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Physiologically relevant iPSC-derived retinal models for ophthalmology research & drug discovery

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Summary

Retinal diseases encompass a diverse group of degenerative and genetically driven conditions that progressively impair visual function. Key pathogenic processes include photoreceptor dysfunction, retinal pigment epithelium (RPE) atrophy, and retinal ganglion cell loss. Age-related macular degeneration (AMD), particularly its dry form, is characterized by oxidative stress, lipid and bisretinoid accumulation, and complement activation within the RPE, leading to gradual central vision decline. Inherited retinal diseases (IRDs), involving more than 300 genes, display substantial phenotypic variability and frequently result in early-onset or progressive visual impairment.

Although anti-VEGF therapies have transformed the management of neovascular retinal disease, no disease-modifying treatments exist for most IRDs, including Stargardt disease. For dry AMD, two anti-complement therapies have recently been approved by the FDA (Syfovre® and Izervay®). While these drugs provide significant benefits, their impact on visual function remains limited, highlighting the need for additional therapeutic options. Development of emerging approaches, including gene- and RNA-based therapies and targeted modulation of inflammatory and metabolic pathways, requires experimental systems capable of capturing human-specific mechanisms and genotype-dependent disease features.

Human induced pluripotent stem cell (iPSC)-derived retinal models provide physiologically relevant platforms for studying retinal biology and disease. These systems retain donor-specific genetics and can be differentiated into mature retinal-relevant cell types, including RPE, photoreceptors, Müller glia, and retinal ganglion cells. Three-dimensional retinal organoids extend this capability by recapitulating retinal development, layered architecture, enabling the study of the interaction between multiple retinal cell types.

Axol Bioscience supports this shift toward human and physiologically relevant research by providing well-characterized iPSC-derived retinal cell models that enable mechanistic studies, toxicity assessment, and therapeutic evaluation across diverse retinal conditions. This whitepaper outlines the development and application of these systems and their role in advancing translational ophthalmology research and drug discovery.

Contents

Retinal therapeutic landscape

Retinal diseases remain a major and growing health challenge driven by aging populations and rising metabolic diseases. Current therapies, including anti-VEGF agents, corticosteroids, and laser-based interventions, have substantially improved outcomes for neovascular AMD and diabetic macular edema. Gene therapy has provided benefit for a small subset of RPE65-associated disease, however, dry AMD, Stargardt disease, and most IRDs still lack disease-modifying treatments[2].

Advances in human-specific retinal biology are reshaping therapeutic strategies. Emerging insights into RPE metabolic dysfunction, photoreceptor susceptibility, and microglial signaling[1] highlight mechanisms that cannot be fully captured by traditional preclinical systems. As therapeutic development progresses toward gene-targeted, cell-based, and pathway-specific approaches, scalable and physiologically relevant human retinal models have become central to mechanistic discovery and translational decision-making.

The complexity of the retina

The retina is a multilayered neural tissue composed of photoreceptors, bipolar cells, horizontal cells, amacrine cells, Müller glia, and ganglion cells, which lies adjacent to a supporting RPE. These layers work together to convert light into neural signals while maintaining a tightly regulated metabolic environment essential for visual function.

Photoreceptors, rods and cones, mediate light detection, with rods supporting scotopic vision and cones, concentrated in the central retina, forming the macula and enabling color discrimination and high-resolution perception[4]. The RPE performs indispensable functions including phagocytosis of photoreceptor outer segments, recycling of 11-cis-retinal, nutrient transport, waste elimination, and regulation of oxidative stress[5]. Disruption of any of these functions contributes to degenerative retinal diseases. Microglia, the resident immune cells of the retina, provide surveillance and homeostatic regulation but can also drive neuroinflammation and synaptic remodeling when pathologically activated[6].

Overview of the retinal diseases

Retinal degenerations arise from diverse etiologies but often converge on photoreceptor degeneration, RPE dysfunction, or retinal ganglion cell (RGC) loss. Age-related macular degeneration (AMD) is the leading cause of central vision loss, with dry AMD characterized by RPE atrophy, lipid deposition, oxidative stress, and complement dysregulation[7].

Inherited retinal diseases (IRDs), including retinitis pigmentosa (RP), Stargardt disease, Leber congenital amaurosis, and Usher syndrome, collectively involve more than 300 genes[2]. While RP is marked by progressive rod-cone degeneration leading to peripheral vision loss, Stargardt disease is caused by *ABCA4* mutations resulting in the accumulation of toxic bisretinoids such as A2E, primarily affecting central vision[5]. Usher syndrome is the most common form of combined deafness and blindness, affecting both the retina and cochlear hair cells in the inner ear[3].

Furthermore, inner-retina disorders, such as glaucoma and optic neuropathies lead to RGC degeneration. iPSC-derived RGC precursors have demonstrated survival and layer-specific integration in preclinical transplantation models, reinforcing their potential in regenerative approaches[9].

Challenges in developing treatments for retinal diseases

Therapeutic development in ophthalmology is shaped by the biological and genetic diversity of retinal disorders. IRDs involve more than 300 genes, requiring models that accommodate patient-specific genotypes and support mechanistic investigation[3]. The limited regenerative capacity of retinal neurons, particularly photoreceptors and retinal ganglion cells, further narrows the therapeutic window and reinforces the importance of early and targeted intervention.

