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Retinal organoids as an emerging tool for *in vitro* pre-clinical testing

Presenting and lead author: Valeria Chichagova[1] (valeria.chichagova@newcellsbiotech.co.uk)

Co-authors: Carol de Santis[1], William Atkin[1], Hannah Steward[1], Carolina Gandara[1]
[1]*Newcells Biotech, Newcastle upon Tyne, United Kingdom*

Abstract

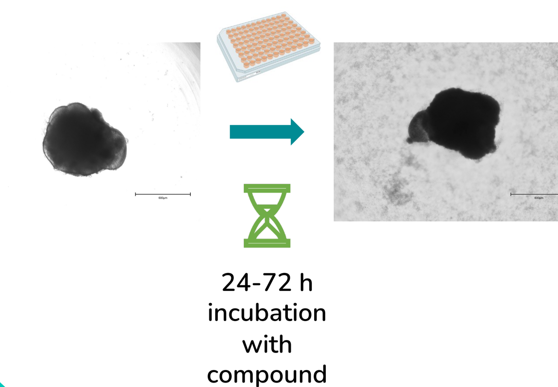
The number of patients with non-treatable visual impairment caused by retinal degeneration is on the rise affecting millions of people worldwide. Application of animal models in the study of disease progression and in drug discovery is limited by the structural and functional differences when compared to human retina. Hence, there is an urgent and unmet need for in vitro models that would recapitulate human physiology and retinal function. We developed methods for production and scaling up of retinal organoids derived from human induced pluripotent stem cells (iPSCs). By 5 months of the differentiation, the organoids contain all the major neural retinal cell types, including photoreceptors, bipolar, horizontal and amacrine cells, and Muller glia. We tested the utility of the organoids for a range of applications, including evaluating their response to a range of known retinotoxins. The organoids were exposed to the compounds for 24 and 72 hours and cell viability was determined used an ATP assay. In addition, we provide evidence for small molecule penetration of retinal organoids confirming suitability of the model for the use with this class of compounds. The development and validation of the retinal organoid model will provide the missing link between compound screening and clinical trials and serve as a model for testing the efficacy and toxicity of drugs thereby providing the in vitro disease models that recapitulate diversity of the human disease, avoiding generation of animal models with targeted mutations as in current practice.

Introduction

The number of patients with non-treatable visual impairment caused by retinal degeneration is on the rise affecting millions of people worldwide. Application of animal models in the study of disease progression and in drug discovery is limited by the structural and functional differences when compared to human retina. Hence, there is an urgent and unmet need for *in vitro* models that would recapitulate human physiology and retinal function. We developed methods for production and scaling up of retinal organoids derived from human induced pluripotent stem cells (iPSCs) and tested them for application in *in vitro* toxicology studies.

