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Challenge 23: Retinal 3D

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Retinal 3D:
A Physiologically-Competent Human Retinal 3D Model

Launch Meeting

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or **Marianne Uteng** (Novartis) (who will be present at the meeting)

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The Challenge

Establish a human 3D retinal cell model:

- Physiologically-competent and predictive of human physiology
- Consist of all major cell types of the retina : Müller- and micro-glia, RPE and neurons (including photoreceptors)
- Enable cellular interplay
- Recapitulates key morphological and functional features
- Provide a panel of relevant readouts for functional testing

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Why was this Challenge developed?
Scientific – Business - 3Rs drivers

- Over 60 million people worldwide are blind
  - Leading causes of blindness in the industrial world is AMD
- Globally, in ophthalmology, more than 600 R&D projects running – with more to come

- Large scientific and business impact of a novel human 3D retina model

- Current in vitro models have major limitations:
  - cells are immature, no interplay between different cell types, functionality limited, lack human relevance etc.

- strong need for better models of human relevance

- majority of studies for efficacy and safety testing in ophthalmological drug development are performed in animals due to lack of relevant in vitro models
  - Ocular safety studies in vivo use up to 20 animals per compound

- strong impact on 3Rs if human relevant model can be applied for R&D

Current state of the art

- **Single cells:**
  - 2D cell line models (human and rodent): ARPE19, Mio-M1, 661W etc.
- **Complex models:**
  - Retinal explants of mouse, rat and pig
- **Stem cell derived models:**
  - retina 3D spheroids and isolated cells thereof

- → Lack of a ***human relevant***, complex retinal 3D model

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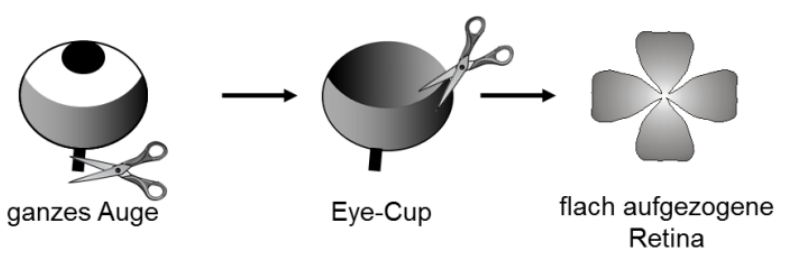
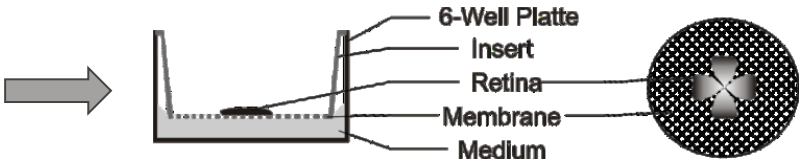
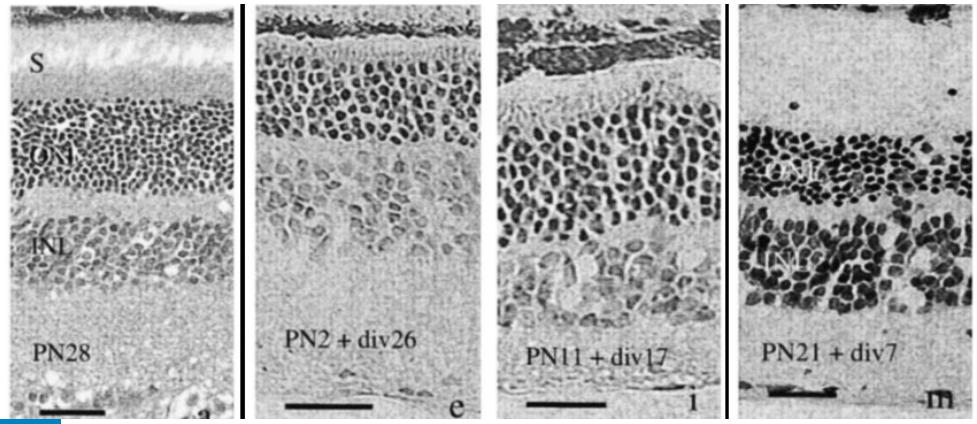
Established complex retinal cell models: Retinal explant cultures



