Development of a human iPSC-derived retinal microphysiological co-culture system for dry age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a leading cause of vision loss. Treatment options remain limited for the most common form, dry AMD, which represents 80–90% of patients[1]. Progress toward therapeutic discovery has been hindered in part by the lack of physiologically relevant *in vitro* models replicating the multiple stress factors driving AMD progression, as well as the crosstalk between retinal cell types in response to these stresses. Here, we describe the development of a microphysiological multicellular co-culture model incorporating retinal pigment epithelium (RPE) cells, the primary cell type affected in AMD, cocultured with either microglia, which play a central role in regulating retinal inflammation[2], or photoreceptors, whose degeneration ultimately leads to vision loss. All cell types were derived from human induced pluripotent stem cells (iPSCs).

Methods

iPSCs were differentiated into retinal pigment epithelium (RPE) cells and microglia, and characterized for lineage-specific markers and functional performance. Neural retinal progenitors (NRPs) were generated via retinal organoid dissociated at day 45. NRPs were subsequently expanded and matured in pro-neural medium.

For RPE–microglia co-culture experiments, RPE cells were first matured in standard flat-bottom culture plates for 21 days. Microglia were then seeded onto 8 µm pore-size transwell inserts and co-cultured with RPE cells for 7 days, in the presence or absence of A2E.

For RPE and retinal progenitor cell co-culture, RPE cells were cultured on transwell inserts for 21 days to establish polarization. Photoreceptors embedded in extracellular matrix were subsequently seeded onto the RPE monolayer and cocultured for 21 days.

Results

All iPSC-derived cell types expressed the expected lineage-specific markers. RPE cells expressed key markers MITF, PMEL17, and MERTK and demonstrated functional phagocytosis of bovine photoreceptor outer segments. Microglial cells responded appropriately to classical pro-inflammatory stimulation such as LPS and exhibited chemotactic responses toward C5a. Approximately 80% of retinal progenitors isolated from retinal organoids expressed CHX10 over two passages, and maturation in pro-neural medium increased the expression of early photoreceptor markers such as CRX.

A culture medium compatible with RPE–microglia co-culture was identified. A2E treatment alone did not induce microglial activation in monoculture. However, in RPE–microglia co-culture, A2E exposure led to increased microglial proliferation and elevated secretion of inflammatory cytokines, indicating activation in response to RPE stress. In parallel, RPE cells displayed increased sensitivity to A2E- and blue light–induced stress when co-cultured with microglia compared to monoculture conditions.

Finally, preliminary data suggest enhanced expression of photoreceptor maturation markers, including NRL and Recoverin, in retinal progenitors co-cultured with RPE cells.