A range of complementary model systems underpins retinal research. *In vivo* models enable evaluation of whole organism-level physiology, delivery strategies, and long-term outcomes, while *in vitro* approaches allow controlled study of cell-type-specific mechanisms. Traditional immortalized RPE lines have supported foundational work, though they do not fully replicate the polarity, phagocytic activity, or metabolic profile of mature human RPE[5]. Primary tissues, iPSC-derived retinal cells, and retinal organoids help address these gaps by offering access to human-relevant cell states and developmental trajectories.

Clinical translation remains challenging in slowly progressive diseases, such as dry AMD and many IRDs, where sensitive and predictive functional readouts are still limited. Together, these factors highlight the importance of well-characterized, physiologically relevant human retinal models that can reproduce disease-specific phenotypes, support mechanistic studies, and strengthen early translational

Modeling retina in the laboratory

Current retinal research relies on a continuum of *in vivo, ex vivo*, and *in vitro* models, each offering distinct advantages and limitations.

In vivo models

In vivo systems, particularly rodent and large-animal models, have been instrumental for studying photoreceptor degeneration, inflammatory responses, synaptic remodeling, and the delivery and biodistribution of gene therapies. However, notable species-specific differences, including variations in photoreceptor ratios, complement activation dynamics, microglial behavior, and the absence of a macula in rodents, limit the translational accuracy of these models[6]. Rabbits and mice remain the most widely used species due to accessibility and established genetic tools, but their anatomical and physiological divergence from the human retina necessitates complementary model systems.

Ex vivo models

Ex vivo approaches, such as human retinal explants, preserve native lamination and cell-cell interactions but are constrained by limited tissue availability, short viability windows, and donor-dependent variability. These features restrict their usefulness for large-scale studies and for long-term disease modeling but make

them valuable for acute mechanistic studies and validation of *in vitro* findings.

In vitro models

• Primary cells and tissues

Primary human retinal cells provide physiologically relevant readouts but are difficult to obtain and maintain with low scalability due to limited proliferation potential and lack of consistency, necessitating the sourcing of cells from multiple donors.

• Immortalized cell lines

Immortalized lines, such as ARPE19 offer reproducibility and ease of use but lack the functionality, metabolic profile, and cell-type specificity of native retinal tissue, reducing their predictive value for degenerative mechanisms.

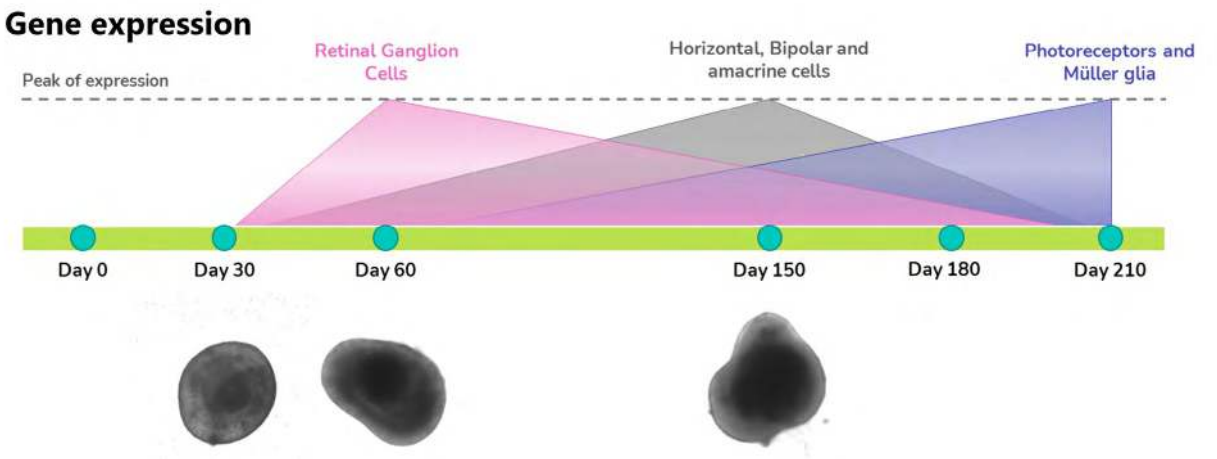
• iPSC-derived retinal cell types

iPSC-derived RPE, photoreceptors, microglia, and retinal ganglion cells retain donor-specific genetic backgrounds and recapitulate essential functional behaviors, enabling mechanistic studies of inherited retinal diseases, neuroinflammation, and cell-type-specific degeneration. However, when cultured separately, they offer only a partial view, limiting insight into the complex cellular interactions that are central to retinal disease progression.

• iPSC-derived organoids

iPSC-derived retinal organoids provide a three-dimensional *in vitro* system that recapitulates key developmental events and the layered organization of the human neural retina, making them well suited for modeling retinogenesis, inherited retinal diseases, and human-specific degenerative mechanisms. Their cellular composition evolves predictably over time, following the sequential order of retinal cell-type specification observed *in vivo* during retinogenesis (figure 1): retinal ganglion cells specified first, around day 60, in the center of the organoids; by day 150, a fully laminated architecture is established, with photoreceptors forming the outer and interneurons the inner nuclear layers, respectively; from day 180 onward, rod/cone-committed photoreceptors, along with more mature bipolar cells, become increasingly abundant, and outer segment development typically begins around day 180 onwards.

