Harnessing the potential of induced pluripotent stem cells to accelerate drug discovery for age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a leading cause of blindness worldwide, affecting more than 200 million people[1]. Unfortunately, therapeutic options for dry-AMD, the most common form accounting for 80-90% of cases, remain limited. A major challenge in developing effective treatments is the absence of reliable *in vitro* AMD models, which has slowed drug discovery efforts. The cell type primarily affected in AMD is the retinal pigment epithelium (RPE). While primary and immortalized RPE lines have provided valuable insights into RPE functions under normal and pathological conditions, they exhibit limitations for drug discovery, including low amplification potential in primary RPE cells and a lack of maturation in immortalized RPE lines[2].

The advent of induced pluripotent stem cell (iPSC) technology has enabled the generation of unlimited quantities of mature, functional RPE cells for large-scale research. Moreover, iPSCs allow the derivation of cell lines from multiple AMD patients, facilitating the study of how genetic background influences disease progression and treatment efficacy.

To establish a highly relevant *in vitro* AMD model, we have developed a protocol for the large-scale differentiation of multiple iPSC lines into RPE cells. These cells are then treated with chronic low doses of A2E and exposed to blue light, simulating lipofuscin accumulation, an aging mechanism implicated in AMD[3]. Our results demonstrated that this combination of stressors induces AMD hallmarks in iPSC-derived RPE cells, including increased oxidative stress, complement pathway activation, increased pro-inflammatory cytokine secretion, and RPE atrophy.

In collaboration with the Hospital Center of Créteil, we collected 26 cell lines from AMD patients, some of whom carry genetic risk alleles such as CFH and HTRA1, which are associated with increased susceptibility to AMD[4]. We are currently differentiating these cell lines into RPE cells and assessing their behavior in our AMD model. Results from the first set of lines suggest that cells carrying a combination of CFH, HTRA1, and ARMS2 risk alleles are more sensitive to combined A2E and blue light stress.

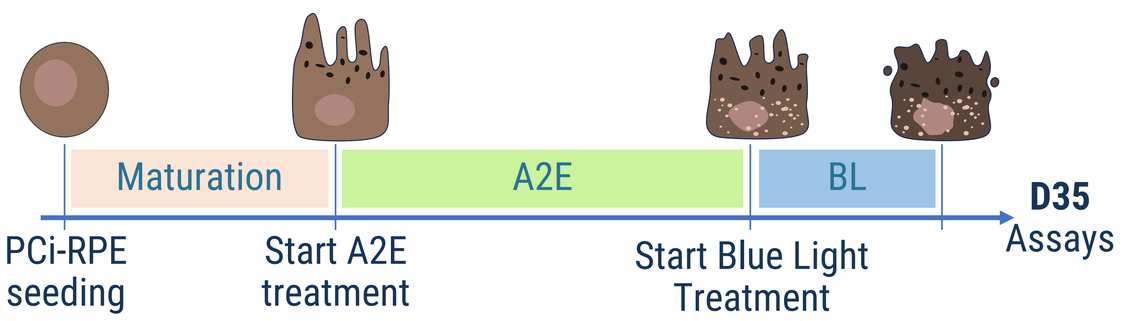


Figure 1. Timeline of the innovative *in vitro* dry AMD model. After a maturation step, RPE cells are exposed to a low, chronic dose of A2E to mimic lipofuscin accumulation. A2E-loaded cells are then exposed to blue light to induce RPE atrophy.

PCi-RPE cells express key RPE markers and are functional

All batches of PCi-RPE cells produced undergo thorough qualification. We ensure that the cells exhibit typical RPE cobblestone morphology and express key RPE markers such as MITF and PMEL17. We also verify their ability to form a tight epithelium by assessing the tight junction marker ZO-1 and we validate their capacity for phagocytosis, which is one of the key functions of RPE cells *in vivo*. These tests ensure the quality and reproducibility across different batches and cell lines for our assays.