Characterization of iPSC-derived RPE and microglial cells

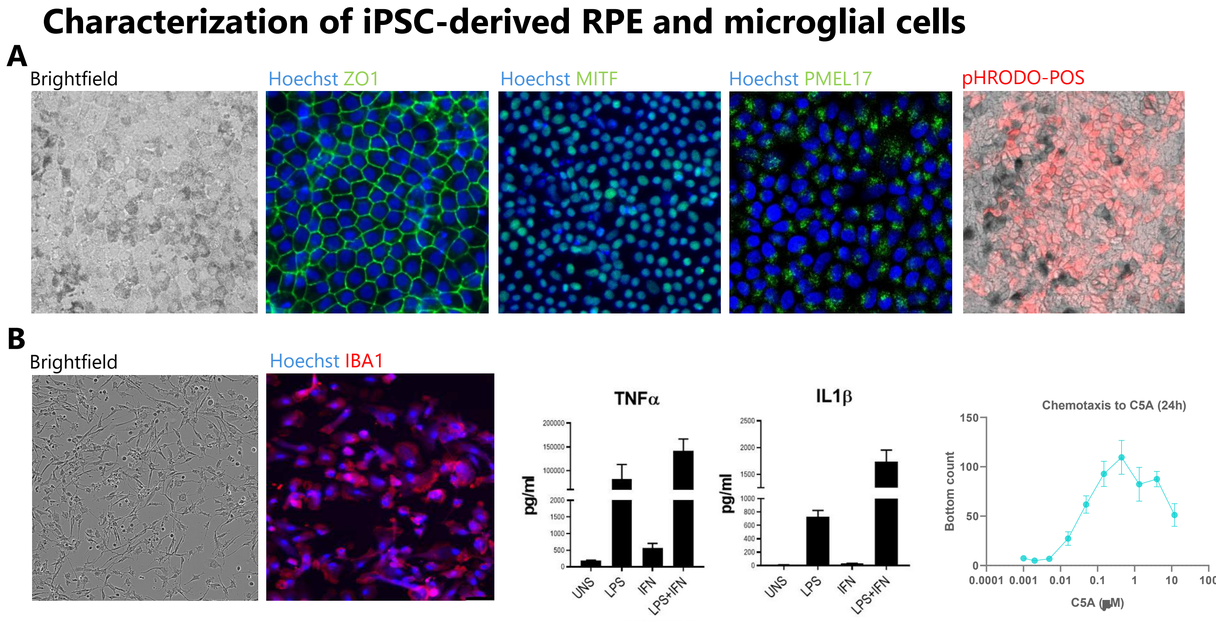

Figure 1. **A.** iPSC-derived RPE cells exhibit typical pigmentation and cobblestone morphology. They express key RPE markers, including ZO1, MITF, and PMEL17. They are also capable of phagocytosing pHrodo-conjugated bovine POS. **B.** iPSC-derived microglial cells show the expected ramified morphology and express the myeloid lineage marker IBA1. They also show increased TNF-α and IL-1β secretion in response to LPS and IFN stimulation. In addition, they show dose-dependent chemotaxis toward C5a.

Isolation, amplification and maturation of iPSC-derived NRPs

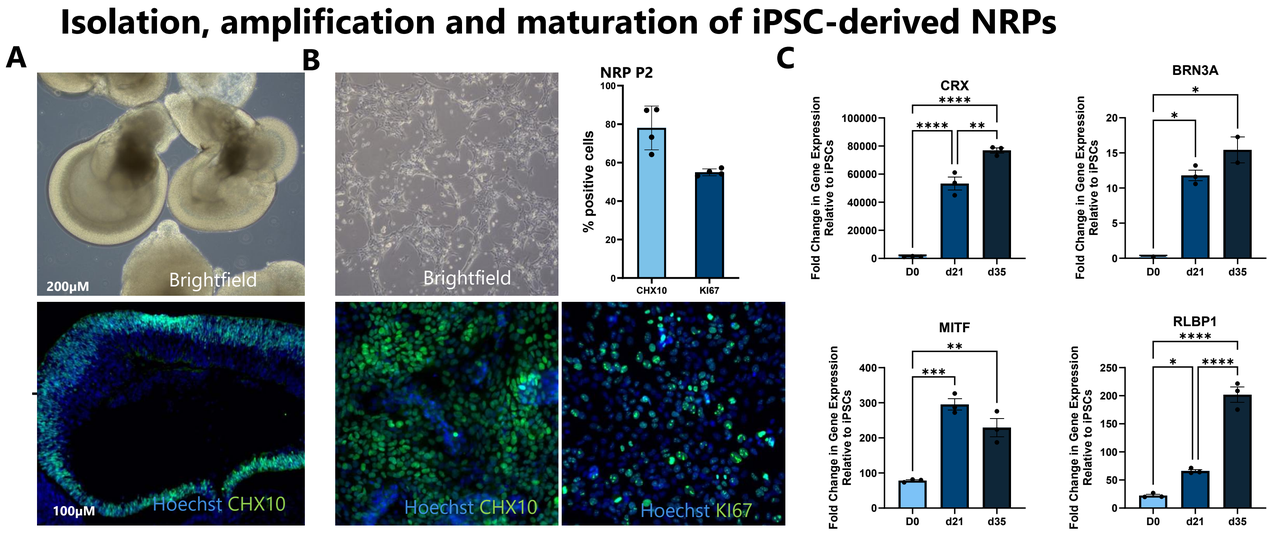

Figure 2. **A.** Retinal organoids at D45 displaying a typical bright-field neuroepithelial layer composed of CHX10-positive neural NRPs. **B.** NRPs obtained from dissociated retinal organoids (ROs) were expanded in optimized culture medium. At passage 2, 78.0% of isolated NRPs remained CHX10-positive and 54.9% were KI67-positive, indicating maintenance of progenitor identity and proliferative capacity. **C.** NRPs matured in pro-neural medium showed increased expression of the photoreceptor marker CRX, the retinal ganglion cell marker BRN3A, the retinal pigment epithelium (RPE) marker MITF, and the RPE/Müller glia marker RLBP1, confirming differentiation potential toward major retinal cell types.