Retinal ganglion cells are not expected to remain abundant after day 60, as they are progressively lost during organoid maturation. This represents a limitation of the model when studying RGC biology at later stages. Consequently, studies focusing on retinal ganglion cells are often better suited to earlier-stage organoids, where RGC populations are better preserved. Nevertheless, this staged maturation enables researchers to select organoids at the relevant developmental window for their study, providing a controlled and physiologically relevant alternative to traditional 2D culture systems and animal models.



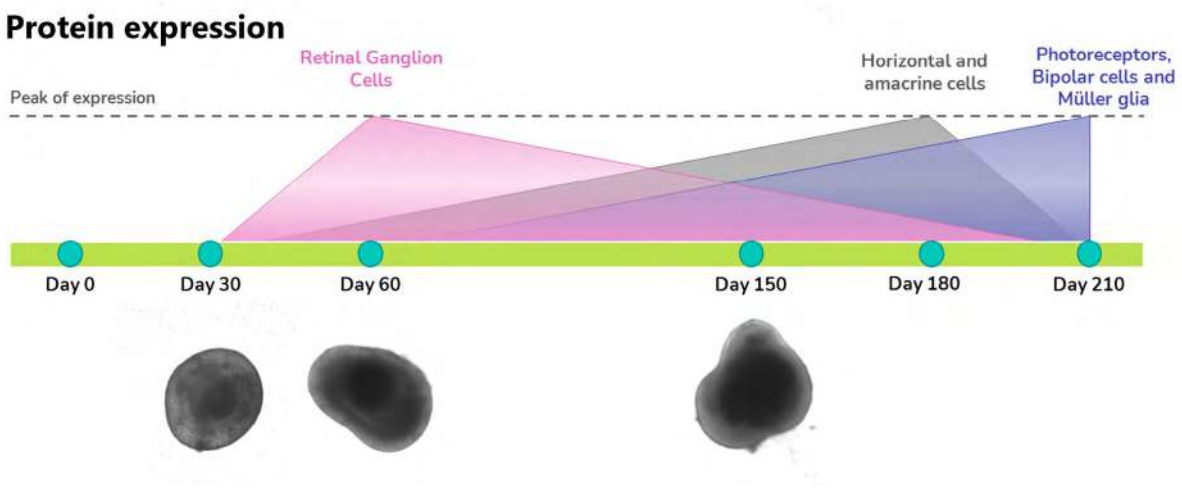


Figure 1. Developmental progression of human iPSC-derived organoids, along with the abundance of retinal cell types throughout the differentiation timeline.

Axol Bioscience ophthalmology portfolio

Retinal disease modeling and toxicology assessment using iPSC-derived cells

Axol Bioscience has developed a suite of iPSC-derived retinal models designed to reproduce key cellular, structural, and functional features of the human retina. These models support disease research, mechanistic studies, and toxicology assessment across multiple retinal cell types.

Phenotypic and functional characterisation of RPE cells and microglia

axoCells™ human iPSC-derived RPE cells display the expected cobblestone morphology and pigmentation when viewed microscopically (figure 2A-B). These cells express characteristic RPE markers, including MITF, ZO-1, and PMEL17 (figure 2C-E), with flow-cytometric analysis showing >95% PMEL17-positive cells relative to isotype controls (figure 2F). Immunocytochemistry further confirms the presence of melanosomal and tight-junction proteins, demonstrating appropriate maturation.

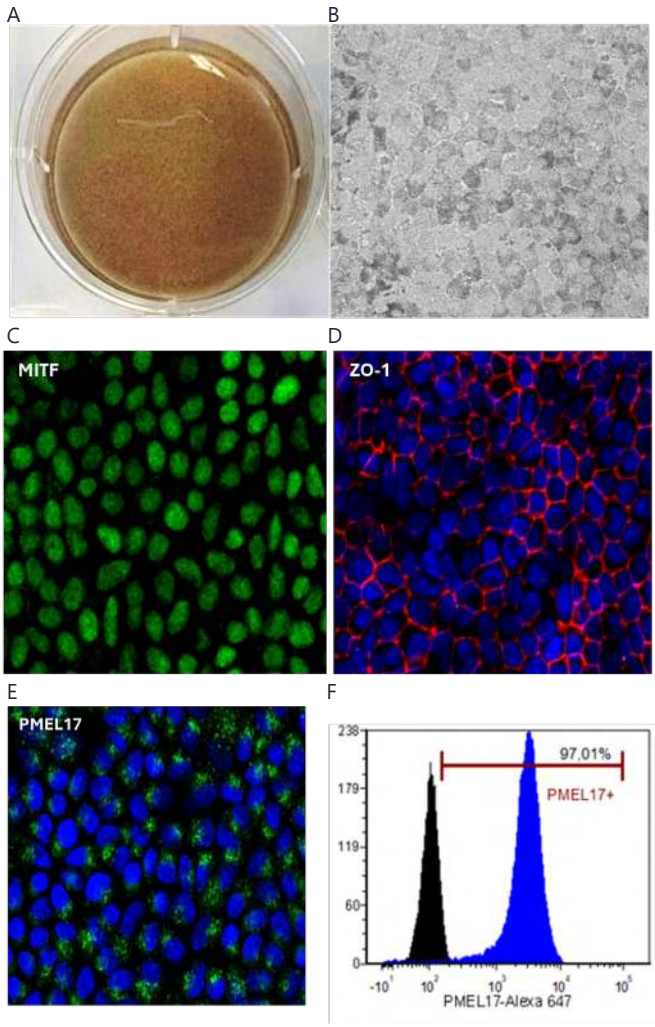


Figure 2. (A-B) Morphology of mature RPE cells in both macroscopic and microscopic views. (C-E) ICC images demonstrating expression of key markers MITF, ZO-1 (tight junction) and PMEL17 (melanosome). (F) Flow cytometry demonstrating >95% PMEL17 expression (blue) against isotype control (black).

