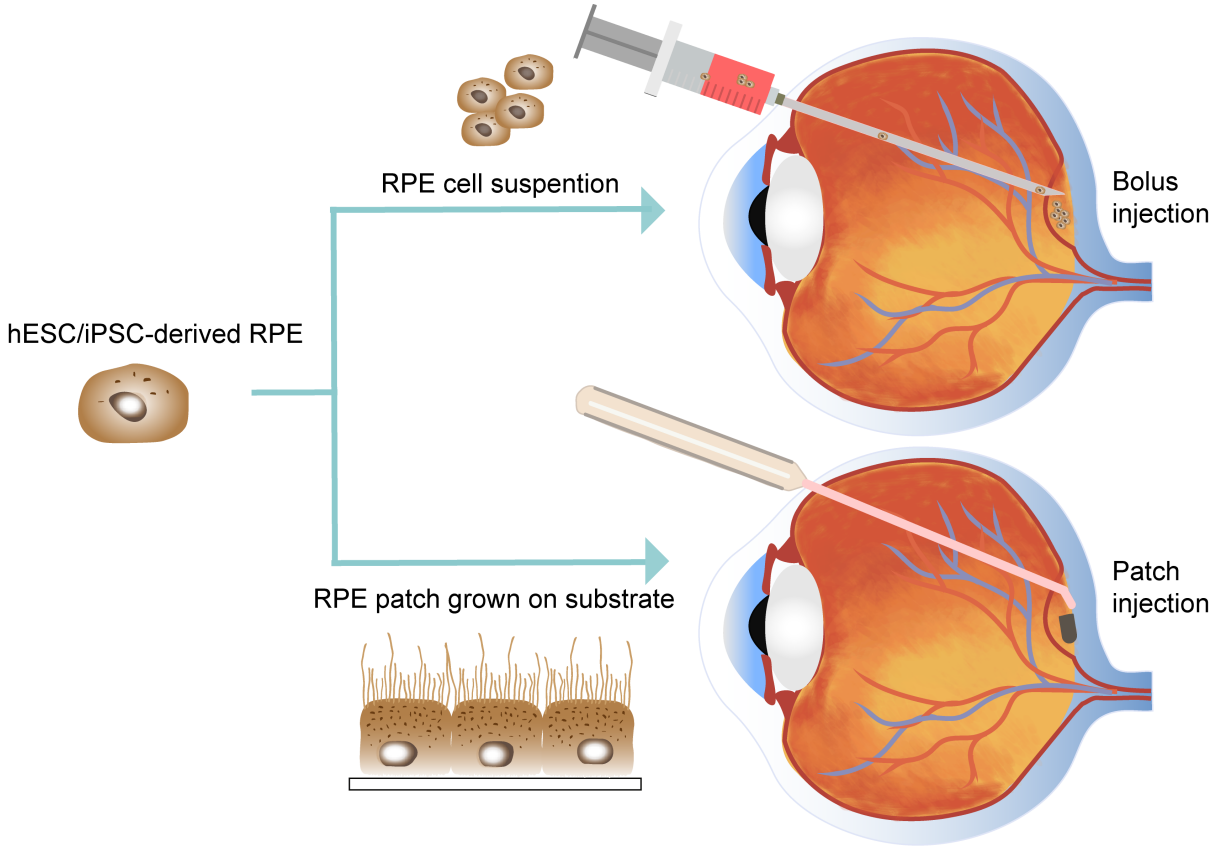
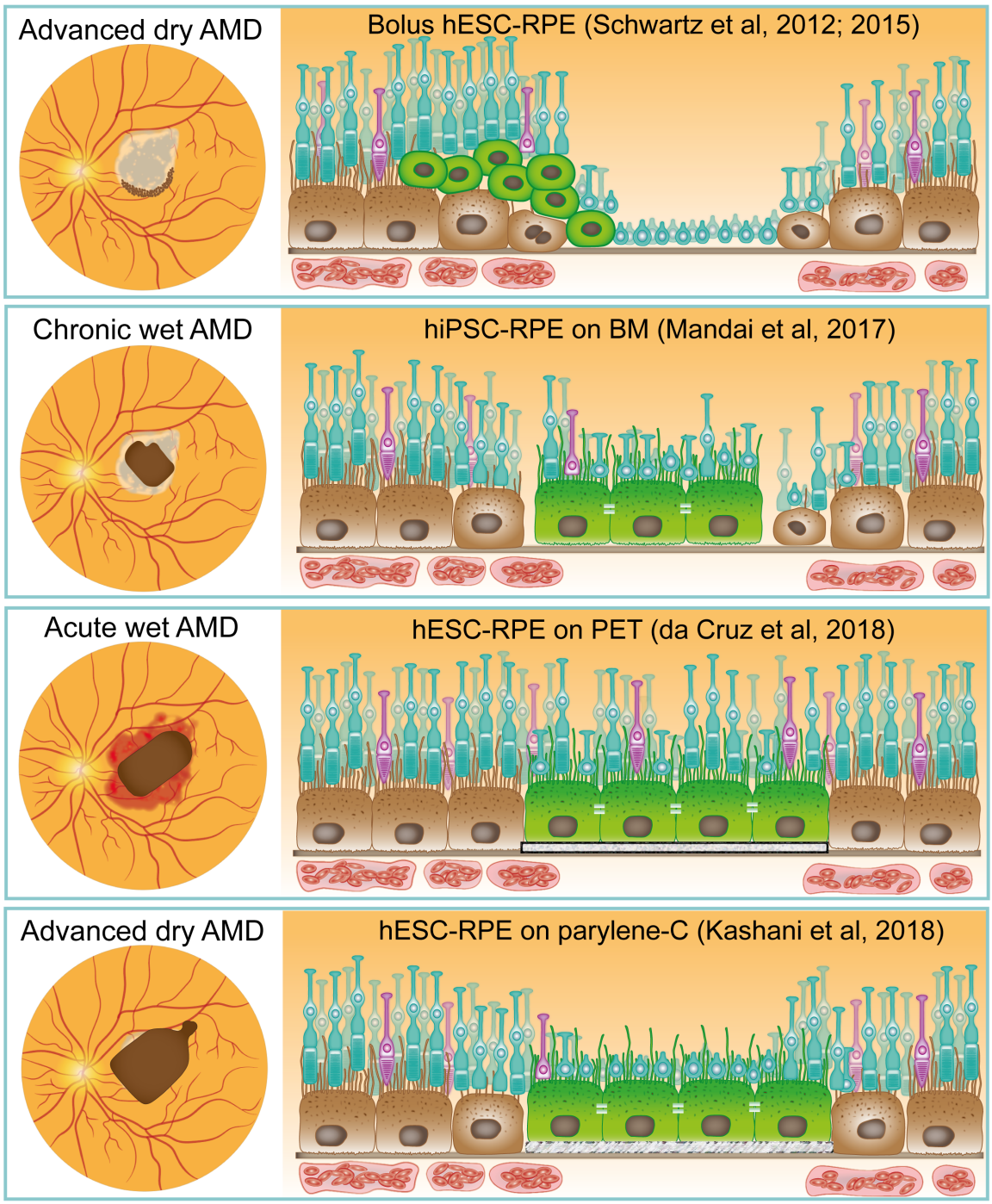