Methods

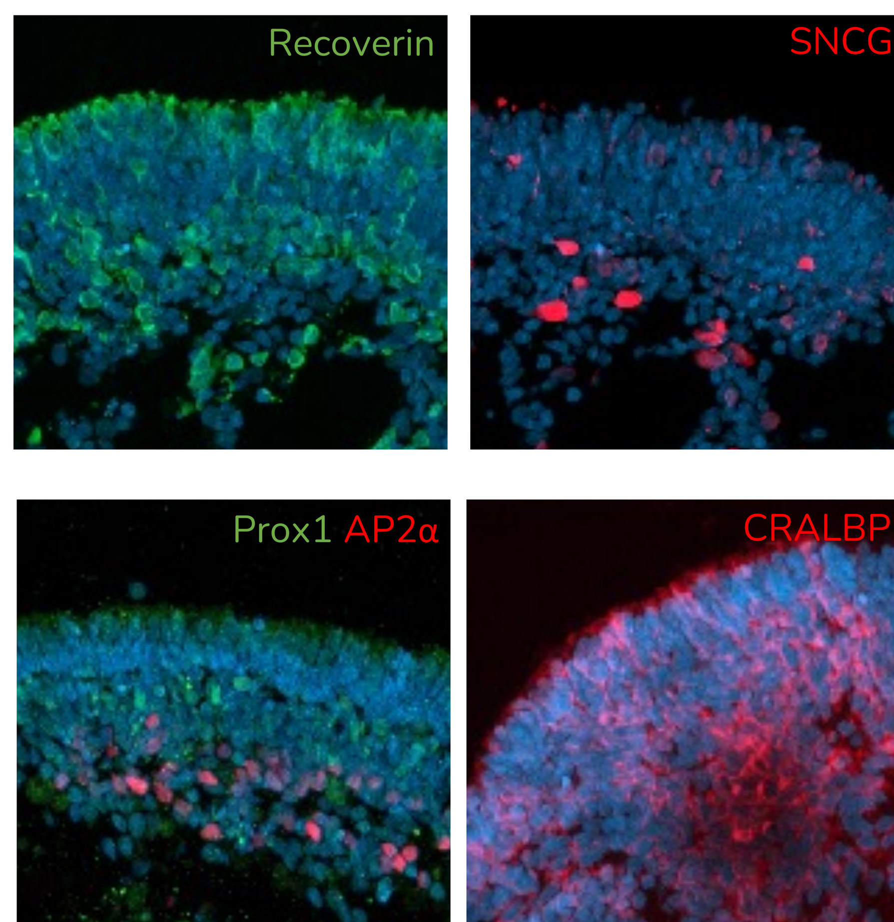
Retinal organoids were differentiated at scale using control human iPSCs in a 96-well plate format to allow higher throughput. After 150 days of differentiation the organoids were assessed for the presence of key retinal cell types using immunofluorescence analysis.



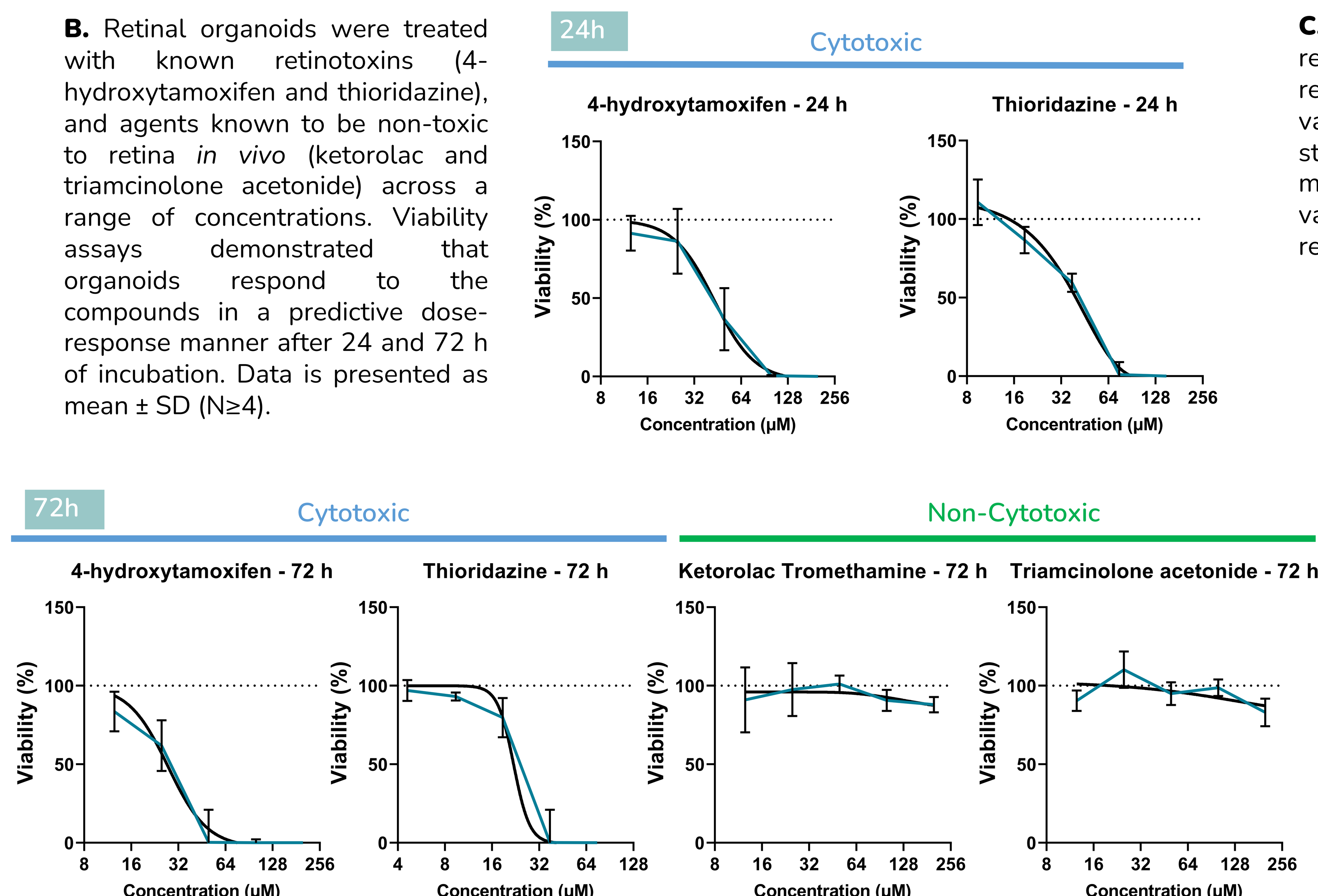
We tested the organoids for their response to agents known to be toxic or non-toxic to retina based on the existing *in vivo* data. After 24 or 72h incubation, viability of the organoids was assessed using CellTiter-Glo® 3D ATP assay.