Functional assays demonstrate that these RPE cells perform essential physiological processes. Following incubation with pHrodo-labelled photoreceptor outer segments (POS) for four hours, the cells exhibit clear phagocytic uptake, a central *in vivo* function of the RPE (figure 3A). Quantitative assessment shows phagocytic activity in approximately 57% of the cell population after 4h incubation (figure 3B). Over time, transepithelial electrical resistance (TEER) increases steadily, indicating the development of a mature epithelial barrier consistent with physiologically relevant RPE monolayers (figure 3C).

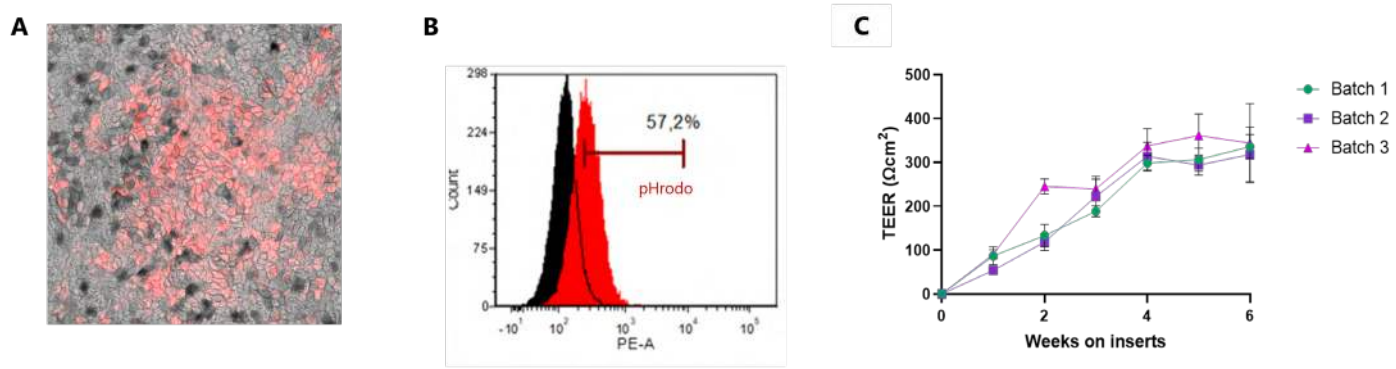


Figure 3. (A) RPE cells phagocytose pHrodo-labelled POS after 4 hours. (B) About 57% of cells are phagocytic at 4 hours. (C) TEER increases over time, demonstrating maturation of a functional epithelial barrier.

axoCells™ iPSC-derived microglia display the expected elongated and ramified morphology characteristic of resting human microglia (figure 4A). After thawing, cells mature over seven days and express canonical microglial markers, including IBA1, TMEM119, CX3CR1, and P2RY12. This marker profile is consistent with a homeostatic microglial identity (figure 4B).

Functionally, these cells model core aspects of human retinal immune activity. Microglia readily internalize pHrodo-labelled substrates over a 24-hour period, a process that is abolished by cytochalasin D, confirming actin-dependent phagocytosis (figure 4C). Chemotactic responsiveness is demonstrated through C5a-directed migration assays, where cells show dose-dependent movement toward chemotactic gradients (figure 4D). Inflammatory profiling following stimulation with LPS, IFN-γ, or both reveals cytokine release patterns (figure 4E) aligned with established microglial activation responses, providing a robust system to study neuroinflammation and immune-retinal interactions.

