Development of photoreceptor outer segments in human iPSC derived retinal organoids produced at large scale

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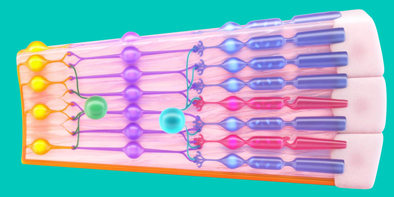


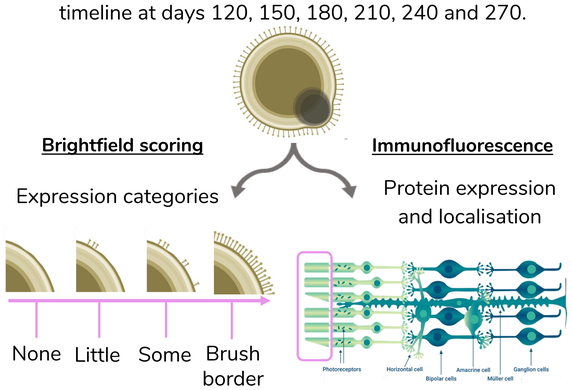
Purpose

Photoreceptor outer segments (POS) play a crucial role in the process of vision, and their dysfunction is implicated in retinal degeneration and eventual vision loss. Retinal organoids (ROs) have become a valuable tool in the study of retinal disease mechanisms and in drug discovery pertaining to their ability to recapitulate aspects of the human retina. Understanding the dynamics of POS development and consistency across multiple batches of ROs is crucial for their wider adoption and routine use.

In this study we have assessed the dynamics, efficiency and batch-to-batch reproducibility of POS development across three batches of wildtype human iPSC-derived retinal organoids produced at large scale.

Methods

- Three independent batches of wild-type ROs were assessed for POS development.
- 100 ROs per batch were monitored over the differentiation timeline at days 120, 150, 180, 210, 240 and 270.



Results

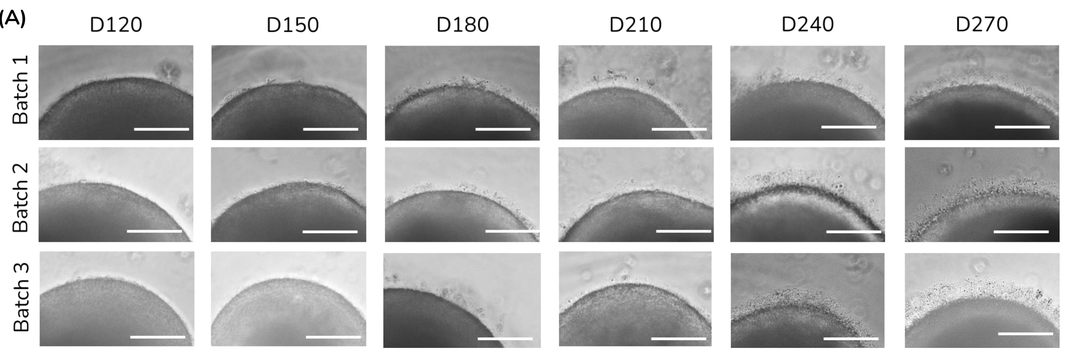
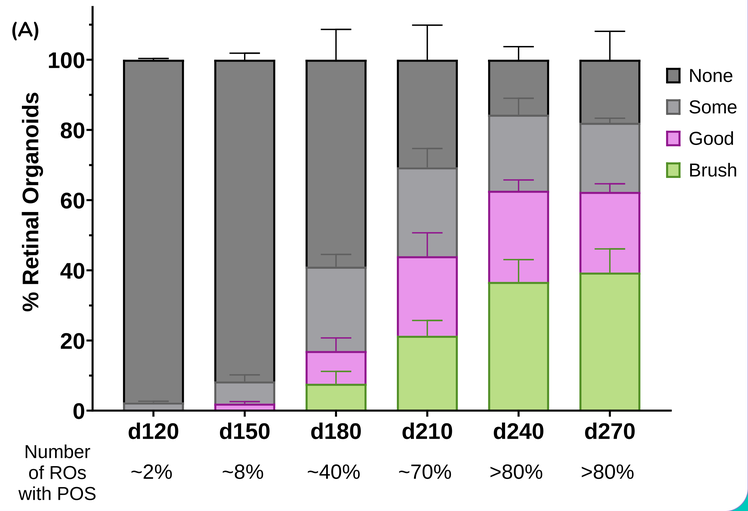


Figure 1. Over maturing timepoints POS increase in number, density and length.

(A) Representative brightfield images of RO POS across 3 biological repeats. Scale bar 150 µm.

Figure 2. POS brush borders start appearing at ~Day 180, increase over time and plateau at Day 240. (A) 100 ROs scored in 4 categories for presence of POS across 3 batches with up to ±10% batch-to-batch variability (mean ± SEM). Percentages (%) indicate total number of ROs with presence of POS.