Results

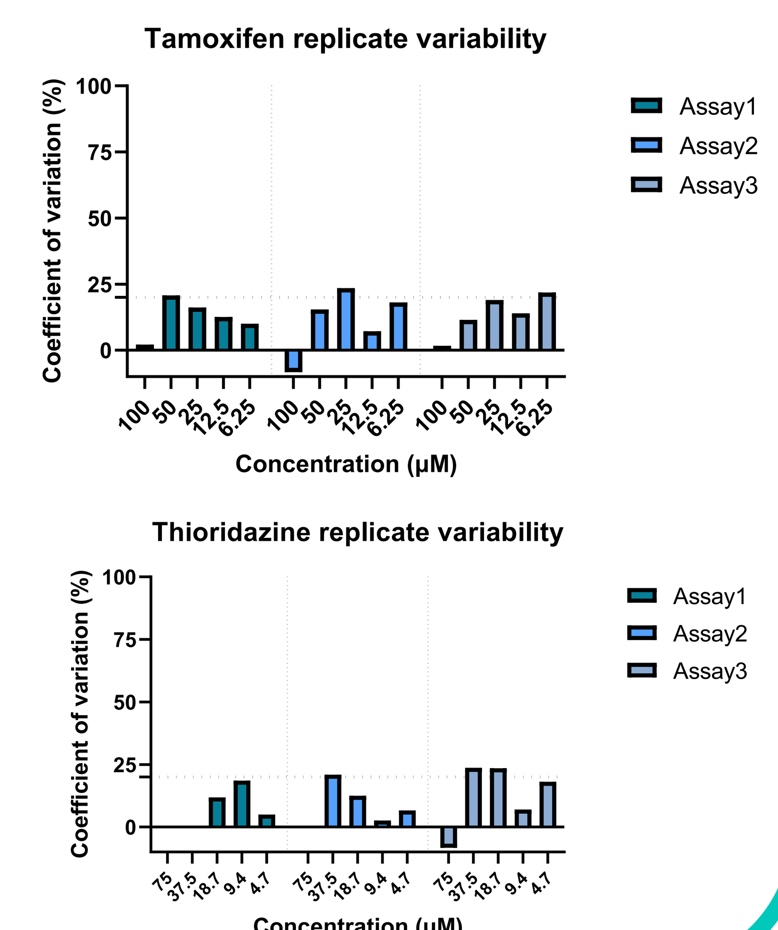
A. At day 150 of differentiation, retinal organoids contain major retinal cell types, including photoreceptors (Recoverin), retinal ganglion cells (SNCG), amacrine cells (AP2α), horizontal cells (Prox1) and Muller glia (CRALBP).



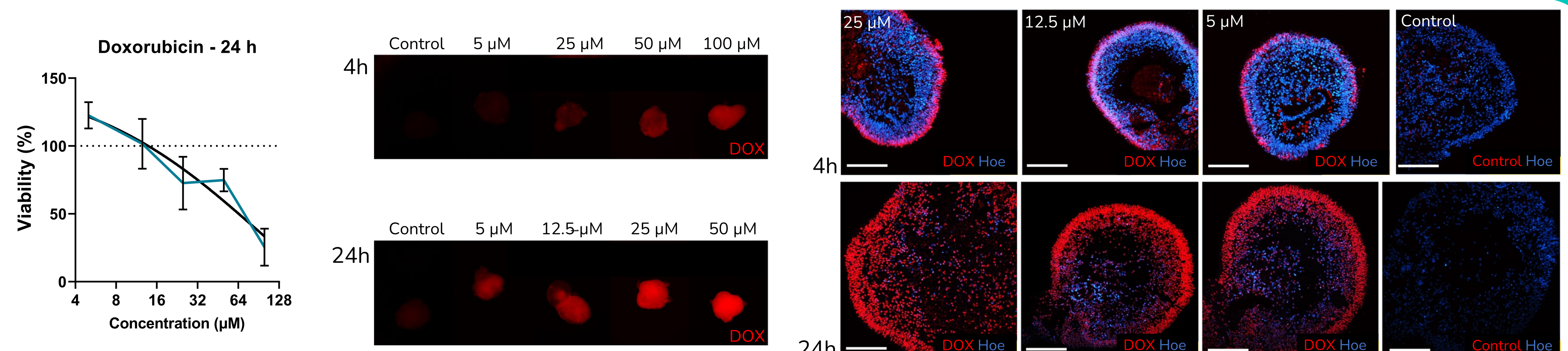
B. Retinal organoids were treated with known retinotoxins (4-hydroxytamoxifen and thioridazine), and agents known to be non-toxic to retina *in vivo* (ketorolac and triamcinolone acetonide) across a range of concentrations. Viability assays demonstrated that organoids respond to the compounds in a predictive dose-response manner after 24 and 72 h of incubation. Data is presented as mean ± SD (N≥4).



C. Organoid viability experiments were repeated across batches to demonstrate result consistency. The coefficient of variation was calculated by dividing the standard deviation (SD) by the replicate mean, and multiplied X100. Coefficient of variation % ≤ 20 % indicates good assay reproducibility.



D. Retinal organoids were assessed for their suitability for studies with small molecules. In order to test whether small molecules penetrate the organoids, doxorubicin was used as a test compound. The intrinsic fluorescence of doxorubicin facilitates visualisation of the drug penetration. Exposure of the iPSC-derived retinal organoids to doxorubicin reduced cell viability in a dose-dependent manner. Immunofluorescence analysis showed that doxorubicin penetrates the organoids after 4 and 24 h incubation.



Conclusion

- Human iPSC-derived retinal organoids are an emerging *in vitro* tool for various applications.
- They are increasingly showing utility in various applications and have a potential of being used in safety screens of new compounds.
- We demonstrate that:
  - Retinal organoids respond to compounds known to induce retinal toxicity in a dose-response manner
  - Known non-toxic compounds have no effect on viability
  - We provide evidence for small molecule penetration of retinal organoids confirming suitability of the model for the use with this class of compounds
  - We demonstrate that our assays are reproducible
- The development and validation of the retinal organoid model will provide the missing link between compound screening and clinical trials and serve as a model for testing the efficacy and toxicity of drugs avoiding using animal models with targeted mutations as in current practice.