3D Retinal Organoids:
New frontiers for regenerative therapies in the eye

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Disclosure: no commercial relationships
The human retina

- Extension of the central nervous system
- 7 main types of neurons and glia organized in 3 cell layers
- Lacks regenerative capacity
Retinal degenerations lead to vision loss or blindness

Retinitis pigmentosa

Age Related Macular Degeneration

Normal retina

Once the retinal neurons die, there’s no treatment available to recover visual function
So what can we do?
The promise of iPS cell technologies

Induced Pluripotent Stem Cells

The promise of iPS cell technologies

- Reprogramming
- Directed differentiation
- Therapeutic strategy
- Cellular differentiation in 2 dimensions
- 3D organoids
- Disease modeling, drug screening, cell therapy

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iPS cells

Gut organoid

Brain organoid

Kidney organoid
Can we use these cells to make a retina?

Zhong et al., *Nature Communications* 2014
Can we use these cells to make a retina?

These 3D retinal “organoids” recapitulate the histological organization and cellular composition of the native retina.

How do they accomplish this?
Retinal organoids closely mimic the timing and progression of human retinal development.

In space and time…
Initiation of Cell Differentiation

hiPSC → neural fate
DMEM/F12/N2
NEAA/hep

neural aggregates

retinal fate
DMEM/F12/B27
NEAA

retinal lineage

Meyer et al, 2009
Recapitulation of the developmental processes leading to the formation of the retina *in vivo*

Eye Field domains

Retinal domains

In vitro

In vivo

Eye Field Specification

Retinal Specification

Optic Cup
Formation of 3D “mini retinas”
Formation of 3D retinal organoids
Cells within organoids follow the spatiotemporal pattern of cell differentiation and lamination of the neural retina.
Cells within organoids follow the spatiotemporal pattern of cell differentiation and lamination of the neural retina.
But are these retinal neurons functional?

Photoreceptors in retinal organoids are able to respond to light!
Retinal organoids for clinical applications

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Reprogramming

Directed differentiation

iPS cells

Cell/ tissue transplantation

3D retinal organoids

Therapeutic strategy

gene-therapy strategies
- gene replacement
- gene correctors / potentiators

nanodelivery strategies
- comparative analysis of delivery efficiency
- cell-type specific targeting
- toxicity studies

drug screening
- restoration of protein function
- cell survival / differentiation / maturation

Disease modeling
Challenges and opportunities

Reprogramming

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• Improving differentiation/survival
  Long production time
  Death of inner neuronal layers at later stages

• Improved disease modeling
  Disease \(\rightarrow\) 6 – 60 years vs. 3D retinal organoids \(\rightarrow\) 6 – ? Months
  Lack of NR/RPE apposition

Directed differentiation

iPS cells

3D retinal organoids

• High throughput capability
  Substantial variability
  Lack of quantitative assays for 3-dimensional models
  Lack of automated technologies
3-D automated reporter quantification technology (3D-ARQ)

- Generation of 3-D retinal organoids
- Automated sorting and handling
- Fluorescence scanning platform
- Drug treatment
- Expression of transgenic fluorescent reporters or fluorescent staining
3D-ARQ

Sensitivity – Reproducibility – Quantitative Power

Fluorescence microplate reader
TECAN infinite M1000

Hoechst: DNA staining dye, nuclear
EGFP: Transgenic protein, cytoplasmic
Calcein: Live cell labeling dye, cytoplasmic
DiI: Cell membrane labeling dye
BodipyTR: Cell membrane labeling dye

Hoechst: S:B ratio
EGFP: S:B ratio
Calcein: S:B ratio
YFP: S:B ratio
DiI: S:B ratio
BodipyTR: S:B ratio

Sensitivity & Reproducibility & Quantitative Power
3D-ARQ
Quantification of transgene expression levels

Assessment of developmental processes

Assessment of the physiological status and response to drugs

Vergara et al., Development 2017
Prominent features of the 3D-ARQ system:

- Facilitates quantitative measurements in complex 3-D retinal organoids
- Meets HTS assay quality requirements
- Versatility of applications as well as fluorophore selection
- Ratiometric strategy accounts for size variability
- Ability to perform longitudinal studies
- Potential for automation

- Possibility to perform drug screening in a human 3-D context that mimics the native histoarchitecture and tissue interactions

- Potential applicability to other organoid systems
Retinal organoids for clinical applications

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iPS cells

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Disease modeling
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Ocular Stem Cell and Regeneration Research Program

Catalyzing Stem Cell innovations to save and restore Sight
CellSight

3D Human Retina Modeling Lab (3DRet Lab)
hiPSC technology to model retinal degenerative diseases
Dr. Val Canto-Soler

Ocular Development and Translational Technologies Laboratory
Mechanisms of retina development and regeneration & drug screening
Dr. Natalia Vergara

Laboratory of Developmental Genetics
Genetic pathways regulating retinal cell differentiation
Dr. Joseph Brzezinski

Laboratory of Advanced Ophthalmic Imaging
Non-invasive functional imaging
Dr. Omid Masihzadeh
CellSight
A multidisciplinary team applying a bench-to-bed-side approach

- Diagnosis
- Phenotyping / Genotyping
- Patient Registry
- Treatment

Sue Anschutz-Rodgers Eye Center

- Patient-specific iPSC
- Disease Modeling
- Gene Therapy Screening
- Drug Screening
- Cell Therapy Strategy
- bench product
- Gates Biomanufacturing Facility
- clinical product
- Quality Control
cGMP Manufacturing

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