Results

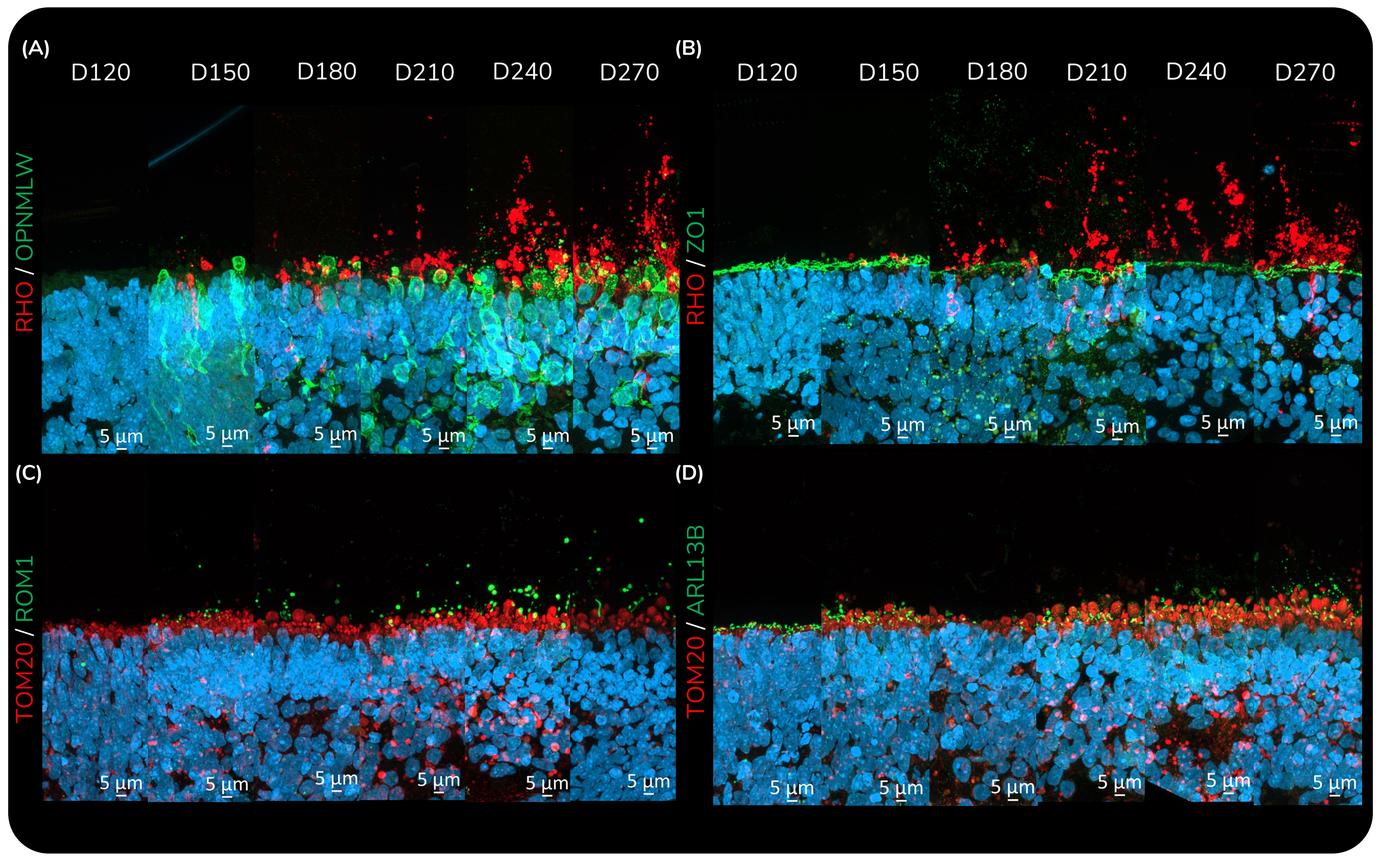


Figure 3. POS increase in presence and length over maturing time points at the protein level.

(A) Expression of cone and rod cells, marked by OPNMLW and RHO respectively, increase over time, with the outer segment portion above the outer nuclear layer (marked by Hoechst). **(B)** Rod cell outer segments, marked by RHO, increase in length cross the outer limiting membrane, marked by ZO1. **(C)** Inner segments, marked by TOM20, protrude above the outer nuclear layer over time with an increase in photoreceptor outer segments marked by ROM1. **(D)** Connecting cilia, marked by ARL13B, increase in number and localise with the protruding TOM20 marked inner segments.

Conclusions

Here we have determined the timeline of POS development in wildtype iPSC-derived ROs both by brightfield appearance and protein expression profiles across multiple batches produced at scale. We observed that large scale batches of ROs reproducibly develop POS and will do so by D240, allowing these more mature ROs to be a suitable model for pre-clinical studies involving disease modelling, drug discovery and gene therapy where POS formation is relevant.

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