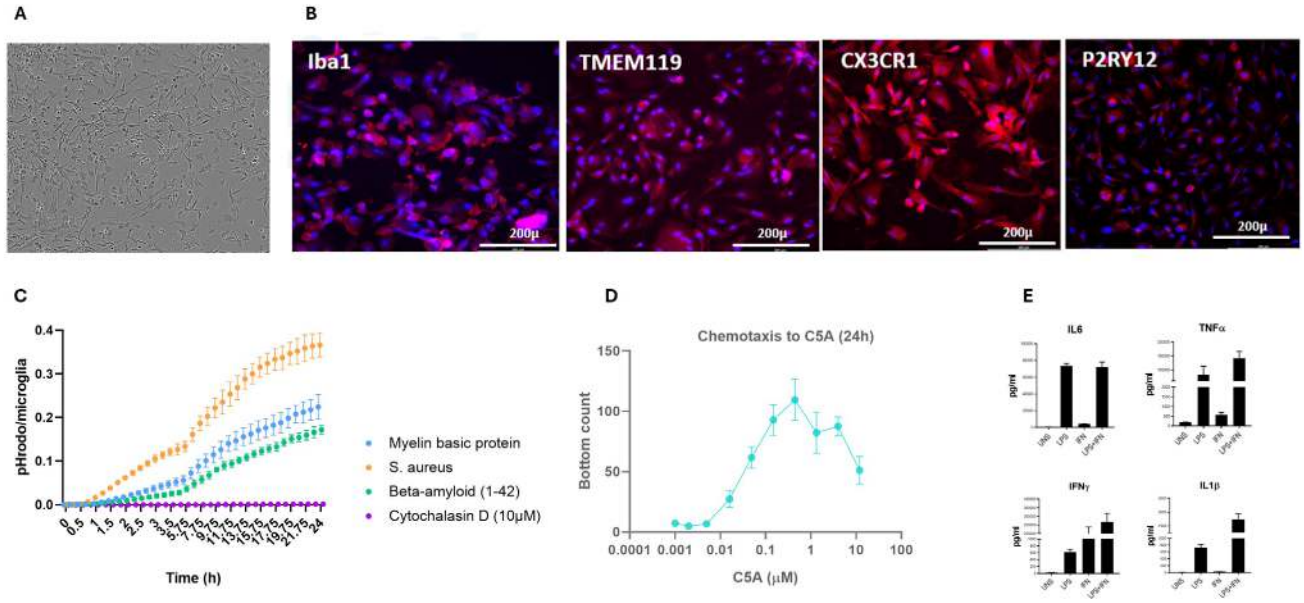
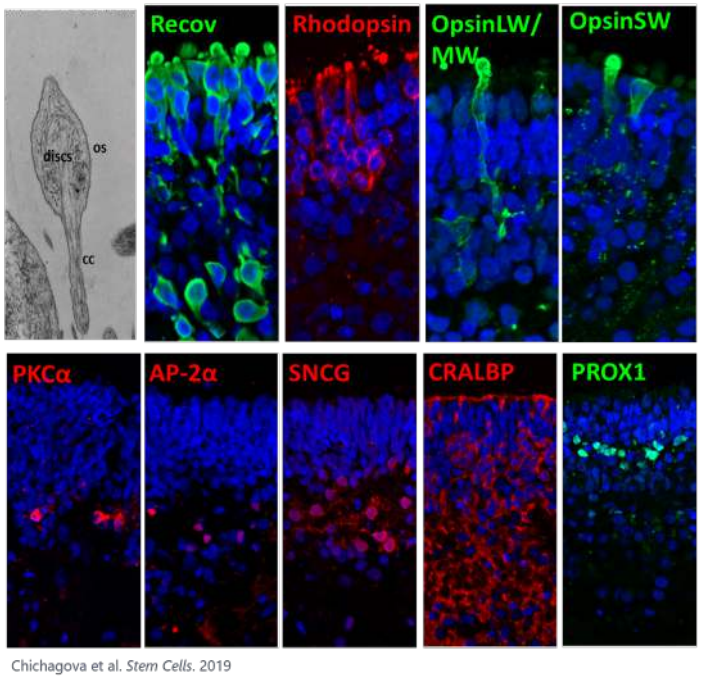


Figure 4. (A) iPSC-derived microglia show resting, ramified morphology. (B) Cells mature over seven days and express IBA1, TMEM119, CX3CR1, and P2RY12. (C) pHrodo substrate uptake is actin-dependent, as blocked by cytochalasin D. (D) C5a induces dose-dependent chemotaxis. (E) LPS and IFN-γ stimulation elicits expected cytokine release, demonstrating utility for modeling retinal immune activity.

Cellular composition of retinal organoids and their application in drug toxicity and efficacy screening

Human iPSC-derived retinal organoids recapitulate the multilayered cellular organization of the human retina. Organoids contain photoreceptors (Recoverin, Rhodopsin, Opsins LW/MW/SW), bipolar cells (PKC-α), amacrine cells (AP-2α), Müller glia (CRALBP), horizontal cells (PROX1), and ganglion cells (SNCG), arranged in a laminated configuration (figure 5).

These organoids respond reproducibly to validated retinotoxic compounds, enabling their use in toxicity screening and mechanistic studies (figure 6A). Exposure to cytotoxic agents produces dose-dependent decreases in organoid viability, accompanied by morphological changes such as darkening and roughened edges under brightfield imaging (figure 6B). For example, ATP-based viability assays conducted across biological replicates confirm consistent, concentration-dependent reductions in metabolic activity following thioridazine treatment. Non-cytotoxic agents, on the other hand, showed no effect on cell viability (figure 6C). This response profile supports the utility of organoids for assessing retinal toxicity and for screening early-stage therapeutic candidates.



Chichagova et al. *Stem Cells*. 2019

Figure 5. Human iPSC-derived retinal organoids reproduce the laminated structure of the human retina, comprising photoreceptors, bipolar, amacrine, Müller, horizontal, and ganglion cells identified by their respective markers.

