NEWCELLS
IN VITRO MODELS TO ACCELERATE CLINICAL TRANSLATION

iPSC-derived Retinal Organoids: best-in-class 3D *in vitro* model to progress for retinal therapy development to the clinic

The go-to 3D model for deeper insights into retina biology

What you can achieve:

- Developmental studies for retina tissue
- Drug safety and efficacy study for lead candidates
- Disease model development with isogenic controls
- Gene therapy vector assessment in vitro

What forms the basis of the study:

- iPSC derived organoids grown individually per well in 96-well plates
- Presence of key retinal cell types
- Formation of neural network owing to physiologically relevant localization of cell types
- Responsiveness to toxins

How can Newcells help

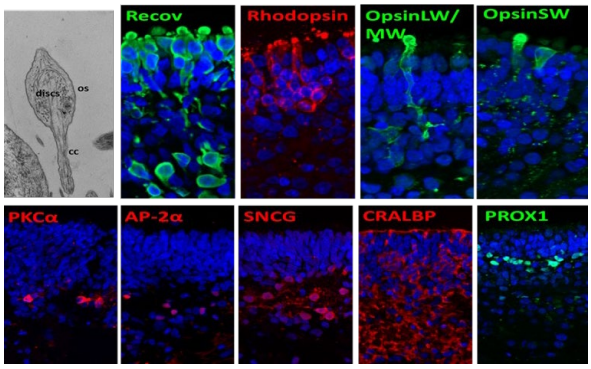
Deliver tailor-made services for retina drug development and gene therapy studies.

Scalable production of retinal organoid products that can be delivered globally for in-house studies

iPSC-derived 3D retinal organoid containing of all key retinal cell types

- Newcells iPSC-derived retinal organoids are physiologically-relevant as they follow the development timeline of retinogenesis in vivo and contain all major retinal cell types.
- The localisation of the key cell types allows to recapitulate the architecture of the human retina.
- The organoids demonstrate functionality as the primitive photoreceptor outer segments are formed
- Retinal organoids are available on-demand through regular batch release every 4-6 weeks as well as through tailor-made projects in our state-of-the art UK facilities.

A:



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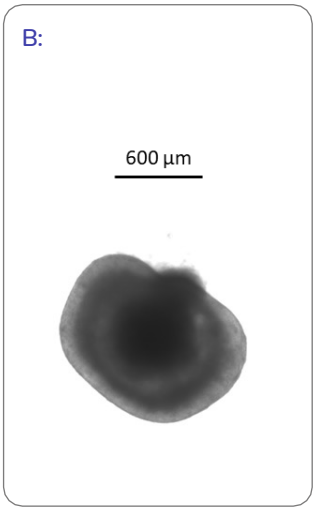

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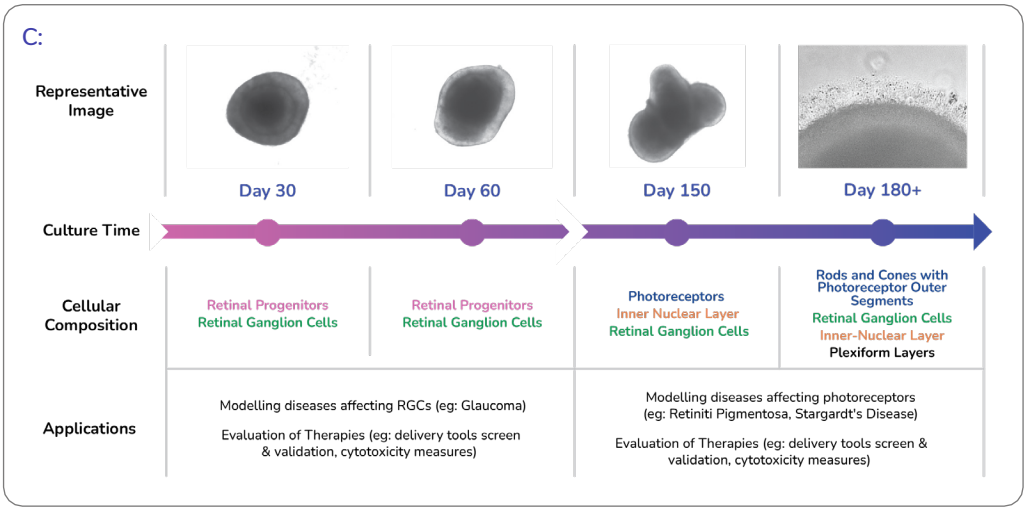

Figure 1: A) Fluorescent labelled cells of human iPSC-derived retinal organoids B) Brightfield image of Day 150 retina organoid C) Cell population in the retinal organoid in different stages of development as per culture timeline