Establishment of an RPE-microglia co-culture system

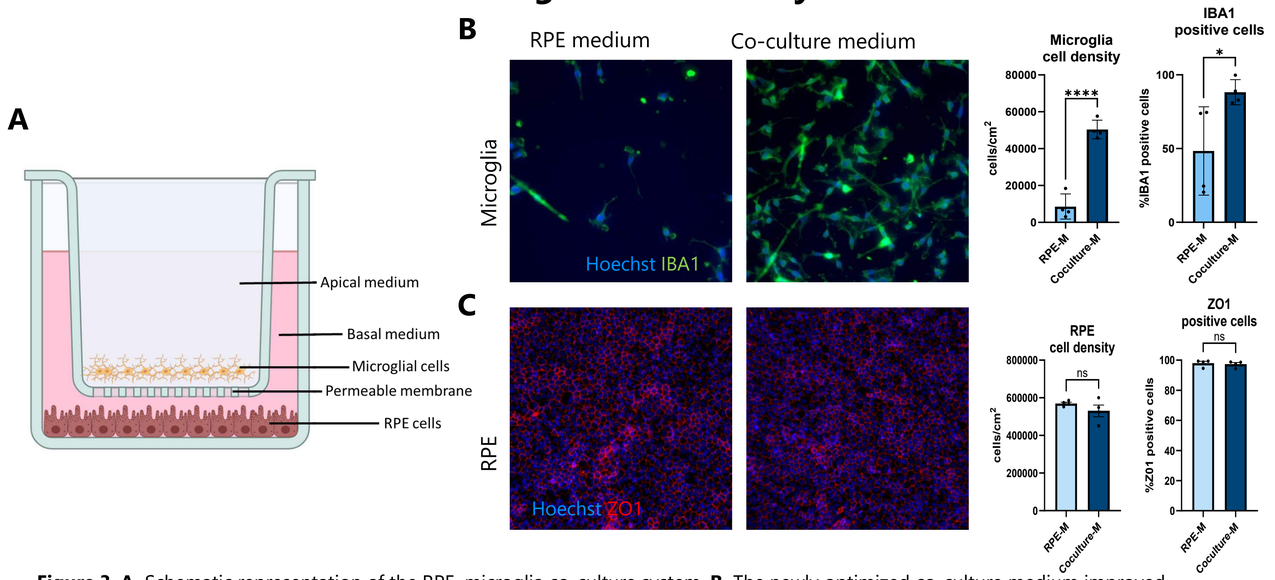

Figure 3. **A.** Schematic representation of the RPE–microglia co-culture system. **B.** The newly optimized co-culture medium improved microglia maintenance, as shown by increased microglial cell density in the insert and a higher percentage of IBA1-positive cells after 7 days of co-culture compared to PhenoCULT-RPE medium. **C.** RPE cell density was maintained in both PhenoCULT-RPE medium and co-culture medium after 7 days of co-culture with microglia. Tight junctions were also preserved under both conditions, as indicated by ZO-1 expression.

