Treating Degenerating Eye Diseases using Stem Cell Derived Eye Tissues

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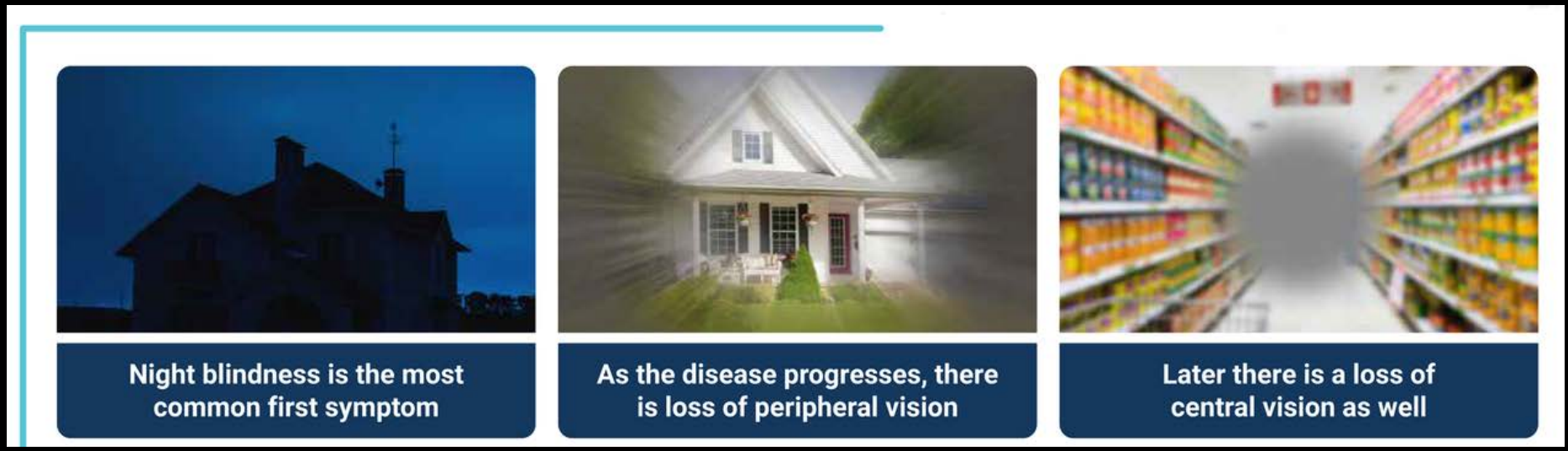
Vision Loss Affects Every Aspect of Life