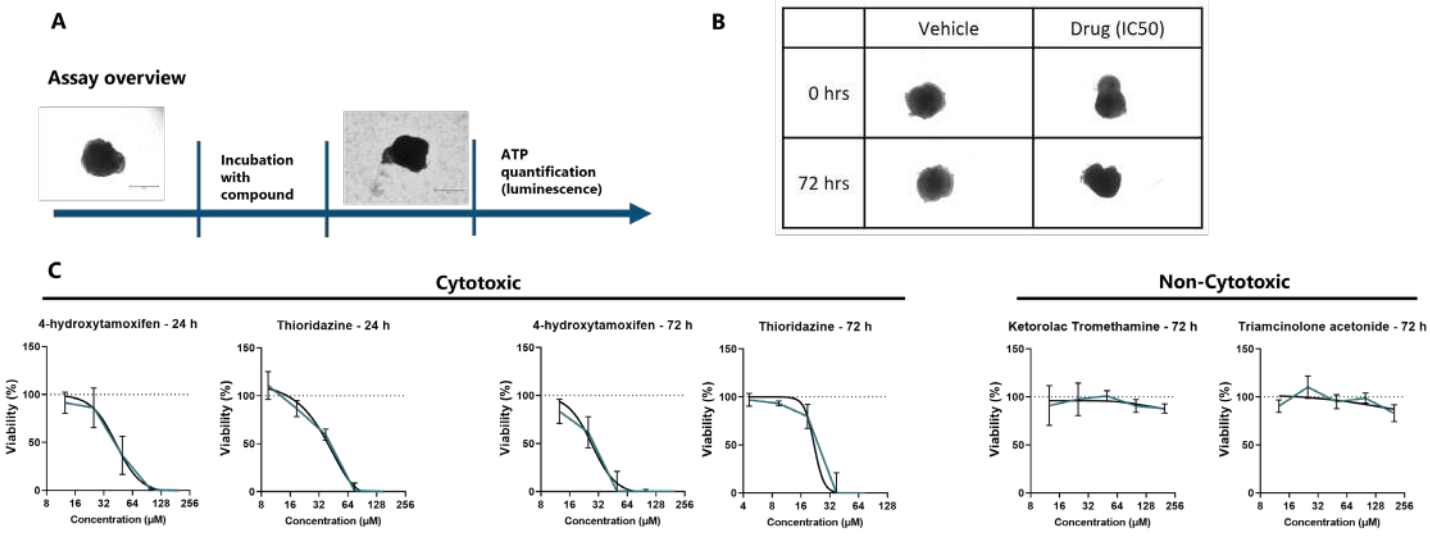


Figure 6. Drug treatment effect on retinal organoid viability. (A) Retinal organoids show reproducible responses to known retinotoxic compounds. (B) Cytotoxic agents cause dose-dependent viability loss with corresponding morphological changes. (C) ATP-based assays confirm concentration-dependent effects of thioridazine, whereas non-cytotoxic compounds show no impact.

Application focus: dry AMD *in vitro* model using relevant stressors

To model dry AMD *in vitro*, Axol employs a workflow that captures the chronic stress environment characteristic of aging RPE cells. Multiple iPSC lines are differentiated into RPE monolayers and matured for 15 days to establish barrier function and characteristic morphology. The cells are then subjected to low dose of N-retinylidene-N-retinylethanolamine (A2E) exposure for another 15 days, followed by blue-light activation for three days (total 33 days), mimicking lipofuscin accumulation and photo-oxidative stress, implicated in AMD pathology (figure 7).

Figure 7. Dry AMD *in vitro* model

This combination of stressors induces the expected RPE atrophy and cell death (figure 8A-B), and key disease-relevant phenotypes, including increased oxidative stress, complement activation and secretion of pro-inflammatory cytokines (figure 9A-D). Complement activity can be further reinforced by supplementing cultures with human serum (figure 10A-C).

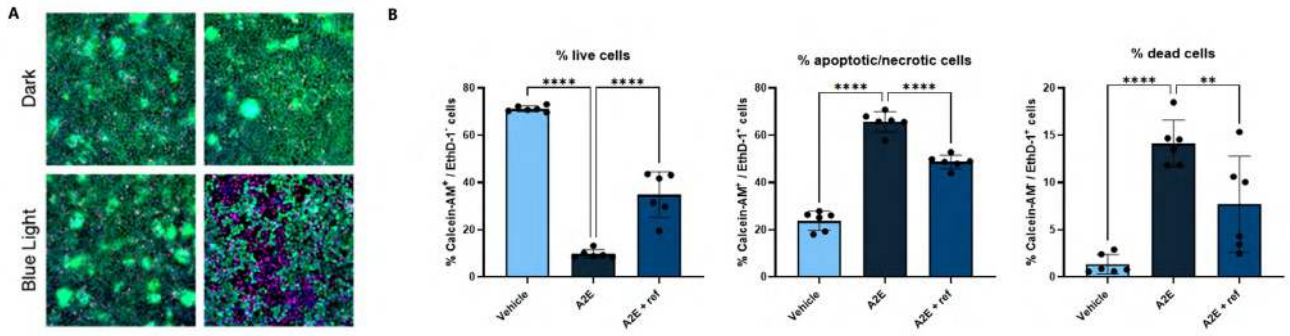


Figure 8. A2E treatment combined with blue light can reproduce RPE atrophy. (A) Images demonstrating enhanced cell death with combination treatment (A2E and blue light) versus vehicle control and blue light. (B) Live/Dead assay demonstrating decreased cell viability in RPE cells treated with A2E and 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. A reference compound is included in all our assays to ensure that the stress applied to RPE cells is in a reversible range. Ns: mean +/- SD, P > 0.05, *P ≤ 0.05. ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001.