Healthy
Dry AMD
Wet AMD
Advanced AMD
Geographic Atrophy
RPE and capillary loss
Photoreceptor death
Healthy RPE
Dying RPE
Tight Junction
Bruch's membrane
Healthy rod (blue) and cone (purple) photoreceptors
Capillary
Dead and dying photoreceptos

Cell source


Human blastocyst
Inner cell mass isolation
Human ESCs
Differentiation and emergence of pigmented foci
Selection and culture of pigmented RPE
Klf4
SOX2
c-Myc
Oct3/4
Culture of RPE sheet
(for either patch or bolus injection)
Human somatic cells
Reprogramming
Human iPSCs
Cobblestone morphology
Secrete PEDF
CRALBP/ ZO1/BEST1/MITF positive


hESC/iPSC-derived RPE
RPE cell suspention
RPE patch grown on substrate
Bolus injection
Patch injection


Advanced dry AMD
Bolus hESC-RPE (Schwartz et al, 2012; 2015)
Chronic wet AMD
hiPSC-RPE on BM (Mandai et al, 2017)
Acute wet AMD
hESC-RPE on PET (da Cruz et al, 2018)
Advanced dry AMD
hESC-RPE on parylene-C (Kashani et al, 2018)
Healthy RPE
RPE Transplant
Dying RPE
Scaffolds
Healthy rod (blue) and cone (purple) photoreceptors
Capillary
Patch
Bolus injection
Tight junction
Bruch's membrane
Dead and dying photoreceptos