Predictive High Throughput Retinal Toxicity Assessment

- The iPSC-derived retinal organoid model can be used for high-throughput screening of retinal toxicity.
- Experiments carried out on these retinal organoids can distinguish between potential toxins and non-toxins for retina as the retinal organoids have been validated with known cytotoxic and non-toxic compounds (Figure 2A)
- Presence of all cell layers allow drug permeation as seen from the use of doxorubicin (Figure 2B) thus allowing evaluation of topically-applied drugs

Gene Therapy Vector Evaluation (Figure 3)

- Rapid *in vitro* evaluation of AAV vectors with highest transduction efficiency of photoreceptor cells .
- Initial safety and efficacy testing of new AAV variants with promoter and transgene previously used in clinical trials (e.g., the GRK1 promoter was used in two clinical trials - NCT03584165 and NCT03872479).
- Screening of AAV gene therapy vectors; a study in collaboration with Professor McLaren at University of Oxford (McClements et al TVST 2022) confirmed robust and efficient transduction of human photoreceptor-like cells by AAV vectors highlighting that highest transduction efficiency was as achieved with AAV2 7m8 and when using the ubiquitous CAG promoter **(Fig 3A).**
- Assessment of tropism of AAV vectors in photoreceptor-type cells. The study above demonstrated that an AAV vector with a CAG-driven transgene transduced a broad range of cell types while vectors with GRK1-driven transgenes showed a more specific targeting of photoreceptors **(Figure 3B).**
- Evaluation of in vitro safety of AAV vector. The work also demonstrated that the viability of the retinal organoids was not affected by AAV transduction **(Figure 3C).**

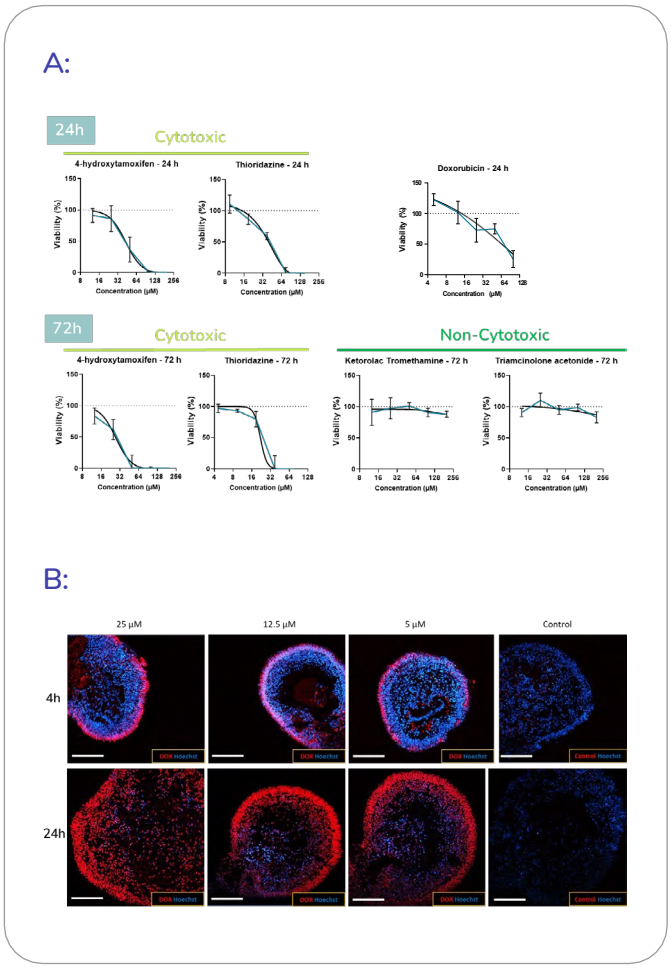

Figure 2: A) Dose response curve for known retinal toxins 4-hydroxytamoxifen, thioridazine and doxorubicin and non-toxic compounds Ketorolac Tromethamine and Triamcinolone acetonide B) Fluorescent imaging for retinal organoids stained with different dilutions of doxorubicin at 4h and 24 h.