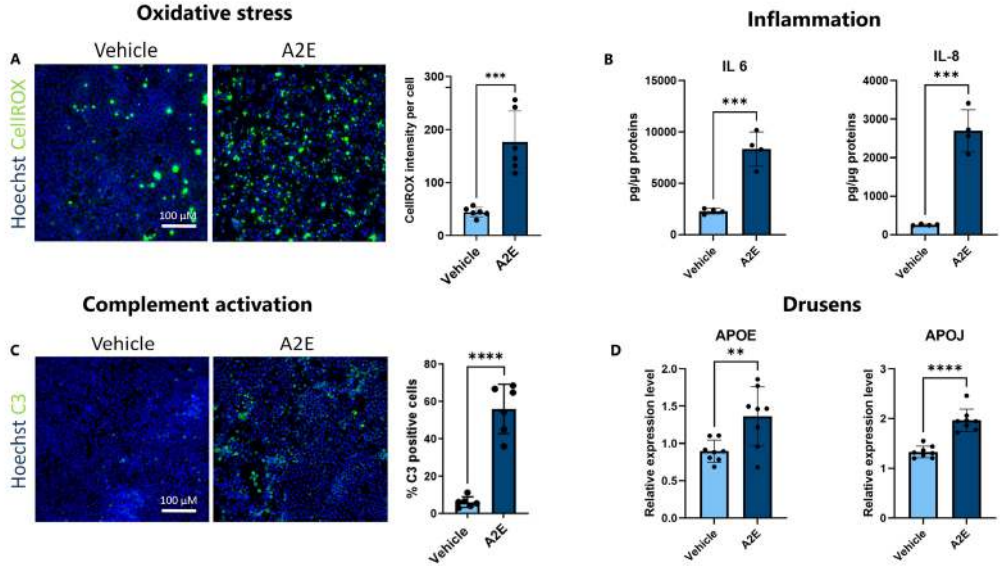


Figure 9. Evolution of important AMD markers in RPE cells treated with A2E and blue light. (A) Intracellular reactive oxygen species levels measured using the CellROX probe. (B) Concentration of IL-6 and IL-8 cytokines in cell culture supernatants quantified by ELISA. (C) Immunostaining for C3 protein. (D) Quantification of APOE and APOJ expression measured by RT-qPCR.

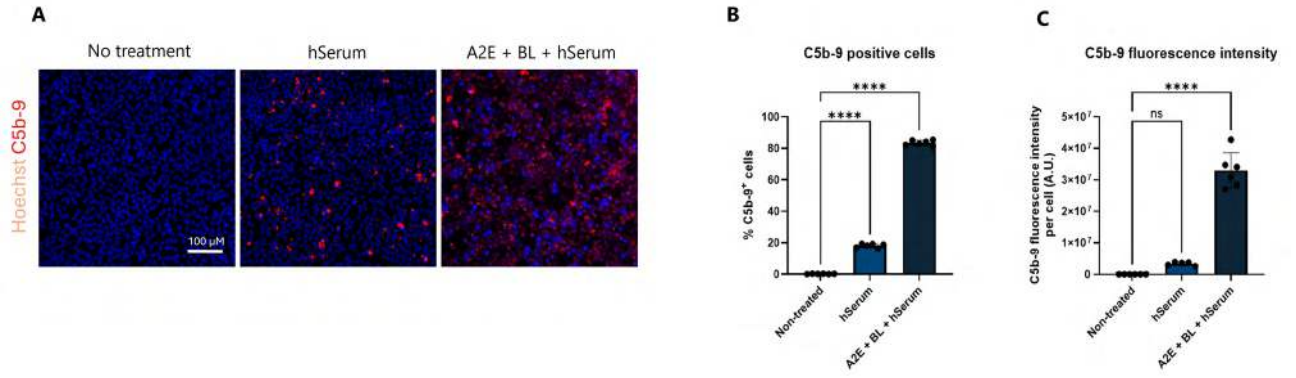


Figure 10. (A) Complement activation in RPE cells treated with human serum alone or a combination of A2E, blue light and human serum. (B-C) C5b-9 (Membrane attack complex, MAC) accumulation in RPE cells quantified by immunostaining.

A high-throughput screening (HTS) version of the model has been developed for 384-well formats. Automated liquid handling supports all stages of cell culture and assay preparation, enabling efficient screening of large compound libraries. The assay achieves Z-factors exceeding 0.5 and inter-plate variability below 10%, indicating robust and reproducible performance. A reference compound included in each experiment demonstrates protective effects against A2E-induced stress, validating the model's capacity to identify compounds with potential therapeutic benefit. With the ability to evaluate up to 6,000 compounds per run, the platform supports rapid assessment of candidate therapeutics for dry AMD (figure 10).

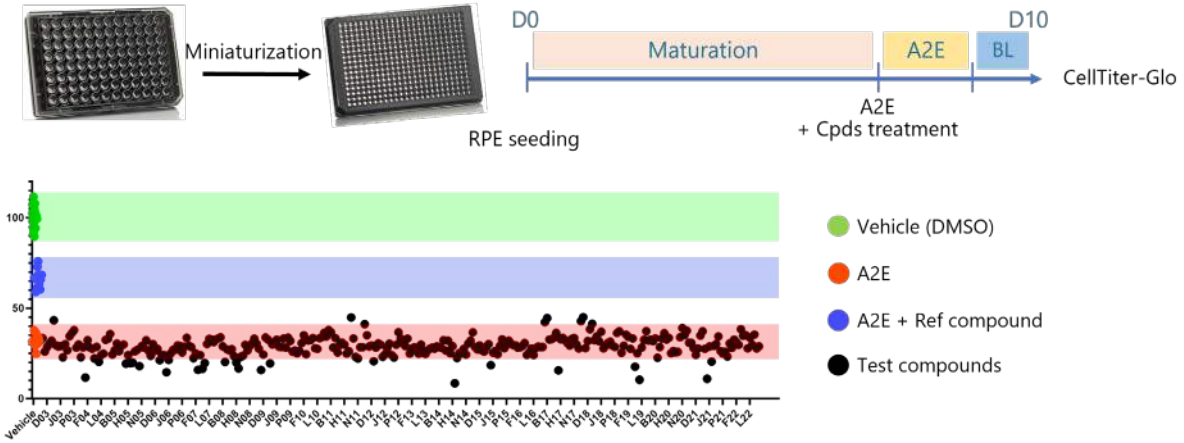


Figure 10. A 384-well high-throughput A2E-RPE assay enables automated screening of up to 6,000 compounds per run. The assay delivers Z-factors >0.5 and inter-plate variability <10%. A reference compound demonstrates protection against A2E-induced stress, confirming assay robustness for dry AMD drug discovery.