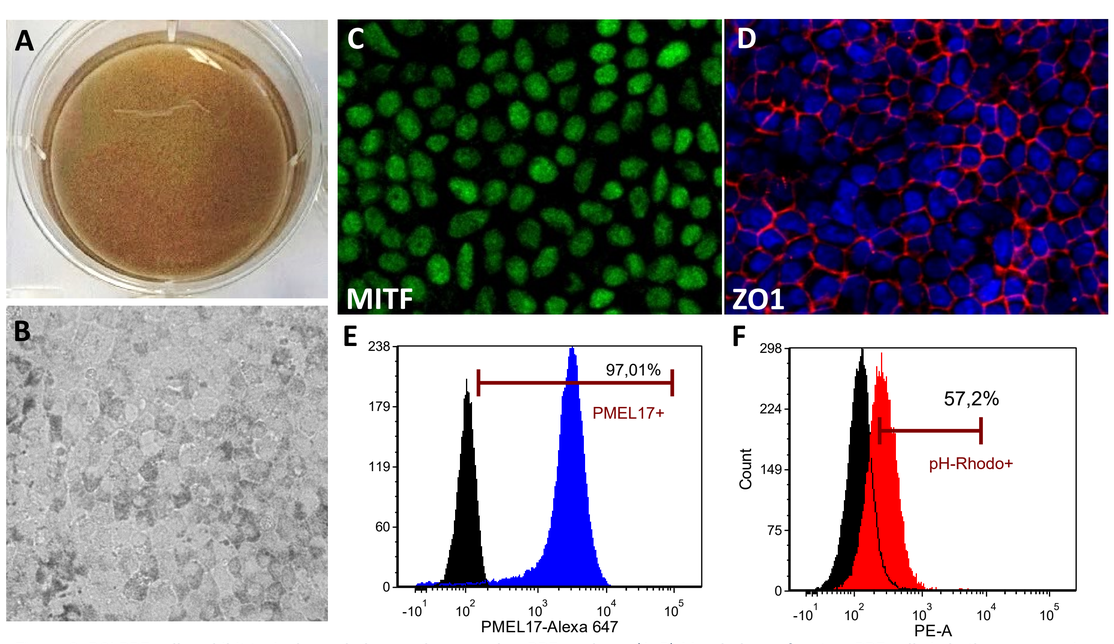
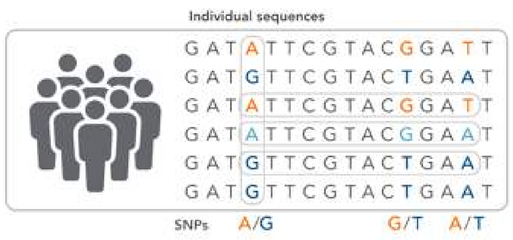


Figure 2. PCi-RPE cells exhibit typical morphology and express key RPE markers. (A, B) Morphology of mature RPE cells in both macroscopic and microscopic views. (C, D) Immunostaining showing MITF and ZO-1 expression in RPE cells cultured for 14 days. (E) Flow cytometry analysis showing that 97% of PCi-RPE cells express the premelanosome marker PMEL17. (F) pH-Rhodo assay showing that PCi-RPE cells are capable of phagocytosis.

Addressing AMD genetic heterogeneity: A 'Clinical Trial in a Dish'

Some people have a strong genetic predisposition to AMD



CFH: SNP rs1061170

ARMS2: SNP rs10490924

HTRA1: SNP rs11200638

Access to patients through a strategic partnership

HÔPITAL INTERCOMMUNAL CRÉTEIL

26 patient cell lines
Age: 66 – 97 years old
Sex: 20 females / 6 males
Clinical description:
Neovascularization
Drusens
Smoker status

3 iPSC-patient cohorts
Patients with "**high risk**" alleles:
40x higher AMD prevalence[4]
Patients with "**protective**" alleles
Patients with "**mixed**" alleles

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An *in vitro* model that faithfully recapitulates the main hallmarks of AMD physiopathology

RPE cell atrophy in the iPSC powered *in vitro* AMD model

Chronic A2E stress combined with blue light illumination induces RPE cell death. We have identified a reference compound that protects RPE cells in our model and include it in all our customers' projects to ensure that the stress induced on RPE cells is within a reversible range.