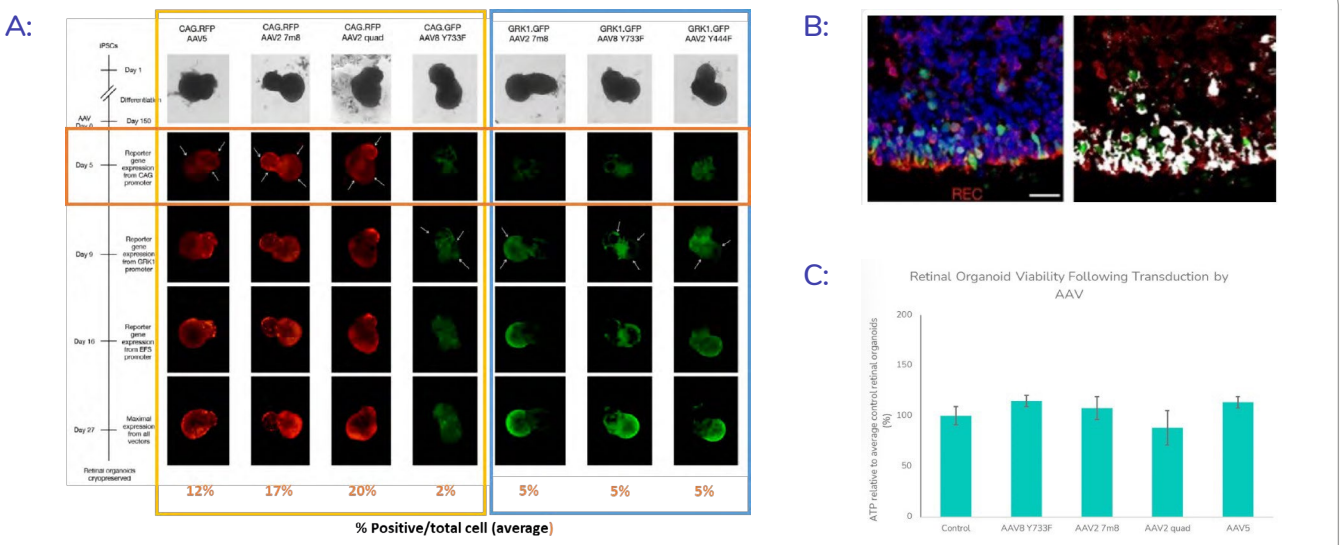

Figure 3 A) Evaluation of the transduction efficiency of iPSC-derived retinal organoids using different recombinant AAV vectors variants to test various capsids (AAV2 7m8, AAV2 quad, AAV2 Y444F, AAV5 and AAV8 7m8) and reporter genes (viz RFP and GFP) under the control of CAG or GRKA1 promoters. B) Recoverin (REC) staining (red) and GFP transgene expression (green) shown on the left panel and signal overlap (white) on the right panel. Each retinal organoid was transduced with 1E+10 genome copies. C) Bar graph data shows that AAV transduction of retinal organoid did not affect organoid viability relative to control untreated retinal organoids.

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Disease Modelling (Figure 4)

Newcells iPSC-derived organoids have been used to model the Retinitis pigmentosa's autosomal dominant mutations; namely the mutation in pre-mRNA processing factor 31 (PRPF31), characteristic of RP Type 11.

- Capture stark differences in control and patient photoreceptor cells in the organoids using TEM (that is not visible in brightfield imaging)
- Replicate 'adaptive survival' in diseased photoreceptor cells in response to oxidative stress, which is known to contribute to the RP disease progression as seen from the apoptotic nuclei (red-dotted circle) and stress vacuoles (blue-dotted circle).

