Retina-on-Chip:
Modeling and treating eye diseases in a dish.

Edwin van Oosten[1,#,*], Devin Veerman[2,#,*], Stijn Berendsen[1,#], Rob Collin[3], Dirk Lefeber[4], Loes Segerink[5], Seba Almedawar[6], Jürgen Prestle[6], Parth Patel[7], Susan Roelofs[7,&], Stefan G. Kauschke[6,&], Andries van der Meer[2,&], Alejandro Garanto[1,3,&]

*[1]Department of Pediatrics, Amalia's Children hospital, Radboud university medical center, Nijmegen, the Netherlands [2]Applied Stem Cell Technologies group, University of Twente, Enschede, the Netherlands [3]Department of Human Genetics, Radboud university medical center, Nijmegen, the Netherlands [4]Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud university medical center, Nijmegen, the Netherlands [5]BIOS lab-on-a-chip, University of Twente, Enschede, the Netherlands [6]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany [7]Locsense B.V., Enschede, the Netherlands [#]Scientific personnel consortium [&]Principal investigators consortium *Contributed Equally*

Background

- The human retina is a highly organized structure consisting of neural retina, retinal pigment epithelial (RPE) cell layer, and vasculature, called the choroid (**Figure 1**).
- Retinal diseases lead to photoreceptor cell death, causing progressive blindness (1).
- We are investigating two particular retinal diseases:
  - Inherited retinal diseases (IRDs): Rare monogenic progressive disease with early age of onset.
  - Age-related macular degeneration (AMD): Multifactorial complex disease with late age of onset.
- Animal models do not completely recapitulate the human disease.
- Current human cell models often lack three-dimensional (3D) complexity of the human retina.

Aim of the project:

Develop a Retina-on-Chip system based on stem cell technology to accelerate disease modeling, therapeutics evaluation, and prevention for retinal diseases.

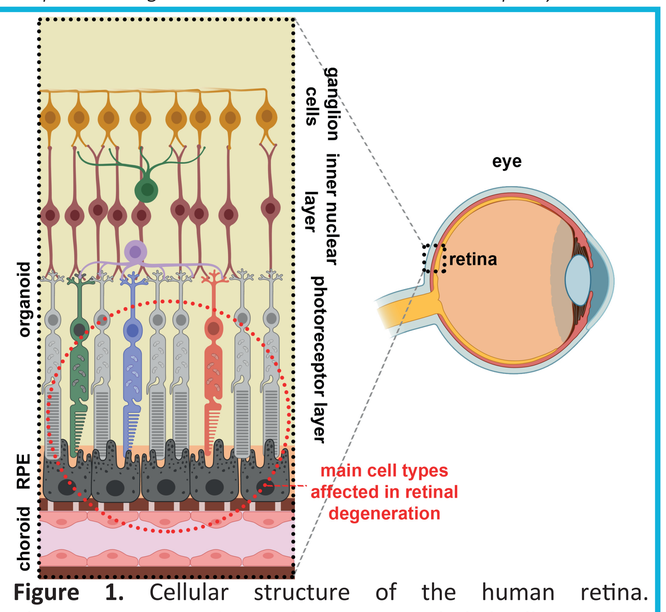


Figure 1. Cellular structure of the human retina. Photoreceptors and retinal pigment epithelial cells are often affected in retinal diseases.

Retinal organoids

- Retinal organoids (ROs) are 3D iPSC-derived retina-like structures.
- All retina-specific cell types are represented in ROs, including photoreceptor cells (2).
- After 140 days *in vitro*, photoreceptor cells in ROs develop outer segments, often referred to as brush borders (**Figure 2**).