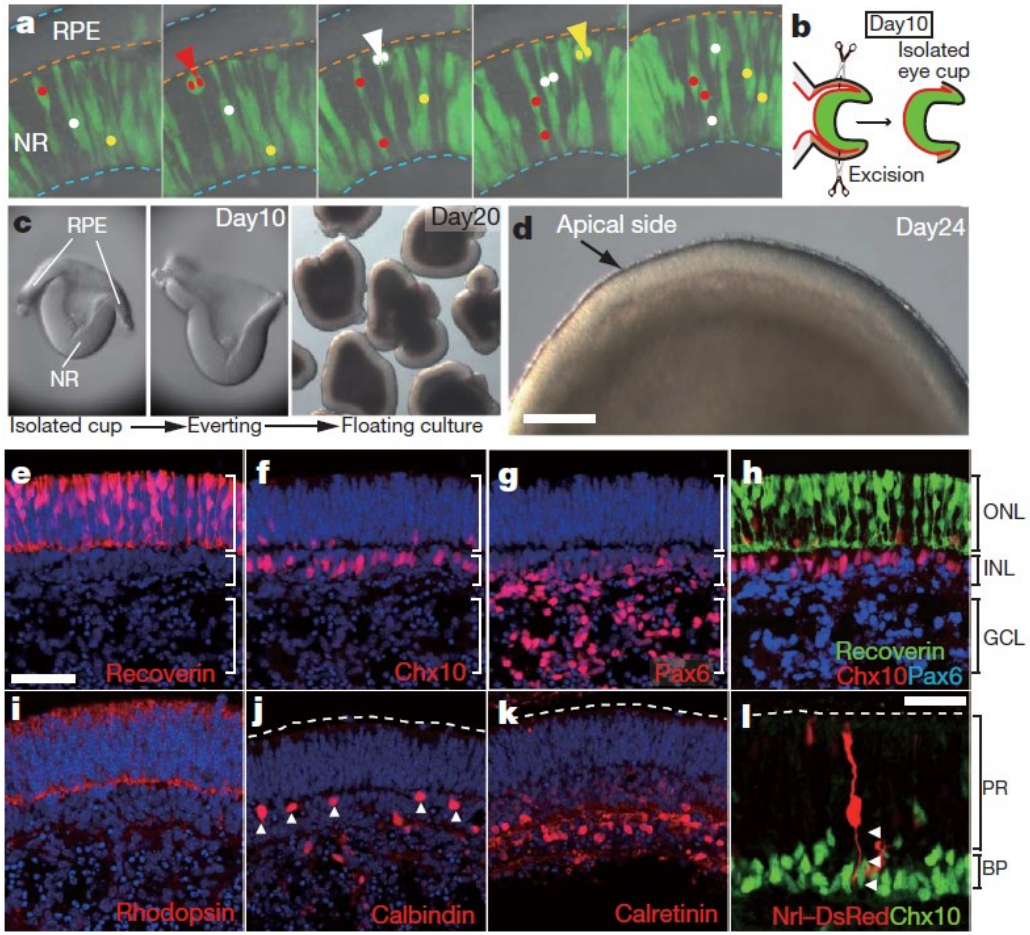




- remains viable as an explant in serum-supplemented growth media for more than 4 weeks
- histotypic development of neonatal as well as preservation of late postnatal mouse retinal structure during long-term culture
- also feasible with:
  - Rat (Pinzon-Duarte et al 2000)
  - Pig (Wang et al 2011)
  - Zebrafish (Kustermann et al 2008)
- **Not feasible with human retina due to lack of tissue**

e.g. Caffé et al. 2001

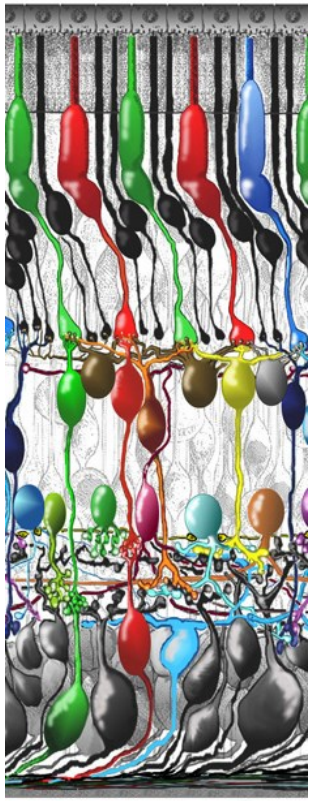
Established complex retinal cell models: Whole embryonic-body culture



- Mouse ES cells generate fully stratified neuro-retinas after 24 days
- Photoreceptors can be isolated and transplanted into eyes in vivo and morphologically integrate
- **Phenotype of cells is pre-mature and long differentiation time for e.g. photoreceptors and glia cells (humans ~6 month)**

e.g. Eiraku et al. 2011; Gonzalez-Cordero et al. 2013

Deliverables **Phase 1**



Mimic the morphology and physiology of the mature human retina:

- Morphological resemblance of layered structure of the retina i.e. plexiform and nuclear layers
- Sustained expression of cell specific (mature) markers by IHC of all implemented cell types
- Basic functional characterization: e.g. synapse formation between neurons by IHC, tight junction expression between RPE cells, phagocytotic activity of microglial cells

What we **don't** want

- A model that requires complex and time consuming set-up, impeding **parallel testing of multiple conditions**
- Incorporate **only** cytotoxicity as a readout
- Incorporate material which is known to show strong **compound adsorption** (e.g. PDMS)
- Require specific **legal work** for acquisition of source cells and materials (i.e. individual informed consent for each study/experiment)

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Deliverables **Phase 2** "Must have"

- **Thorough functional and morphological characterisation of the retinal model including recapitulation of drug induced retinal toxicities:**

- Development of additional methods to address function and/or phenotypic changes of the different cell types in culture

- Provision of accessible morphological and functional readouts and be compatible with standard microscopes

- Recapitulation of retinal toxicities of known drugs (e.g. Chloroquine, …)

- Amenity to the testing of compounds in parallel (multi-well-plate setting)

- Easy to implement in an industry laboratory setting, reproducible and easily transferrable between laboratories

- Assessment of inter/intra-laboratory reproducibility

- Adaptability to other species relevant for safety assessment (rodent and /or non-rodent)

- Evidence of cost efficiency and applicability to individual and longer term experiments

Deliverables **Phase 2** “nice to have”

- Demonstration of the barrier function of outer and/or inner-limiting membrane
- A functional blood-retina barrier
- Vasculature (endothelial cells) to mimic neo-vascularization and leakage of blood vessels to model disease phenotypes such as Diabetic Macular Edema
- Clear strategy how to commercialise the results into a product or service

Sponsors' contributions

- Expertise in ophthalmology and *in vitro* models including specifications for an *in vitro* model which is fit for purpose for drug testing in an industry setting
- Compounds and knowledge of compounds where available for evaluation of both the pharmacological and toxicological performance of the *in vitro* retinal test system
- Potential for in-house testing using the system to test transferability and reproducibility of the 3D retinal *in vitro* model

Thank You

The Sponsors are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

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