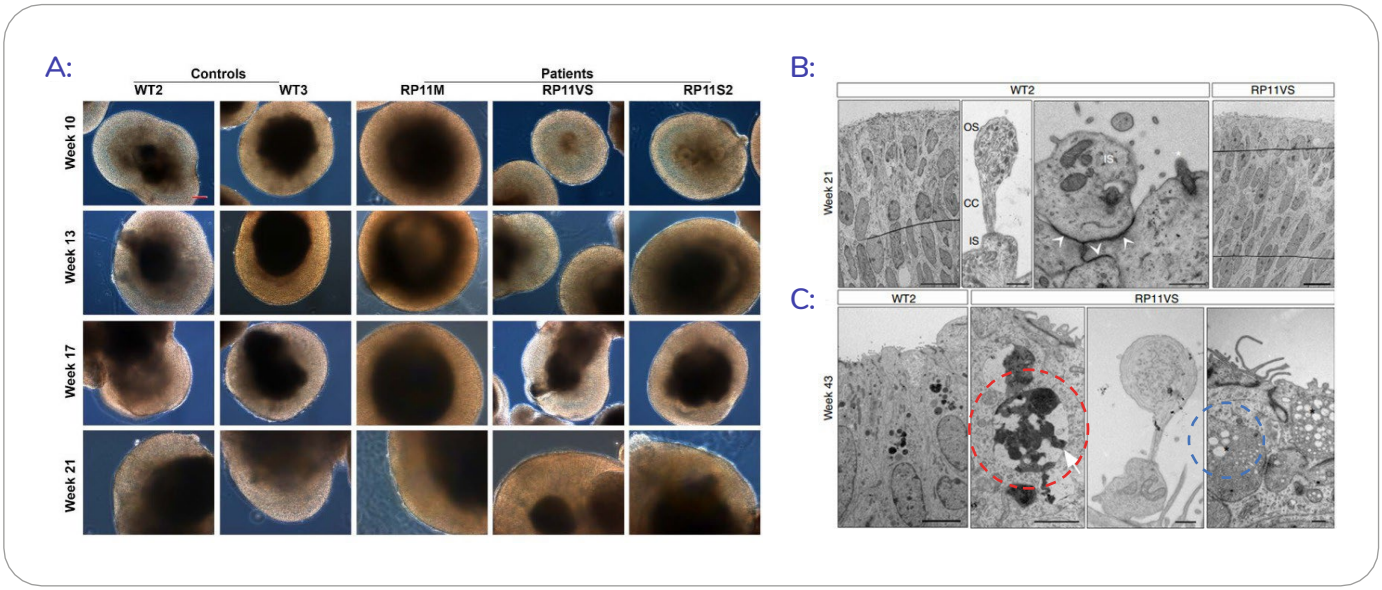

Figure 4 A) Brightfield images of iPSC-derived retinal organoids from healthy donors (WT2 and WT3) and RP patients (RP11M, RP11VS and RP11S2) (B) TEM revealed the presence of outer limiting-like membrane (white arrows), inner segments (IS), connecting cilia (CC) and developing outer segments (OS) in retinal organoids after 21 weeks in culture, scale bars: 10 µm, 500 nm, 500 nm and 10 µm C) At 43 weeks in culture, TEM showed that patient photoreceptors contained apoptotic nuclei with electron dense structures of condensed chromatin (white arrow) and stress vacuoles (black stars) scale bars: 5 µm, 2 µm, 500 nm, 500 nm.

If you would like further information, please contact our experts or visit our website:

info@newcellsbiotech.co.uk
or visit: www.newcellsbiotech.co.uk/RO

Scan the QR code to download the flyer

Retinal Organoid Services			
SKU No.	Offering	Readouts	Inclusions
Retinal Organoid Services			
RST00000RO	Retinal Toxicity	Brightfield Imaging. ATP/LDH (basic)	3 drugs at 5 concentrations
		Brightfield Imaging, ATP/LDH. Qualitative IF (3 markers). TUNEL (comprehensive)	1 drug at 5 concentrations
RSD00000RO	Retina Disease Modelling	Brightfield imaging Quantitative IF cell viability assay Gene expression Photoreceptor degeneration SEM and TEM	Retinal organoids from healthy donor for relative comparison
RSG00000RO	Retina Gene Therapy Evaluation	Brightfield imaging Quantitative IF cell viability assay Gene expression Photoreceptor degeneration SEM and TEM	1 gene therapy vector at 3 concentrations

Retinal Organoid Products				
SKU No.	Offering	Readouts	Time-points	Inclusions
Live Retinal Organoids Product				
RP000D60RO	Human iPSC-derived retinal organoids (n=10) in 5 ml vial filled with organoid culture medium	N/A	Day 60	Pasteur pipettes (n=3), 96 well plates (n=1) and organoid culture medium (serum-free)(135 ml)
RP00D150RO			Day 150	
RP00D180RO			Day 180	
Retinal Organoid Frozen Pellets				
RP0D30ROFP	Human iPSC-derived retinal organoids (N=16) lysed and frozen in 5 ml microcentrifuge tube	N/A	Day 30	Packed & Shipped in dry ice
RP0D60ROFP			Day 60	
RP0D90ROFP			Day 90	
RPD120ROFP			Day 120	
RPD150ROFP			Day 150	
RPD180ROFP			Day 180	
RPD210ROFP			Day 210	
Retinal Organoid Frozen Sections				
RP0D60ROFS	Human iPSC-derived retinal organoids frozen sections from > 36 organoids (10 µm thickness, 6 sections/slide, the sections are grouped in 3 areas containing at least 12 organoids per section) on a microscopic slide, 6 sections of a pool of organoids)	N/A	Day 60	Shipped at -20°C
RP0D90ROFS			Day 90	
RPD120ROFS			Day 120	
RPD150ROFS			Day 150	
RPD180ROFS			Day 180	
RPD210ROFS			Day 210	
Retinal Organoid Culture Medium				
RP0000M500	Human iPSC-derived retinal organoid culture medium. 500 mL in 1 bottle	N/A	N/A	N/A

iPSC-derived Retinal Organoids

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