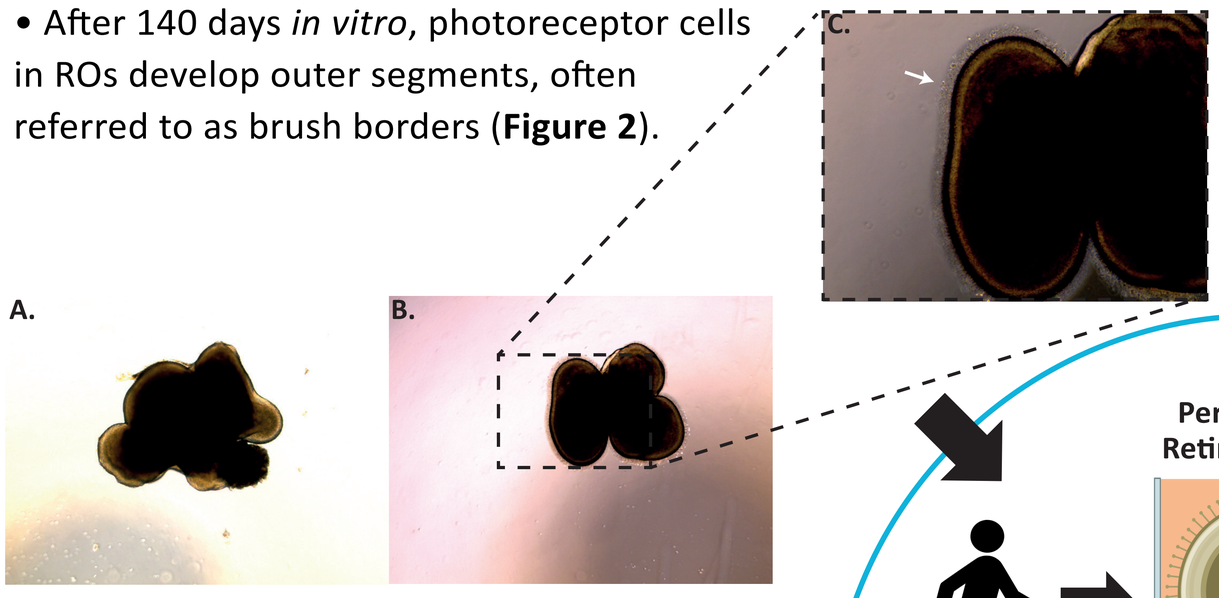
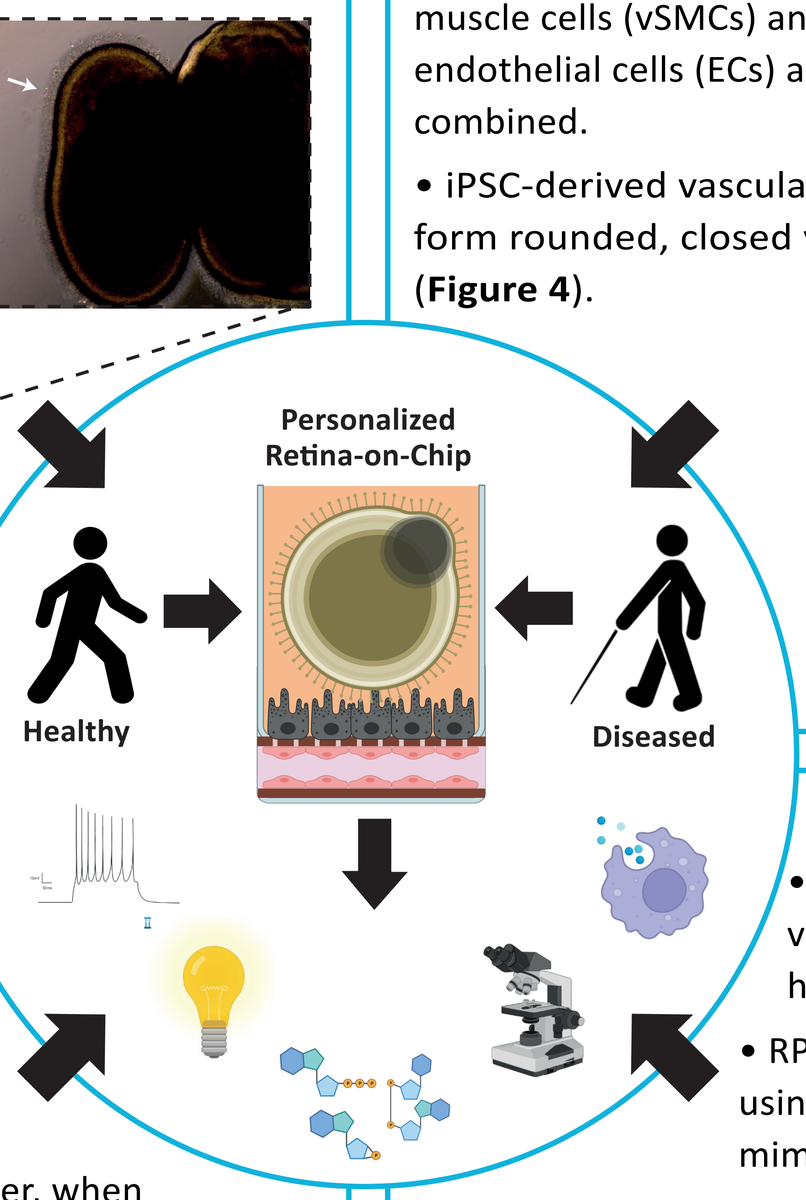