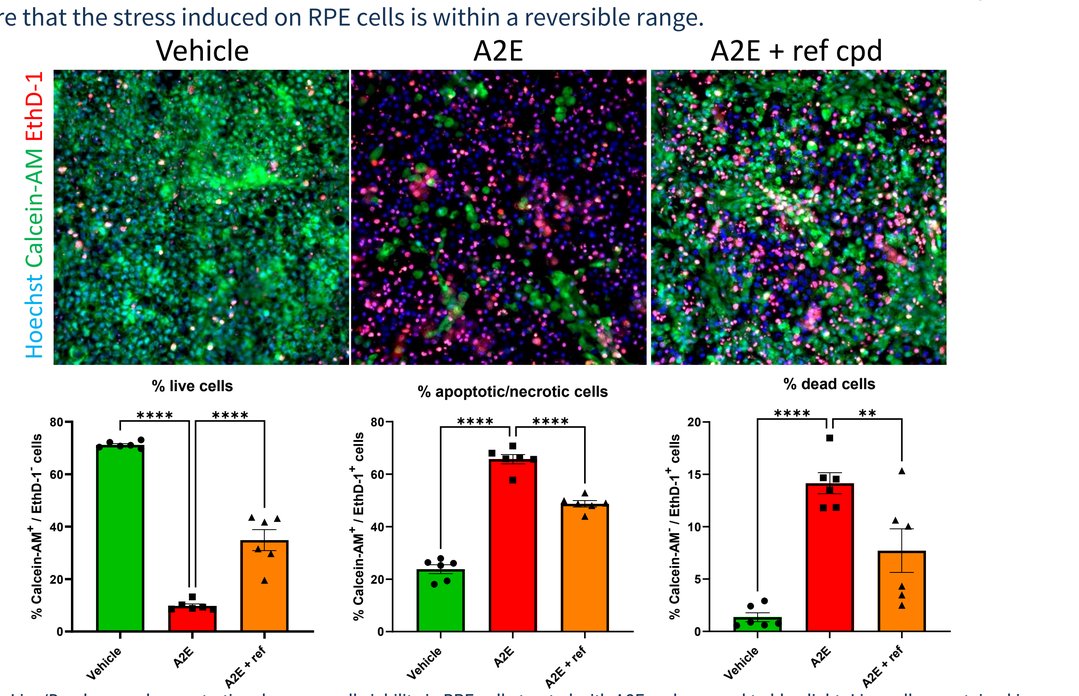

Figure 3. Live/Dead assay demonstrating decrease cell viability in RPE cells treated with A2E and exposed to blue light. Live cells are stained in green using calcein-AM, while dead cells are stained in red using ethidium homodimer (EthD-1). Cells labelled by both calcein-AM and EthD-1 are considered apoptotic or necrotic.

Evolution of AMD markers after A2E and blue light exposure

RPE cells exposed to A2E chronic stress followed by blue light illumination exhibit the main AMD markers with increased level of oxidative stress, elevated secretion of proinflammatory cytokines IL-6 and IL-8, increased expression of proteins involved in drusen formation and activation of the complement cascade.

