Comparison of multiple batches of human iPSC-derived retinal organoids produced at large scale

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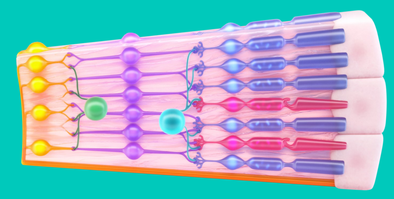


Purpose

Due to well-known limitations of *in vivo* and existing *in vitro* retinal models, a 3D *in vitro* model of the human retina which is reproducible and able to accurately predict *in vivo* outcomes is highly desirable.

Our aim was to investigate the consistency of human iPSC-derived retinal organoids (RO) produced at large scale by quantifying the gene and protein expression levels of key retinal cell markers across differentiation in multiple batches.

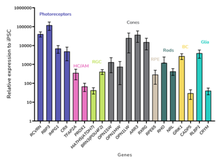
Methods

Retinal organoids selected at random

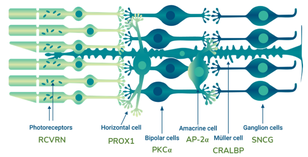
4 biological replicates

0 – 210 days in culture

RT-qPCR **IF**



Gene expression relative to iPSCs



Protein expression and localization

Results

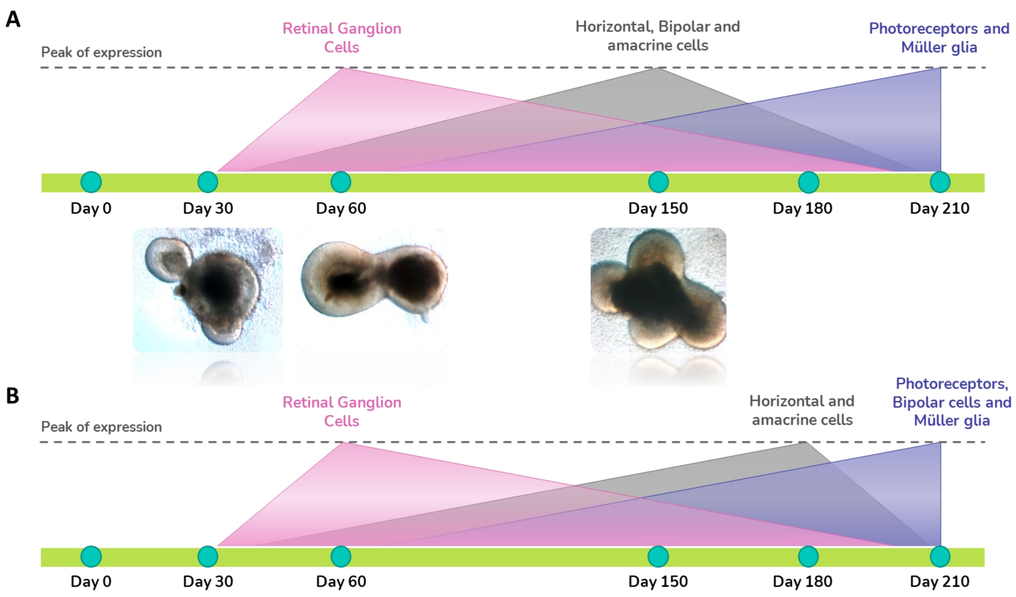


Figure 1. Photoreceptor (PR; Genes: *RCVRN, RBP3, IMPG1, CRX;* Protein: RCVRN), bipolar (BC; Genes: *GRIK1, CADPS;* Protein: PKCα), Müller glia (MG; Genes: *RLBP1, CRYM;* Protein: CRALBP), retinal ganglion cell (RGC; Genes: *MATH5, BRN3;* Protein: SNCG), horizontal and amacrine (HC/AC; Genes: *PROX1, TFA2A;* Protein: PROX1, AP2α), Cone PR (Genes: *OPN1SW, OPN1MW, OPN1LW, ARR3, RXRG;* Protein: OPN1MW/LW) and Rod PR (Gene: *RHO, NRL;* Protein: RHO) cell markers are expressed at different times throughout RO development which resembles *in vivo* development. **(A)** Gene expression and **(B)** protein expression of RGC markers peak between day 30-90, when PR progenitor cells start differentiating. Expression of cone and rod PRs initiate from day 120 and achieve highest expression and maturation levels after 210 days in culture.