Figure 2. Brightfield image of iPSC-derived ROs. **A.** ROs at 140 days *in vitro*. **B.** ROs at 189 days *in vitro*. **C.** At this stage, a clear brush border, representing the outer segments of photoreceptor cells, can be seen along the edge of the ROs.



Vasculature

- The interaction between the RPE and choroidal vasculature is vital for normal eye function (3).
- To create a choroidal-like vasculature, iPSC-derived vascular smooth muscle cells (vSMCs) and endothelial cells (ECs) are combined.
- iPSC-derived vasculature form rounded, closed vessels (**Figure 4**).

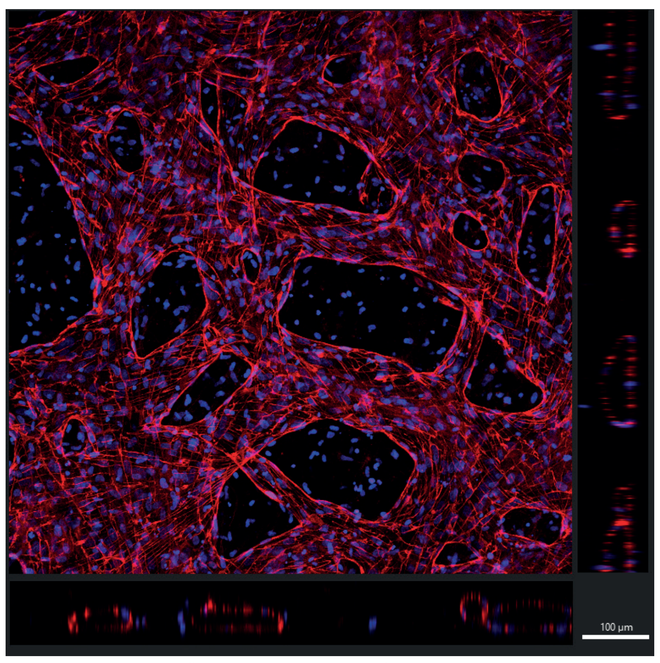


Figure 4. Immunofluorescent image of iPSC-derived vasculature using vSMCs and ECs. Red = VE-cadherin, Blue = DAPI, scalebar= 100 µm

RPE cells

- RPE cells have several functions in the human retina (3), including:
  - Formation of the blood-retinal barrier
  - Photoreceptor outer segment renewal
  - Important role in the visual cycle
  - Nutrient and metabolite transport
- iPSC-derived RPE cells form a tight, pigmented barrier, when cultured in transwell inserts. (**Figure 3**)