Emerging trends and future directions

Advances in retinal modeling are moving toward more integrated, patient-specific, and translationally predictive systems. Although retinal organoids allow the study of interactions between the various cell types present in the neural retina, one of their main limitations is the absence of appropriately spatially organized RPE cells, as well as the lack of microglia, which limits the investigation of retinal inflammation.

Axol Bioscience is currently developing multi-lineage co-cultures combining RPE, microglia, and photoreceptors to address these limitations and offer a model enabling the study of the interaction between RPE and photoreceptor cells as well as the impact of inflammation on disease phenotype.

The development of microfluidic retinal-on-chip systems, offering precise control of nutrient flow, oxygen gradients, and mechanical stress while enabling long-term culture and real-time monitoring, is also being investigated. The use of iPSC-derived retinal ganglion cells combined with microfluidic devices to mimic optic nerve-like structures has recently been reported[12] and could represent an attractive option for modeling glaucoma. These innovations converge to support personalized medicine approaches, molecular signatures, or predicted therapeutic responses.

Conclusion

Retinal diseases remain challenging to treat due to their biological complexity, genetic heterogeneity, and the tightly integrated structure of the neural retina. As therapeutic strategies advance toward gene-targeted, cell-based, and pathway-specific interventions, the need for human-relevant experimental systems has become central to progress.

iPSC-derived retinal models offer a physiologically meaningful way to study photoreceptor degeneration, RPE dysfunction, and microglial activation.

Within this evolving research landscape, Axol Bioscience provides rigorously characterized iPSC-derived retinal cell types and organoid systems that enable controlled, reproducible investigation of disease mechanisms and therapeutic responses. These platforms support mechanistic discovery, help align preclinical studies with human biology, and contribute to more predictive translational workflows. While regulatory agencies such as the FDA are advocating for the reduction of animal model use, the field will need to quickly develop multi-lineage models, advanced co-cultures, and higher-order in vitro retinal systems. In this contecxt, human iPSC-derived platforms will become an essential tool for accelerating the development of effective treatments for currently untreatable retinal degenerations.

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Glossary

Abbreviation / Term	Definition
AMD	Age related macular degeneration, progressive degeneration of macula. Huntingtin gene – the gene where CAG repeat expansion causes HD.
RPE	Retinal pigment epithelium, supports photoreceptors and maintains outer BRB.
BRB	Outer blood-retinal barrier, regulates the movement of molecules between the bloodstream and the retina.
IRDs	Inherited retinal diseases; monogenic disorders causing retinal degeneration.
iPSC	Induced pluripotent stem cells
RGC	Retinal ganglion cell, output neuron transmitting visual signals to the brain
A2E	Bisretinoid lipofuscin component causing RPE stress and toxicity..
PPCs	Photoreceptor precursor cells; iPSC derived cells for transplantation studies.
TEER	Transepithelial electrical resistance; metric of RPE barrier tightness.
RPE Atrophy	Loss of RPE integrity leading to secondary photoreceptor degeneration.
ROS	Reactive oxygen species; drivers of oxidative stress in retinal degeneration.
RPE Organoids / RO	iPSC derived retinal organoids modeling 3 D retinal development.

Axol Bioscience

Axol Bioscience is a leading provider of human induced pluripotent stem cell (iPSC) technologies, specializing in the manufacture of high-performance iPSC-derived cells and the delivery of industry-leading laboratory services. With over a decade of expertise, Axol partners with global pharmaceutical companies, contract research organizations (CROs), biotechnology firms, start-ups, and academic institutions to advance the use of iPSC-based models in drug discovery and disease research.

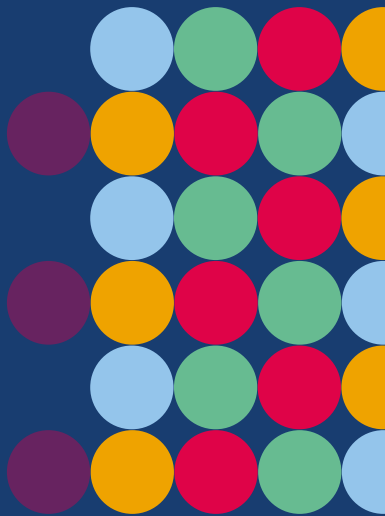
The company operates across four key areas, neuroscience - including ALS, Alzheimer's, Huntington's, Parkinson's - neuroinflammation, ophthalmology dermatology and cardiovascular.

Axol offers scalable solutions including iPSC banking, reprogramming, gene editing, cell manufacturing, and quality control. The company is committed to closing the translational gap and developing physiologically relevant *in vitro* systems that improve consistency and relevance in human disease modeling and compound testing.

Axol is recognized for its scientific excellence, transparent collaboration and rigorous quality standards. Manufacturing operations are ISO 9001:2015 certified.

eBook

Physiologically relevant *in vitro* retinal models for ophthalmology drug discovery and safety testing



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