Results

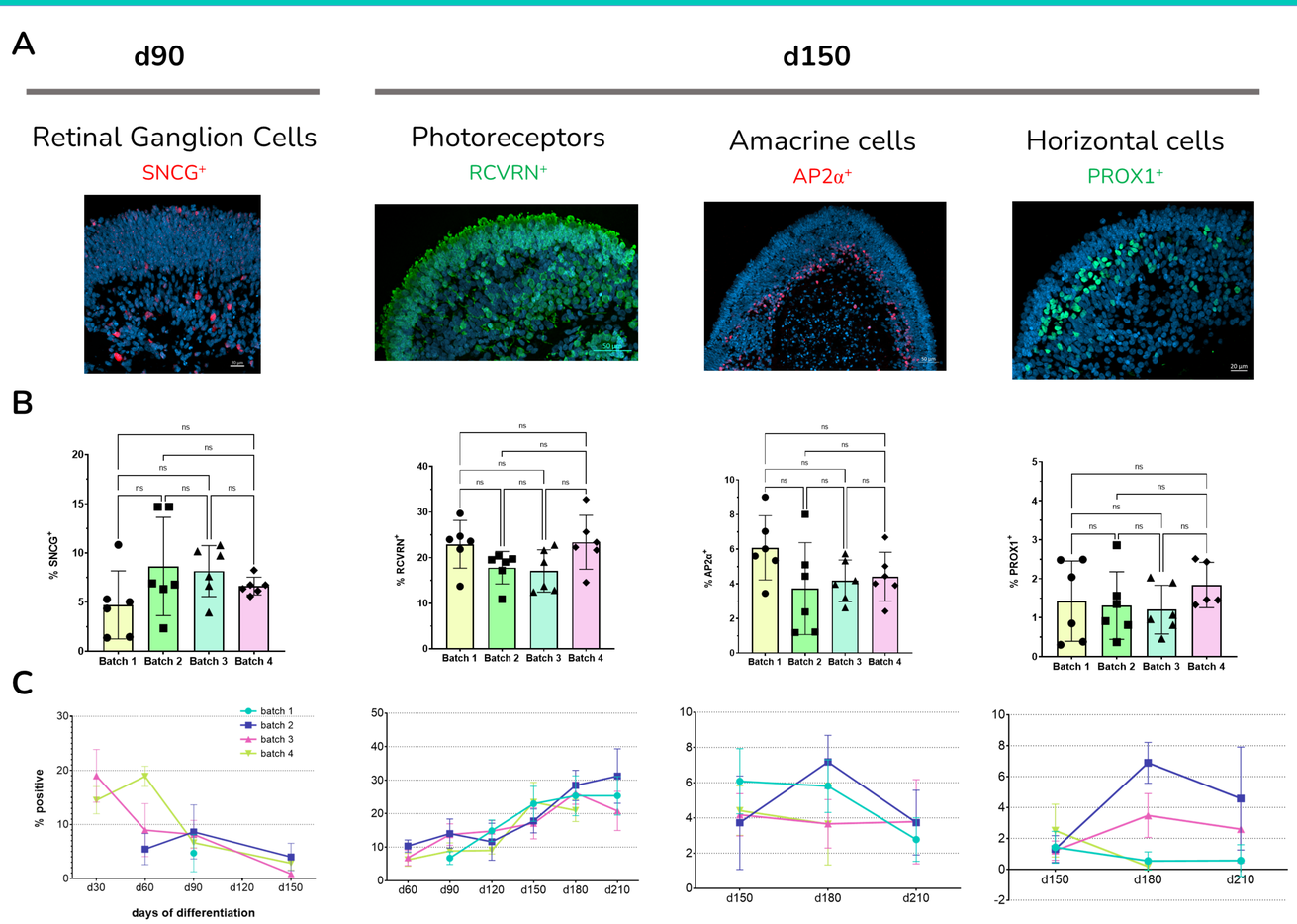


Figure 2. (A) Distribution and localization of key retinal cells in RO at day 90 and 150 of differentiation. **(B)** Quantification of cell populations revealed that at day 150 of development, PRs, AC, RGC and HC represent on average 20%, 4.6%, 3% and 1.6% of the total number of cells. This average was consistent across 4 biological replicates ($p>0.05$; error bars = SD) for PR, AC and HC at d150, and RGC at d90 (mean 7%). **(C)** RGCs are more abundant in early developmental stages, whereas the appearance of PRs gradually increases throughout RO development and plateaus at day 210 with nearly 30% RCVRN$^+$ cells.

Conclusions

We analysed the gene and protein expression profile of key retinal cell markers across differentiation in four batches of human iPSC-derived ROs. We observed that PRs, HCs and ACs, at later stages of development, and RGC at early stages, appear at consistent levels across multiple batches. This data set provides crucial information for pre-clinical studies in ROs with application in drug discovery, disease modelling and gene therapy.

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