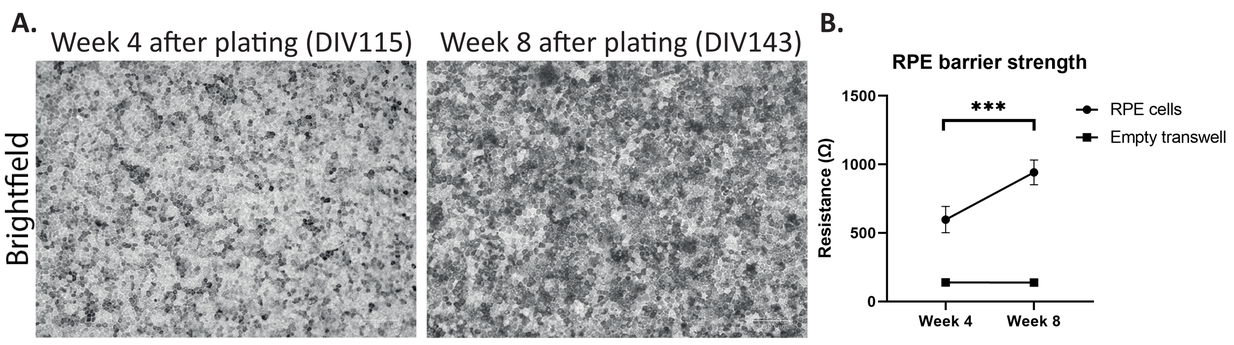


Figure 3. RPE form a pigmented monolayer. **A.** Brightfield image of RPE cells cultured on transwell inserts 4 and 8 weeks after plating at P3 (115 days and 143 days *in vitro*, respectively). RPE increase in pigmentation over time. **B.** TEER measurements show that RPE form a tight barrier which increases in strength over time. Data represents mean ± SD ***$p < 0.001$

Microfluidic chip

- The Retina-on-Chip (RoC) combines ROs, RPE, vasculature, and microfluidics to simulate the human retina.
- RPE cells are separated from the vasculature by using a membrane of 2 µm thickness in order to mimick the Bruch's membrane (4).
- The microfluidic chip allows for cultivation and maturation of both iPSC-derived ROs as well as RPE cells (**Figure 5**).

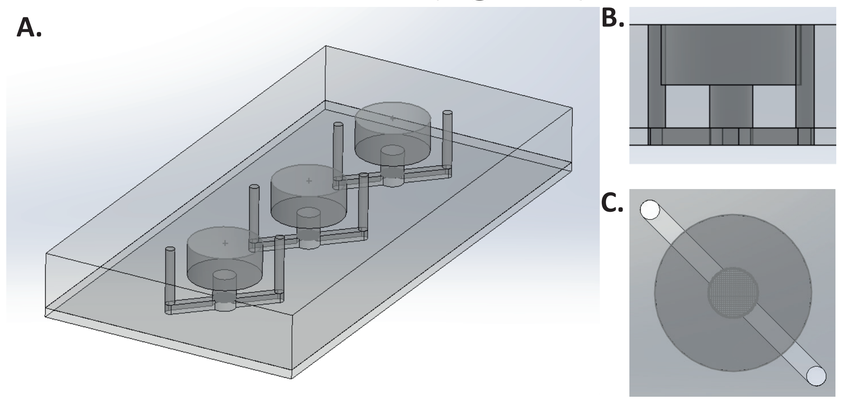


Figure 5. Microfluidic chip design. **A.** Three-dimensional view of the microfluidic chip. **B.** Cross-section and top view (**C.**) of the microfluidic chip design.

Conclusion

- We have reprogrammed patient-derived cells into iPSCs.
- Successful generation of ROs, ECs, vSMCs, and RPE cells from iPSCs.
- First functional readouts are established and being analyzed.
- The first prototype of the microfluidic chip has been designed.

Future plans

- Further development of the microfluidic chip.
- Establishing additional functional readouts.
- Validation of functional readouts in disease models.
- First steps in combining cellular models on the Retina-on-Chip.

(1) Veleri, S., Lazar, C., Chang, B., Sieving, P., Banin, E., & Swaroop, A. (2015). Biology and therapy of inherited retinal degenerative disease: insights from mouse models. Disease Models & Mechanisms, 8(2), 109-129.
(2) Afanasyeva, T., Corral-Serrano, J., Garanto, A., Roepman, R., Cheetham, M. and Collin, R., 2021. A look into retinal organoids: methods, analytical techniques, and applications. Cellular and Molecular Life Sciences, 78(19-20), pp.6505-6532.
(3) Lehmann, G., Benedicto, I., Philp, N., & Rodriguez-Boulan, E. (2014). Plasma membrane protein polarity and trafficking in RPE cells: Past, present and future. Experimental Eye Research, 126, 5-15.
(4) Murphy, A., Truong, Y., O'Brien, C., & Glattauer, V. (2020). Bio-inspired human in vitro outer retinal models: Bruch's membrane and its cellular interactions. Acta Biomaterialia, 104, 1-16.

Support: