

Chapter 76

Stem Cell-Based RPE Therapy for Retinal Diseases: Engineering 3D Tissues Amenable for Regenerative Medicine



Karim Ben M'Barek, Walter Habeler, and Christelle Monville

Abstract Recent clinical trials based on human pluripotent stem cell-derived retinal pigment epithelium cells (hPSC-RPE cells) were clearly a success regarding safety outcomes. However the delivery strategy of a cell suspension, while being a smart implementation of a cell therapy, might not be sufficient to achieve the best results. More complex reconstructed tissue formulations are required, both to improve functionality and to target pathological conditions with altered Bruch's membrane like age-related macular degeneration (AMD). Herein, we describe the various options regarding the stem cell source choices and the different strategies elaborated in the recent years to develop engineered RPE sheets amenable for regenerative therapies.

Keywords Human pluripotent stem cell · Human-induced pluripotent stem cell · Human embryonic stem cell · Stem cell · Tissue engineering · Retinal pigment epithelium · Age-related Macular degeneration · Retinitis pigmentosa · Cell therapy · Regenerative medicine

K. Ben M'Barek · W. Habeler
INSERM UMR861, I-Stem, AFM, Corbeil-Essonnes, France

UEVE UMR861, I-Stem, AFM, Corbeil-Essonnes, France

CECS-I-Stem, AFM, Corbeil-Essonnes, France

C. Monville (✉)

INSERM UMR861, I-Stem, AFM, Corbeil-Essonnes, France

UEVE UMR861, I-Stem, AFM, Corbeil-Essonnes, France

e-mail: cmonville@istem.fr

76.1 Introduction

The retinal pigment epithelium (RPE) has a pivotal role in maintaining photoreceptor survival, integrity, and functionality (Strauss 2005; da Cruz et al. 2007; Ben M'Barek et al. 2015). RPE cells organize as monolayered epithelium in tight contact with the outer segment of photoreceptors (Strauss 2005). Dysfunctions or degeneration of RPE cells is associated with AMD and retinitis pigmentosa (RP). These diseases that might ultimately lead to blindness are estimated to affect about 30 millions of people worldwide (Gehrs et al. 2006). In addition, AMD disease burden will dramatically increase with a projection of 288 millions of people to be affected by 2040, due to prolonged life expectancies (Wong et al. 2014).

RP is a heterogeneous group of rare diseases caused by diverse genetic mutations (<https://sph.uth.edu/retnet/disease.htm>). Some of these mutations affect specifically RPE functions like the mutations in *MERTK*, *RPE65*, *CRALBP*, *BEST1*, or *LRAT* genes (Sparrow et al. 2010). *MERTK*, *RPE65*, *CRALBP*, and *LRAT* mutations correspond to about 5% of autosomal recessive RP (Hartong et al. 2006), and a prevalence of 1.5 case in 100,000 births for *BEST1* gene mutations was estimated (Bitner et al. 2012). At the opposite, AMD is a complex pathology caused by the combination of genetic and environmental factors (Ambati et al. 2003). Two forms of AMD are typically described: a dry form (atrophic) that account for 90% of all cases and a wet form (neovascular). RPE degeneration and damages of the Bruch's membrane associated with the formation of protein and lipid deposits, called drusen, are characteristics of the dry form. Wet AMD involves choroidal neovascularization with potential hemorrhages that are susceptible to damage the macula (Ambati et al. 2003).

Treatment options for AMD and RP are extremely limited (Gehrs et al. 2006). Only wet AMD benefits from the recent advance of anti-angiogenic treatments (anti-VEGF therapy). In light of this unmet medical need, regenerative medicine appears as an attractive therapeutic alternative to cure patients.

76.2 Stem Cell-Based RPE Production

Replacement of dead/dysfunctional RPE cells by new ones is a strategy that was first proposed in early 1980s (Gouras et al. 1985). Initially, RPE sources consisted of autologous RPE from the periphery, allogeneic RPE from cadavers, or fetal RPE (Binder et al. 2007; Radtke et al. 2008). These sources have a lot of limitations in terms of supply chain, donor to donor variability, and scale-up (limited cell amplification). With the emergence of hPSCs, efforts are now directed to stem cells sources (Thomson et al. 1998; Takahashi et al. 2007).

76.2.1 Sources

Three main sources for stem cell therapy are preferred regarding the following criteria: ability to differentiate into RPE cells, ease of culturing, and suitability for a clinical application.

The first one is the recently described human adult RPE stem cells (RPESCs) (Salero et al. 2012). About 2–3% of human RPE cells, harvested from human eye cadavers, could self-renew in vitro, a prominent characteristic of stem cells. Besides their capacity to differentiate into other lineages, they are able to generate new RPE cells that keep their epithelial morphology after transplantation (Stanzel et al. 2014). This source remains an option that will be tested in clinical trials but amplification and isolation remain difficult.

The second stem cell source is based on human embryonic stem cells (hESCs), which were first derived in 1998 (Thomson et al. 1998). These cells retain pluripotency, meaning that they can be differentiated in any cell type of the adult body (including RPE cells) if the required signaling cues are provided. In addition, they can be amplified for a virtually unlimited number of passages, allowing the possibility of industrialized manufacturing processes.

Human-induced pluripotent stem cells (hiPSCs) represent the third stem cell source (Takahashi et al. 2007). While not being completely identical, hiPSCs share the same pluripotency and self-renewal potentials than hESCs (Wu et al. 2016). hiPSCs differentiated into RPE cells are functional in vivo and restore visual functions of rodents with dystrophic retinas (Kamao et al. 2014) to a similar level than hESC-RPE (Riera et al. 2016).

76.2.2 Protocols for the Generation of RPE from hPSCs

hPSCs (hESC and hiPSC) are able to spontaneously differentiate into RPE cells in adherent cultures after FGF2 withdrawal (Klimanskaya et al. 2004; Lustremant et al. 2013; Leach and Clegg 2015). Protocol improvements were undertaken to reduce the culture duration or to improve the yield. Purity of RPE cells can be achieved by manual selection or using growth factors/small molecules (Bharti et al. 2011; Leach and Clegg 2015). These hPSC-RPE cells display characteristics similar to primary fetal RPE cells. In addition, hPSC-RPE cells could also be obtained concomitantly with the formation of self-forming neural retinas in vitro (Meyer et al. 2011; Reichman et al. 2014).

76.2.3 Manufacturing Strategy

From an industrial perspective, clinical hPSCs should be manufactured in a good manufacturing practice (GMP) and compliant establishment and preserved as frozen cell banks (either a unique master cell bank (MCB) or a MCB and derived working cell banks). This MCB has to be characterized based on regulatory standards that include safety analysis (virology, fungi, mycoplasma, bacteria, and endotoxins), purity, potency (embryonic body formation and alkaline phosphatase activity), and stability. From this MCB of hPSCs, a bank of hPSCs-RPE is derived and banked according to GMP principles. Cryovials of this qualified hPSC-RPE bank could be sent straight to the surgical rooms in order to be thawed and transplanted as a cell suspension without prior additional culture in vitro (Schwartz et al. 2012, 2015). This formulation represents the most practical implementation for the handling and delivery to the surgical site with a simplified logistic. A formulation as a reconstructed epithelial sheet might require an additional culture period of hPSC-RPE cells from the bank in order to organize them as an epithelium. The graft should then be transferred in a closed and sterile packaging to the surgical room. The stability of the graft is therefore limited in time, and scheduling should be carefully prepared to have the graft ready for the day of the surgery. To overcome this limitation, freezing steps might be necessary.

76.3 Cell Delivery Strategy: Cell Suspension Versus Reconstructed Tissue

The formulation remains a crucial aspect depending on the nature of the pathology in particular when the Bruch's membrane is altered. First approaches were based on cell suspension, but the field has moved to a more elaborated tissue formulation. The idea is that safety and clinical outcomes might be better as RPE functions depend also on their polarized epithelial organization (Stanzel et al. 2014; Hsiung et al. 2015; Nazari et al. 2015). In addition, the survival of the transplanted cells was improved when transplanted as a cell sheet (Diniz et al. 2013). This paradigm was recently demonstrated to improve the functionality of the graft in vivo in animal models (Ben M'Barek et al. 2017).

76.4 Types of Support for Tissue Reconstruction

The delivery of an already formed epithelium requires the use of a supportive matrix allowing the sheet to be safely removed from the culture plate to be then loaded on the transplantation device. In addition, this support might replace damaged Bruch's

membrane in patients. Thus specific qualities are required to support hPSC-RPE functions and survival: thickness, mechanical properties (flexibility, ease of handling), permeability, and eventually biodegradation (nontoxic by-products) (Hynes and Lavik 2010; Kador and Goldberg 2012; Nazari et al. 2015).

76.4.1 Synthetic Polymers

Synthetic polymers have the main advantage of being structurally and chemically precisely defined, with consistency, homogeneous properties, and a high potential for industrial processing (Diniz et al. 2013; Stanzel et al. 2014; Ilmarinen et al. 2015). Among others, ultrathin parylene substrates have proven their safety and efficacy (Hu et al. 2012; Diniz et al. 2013; Pennington and Clegg 2016). They will be tested in phase I/II clinical trial for dry AMD in the USA (clinical trial reference: NCT02590692). Poly-lactic-co-glycolic acid or PLGA is also an attractive substrate that is biodegradable (Song and Bharti 2016). hPSC-RPE can be cultured on such substrate and form an epithelium that can be transplanted (Bharti et al. 2014; Song and Bharti 2016). This combination will also be tested in a USA-based clinical trial (Song and Bharti 2016). Finally, a porous polyester scaffold is currently tested in the UK (NCT01691261) (Ramsden et al. 2013).

76.4.2 Biological Materials

Various biological scaffolds have been proposed like Descemet's membranes (Thumann et al. 1997) or human amniotic membranes (hAMs) (Kiilgaard et al. 2012). hAMs, obtained from cesarean sections of normal births (Capeans et al. 2003), are well-tolerated in the subretinal space (Kiilgaard et al. 2012). Our group, in collaboration with the Institut de la Vision and St Louis Hospital (France), is developing the use of this scaffold for a phase I/II clinical trial targeting RP caused by a RPE dysfunction.

76.4.3 No Support

A Japanese group developed a strategy without additional scaffold (Kamao et al. 2014). RPE are allowed to form an epithelium on a collagen coating. Then the collagen coating is digested to liberate the sheet. So far, one patient has been transplanted using this strategy (Mandai et al. 2017).

76.5 Conclusion

First clinical trials have demonstrated the safety of hESC-RPE implantation and have paved the way for optimized systems. Future clinical trials with engineered tissue based on the various scaffolds described herein will give clues about the formulations that provide the best results to patients.

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