Microglia increase RPE sensitivity to A2E and blue light–induced stress

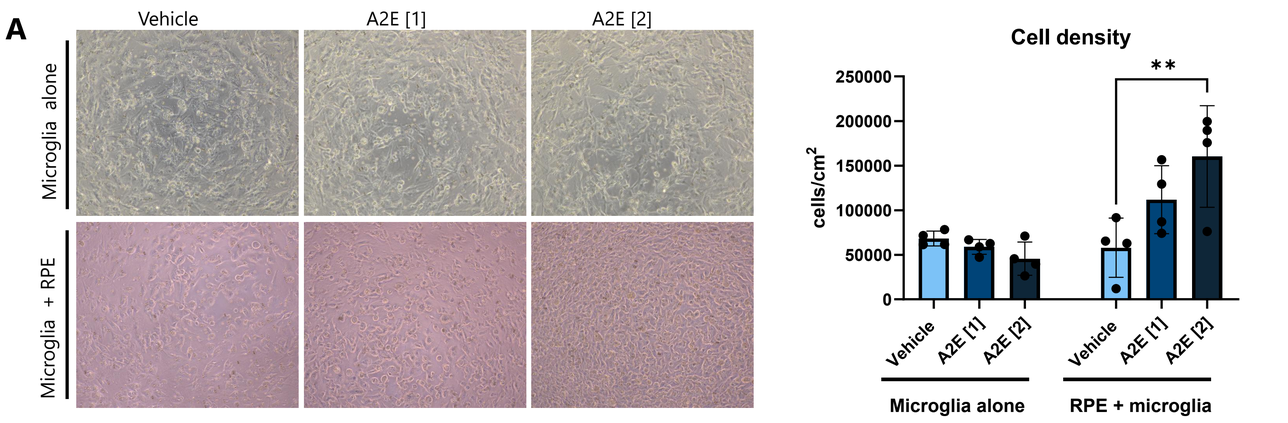

Figure 4. A. Microglial morphology and cell density was not affected by A2E treatment in monoculture. In contrast, an increase in microglial cell density was observed in RPE–microglia co-culture in an A2E dose-dependent manner. **B.** A2E treatment in RPE–microglia co-cultures is associated with increased secretion of inflammatory cytokines (IL-1β, TNF-α, IL-10, and IL-6), as measured by LegendPlex analysis. **C.** A decrease in RPE cell viability is observed following A2E and blue light exposure, with a greater reduction in co-culture compared to monoculture at equivalent A2E concentrations.

Establishment of an RPE-NRP co-culture system – preliminary data

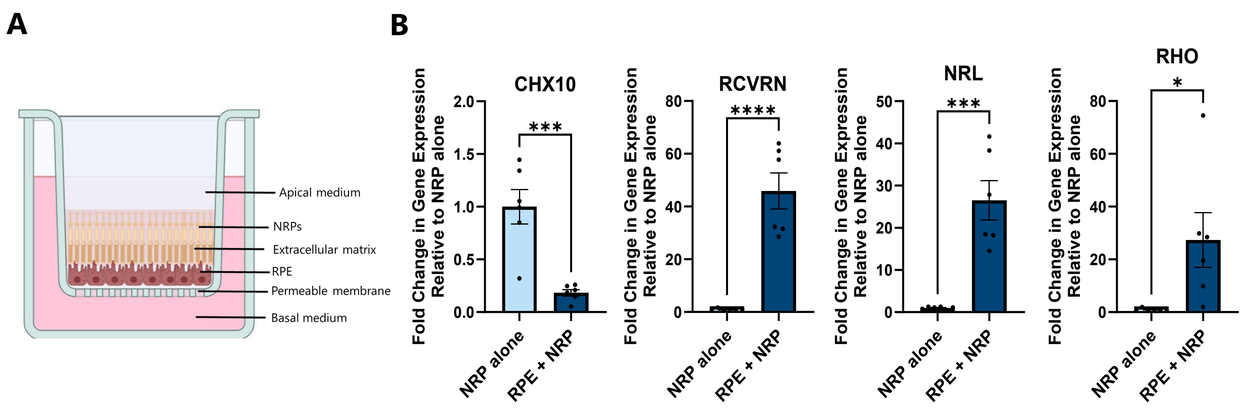

Figure 5. A. Schematic representation of the RPE–NRP co-culture system. B. NRPs co-cultured with RPE cells for 21 days in pro-neural medium exhibited increased expression of the photoreceptor maturation markers RCVRN, NRL, and RHO, alongside decreased expression of the retinal progenitor marker CHX10, compared to NRPs cultured alone under the same conditions.

Conclusion

The establishment of multicellular co-culture models represents an important step toward the development of more physiologically relevant *in vitro* systems for dry AMD. Future work will focus on further optimizing the maturation of NRPs toward the photoreceptor lineage, as well as developing a triculture model incorporating RPE, microglia, and photoreceptor cells.

References

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2. Kaur ,G. & Singh , N. The Role of Inflammation in Retinal Neurodegeneration and Degenerative Diseases. Int J Mol Sci. 2021 Dec 30;23(1):386