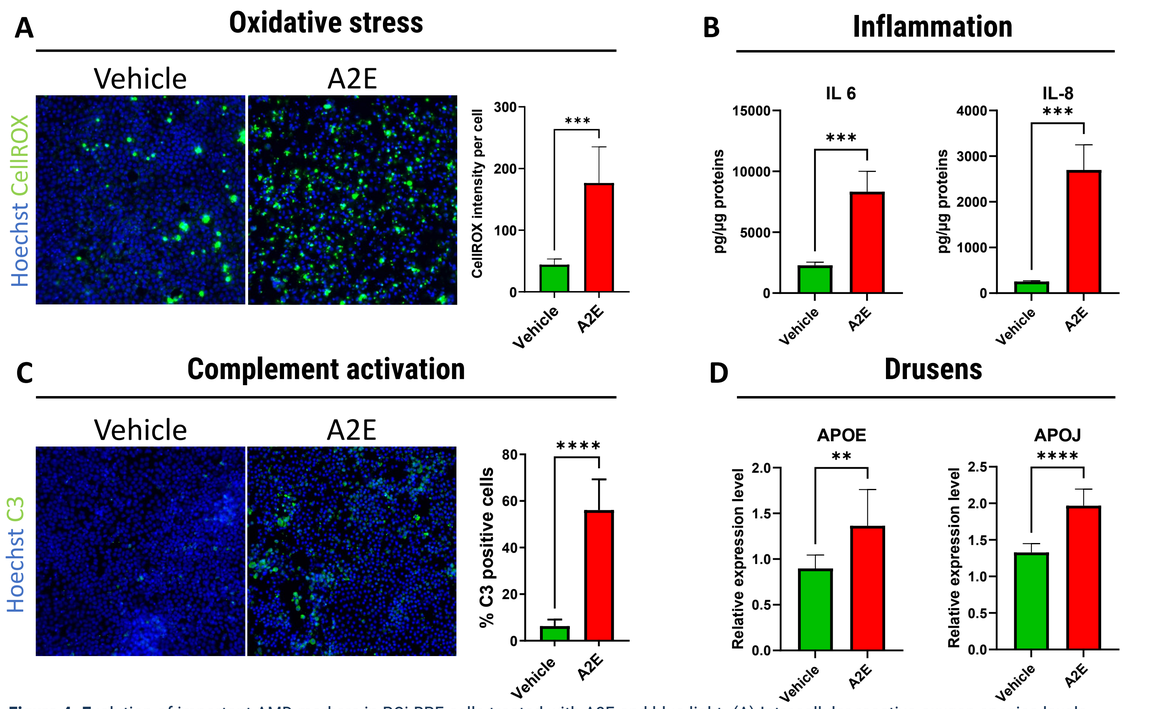

Figure 4. Evolution of important AMD markers in PCi-RPE cells treated with A2E and blue light. (A) Intracellular reactive oxygen species levels measured using the CellROX probe. (B) Concentration of IL-6 and IL-8 cytokines in cell culture supernatants quantified by ELISA. (C) Immunostaining for C3 protein. (D) Quantification of APOE and APOJ expression measured by RT-qPCR.

AMD-risk PCi-RPE cells exhibited increased sensitivity to our *in vitro* AMD model

PCi-RPE cells carrying CFH, ARMS2, and HTRA1 alleles associated with increased risk of AMD showed a further decrease in cell viability after exposure to A2E and blue light stressors, compared to cells carrying protective versions of these alleles.

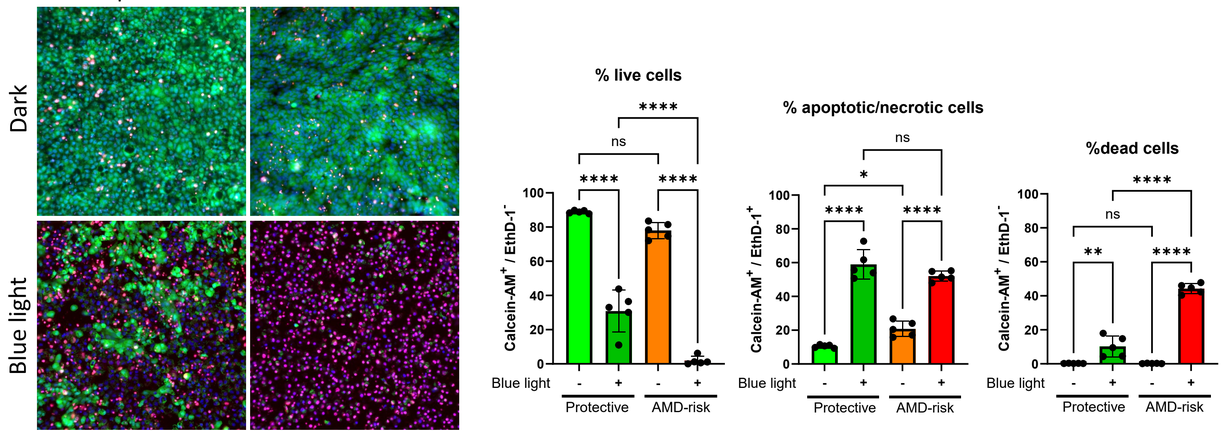

Figure 5. Live/Dead assay demonstrating a further decrease in cell viability in RPE cells carrying alleles associated with increased risk of AMD. Live cells are stained in green using calcein-AM, while dead cells are stained in red using ethidium homodimer-1 (EthD-1). Cells labeled by both calcein-AM and EthD-1 are considered apoptotic or necrotic.

Conclusion

By combining iPSC-derived RPE cells with chronic A2E stress followed by blue light illumination, we have created the first *in vitro* model that faithfully recapitulates key mechanisms involved in RPE atrophy associated with AMD. Moreover, we collected multiple patient cell lines to be able to account for the impact of genetic backgrounds on the efficacy of potential treatments. Our preliminary results suggest that PCi-RPE cells carrying alleles associated with increased risk of having AMD are more sensitive to the stressors used in our model.

Future work will focus on incorporating other cell types involved in AMD pathogenesis in our model, such as photoreceptors and microglial cells.

References

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