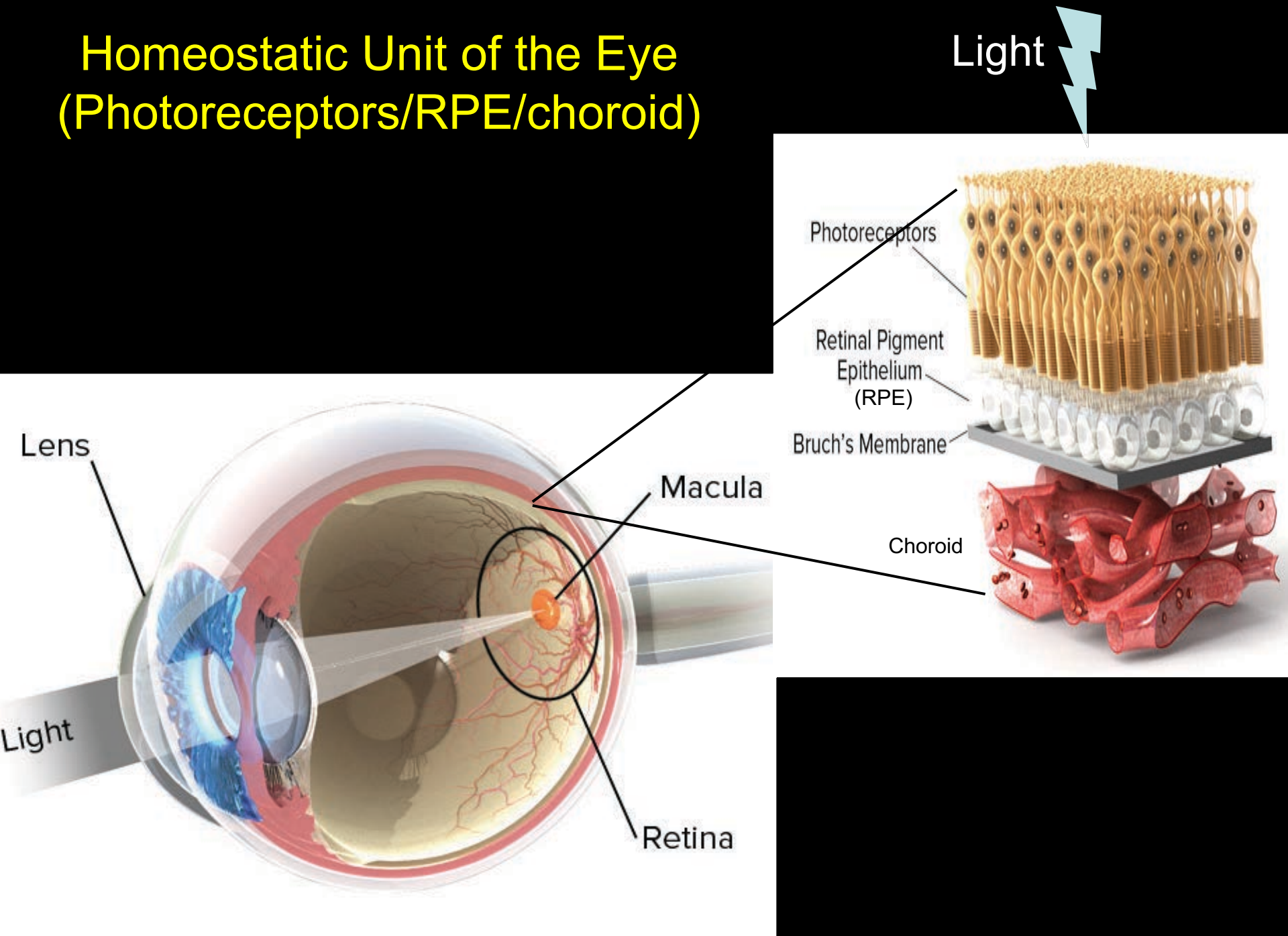


Bardet-Biedl syndrome, choroideremia, retinitis pigmentosa, Best disease, macular degeneration (AMD)

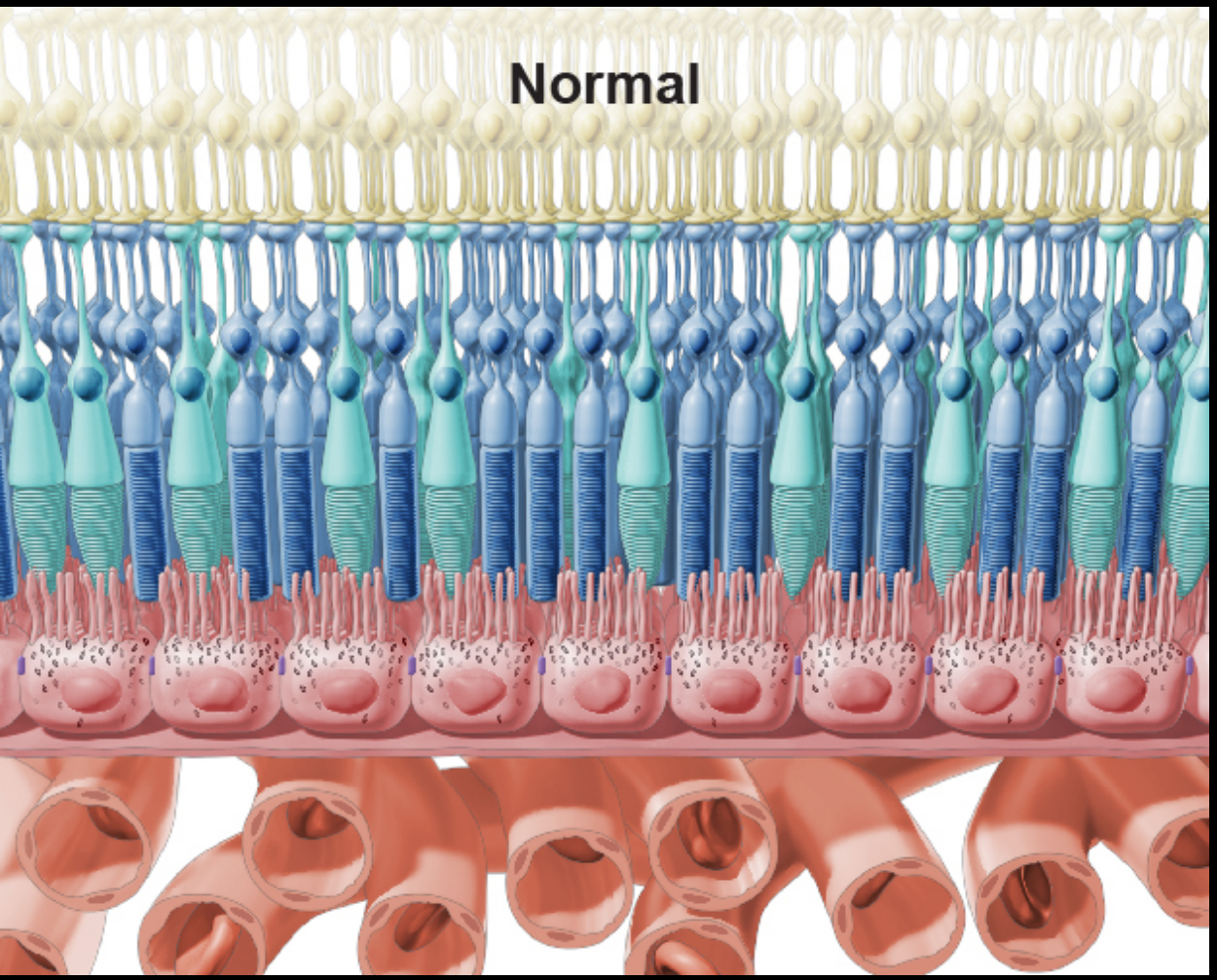
- There are over 350 different genetically manifested eye conditions that affect millions of people world-wide

Sources: https://www.fightingblindness.org/
https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases

Homeostatic Unit of the Eye (Photoreceptors/RPE/choroid)



Healthy Retina Anatomy



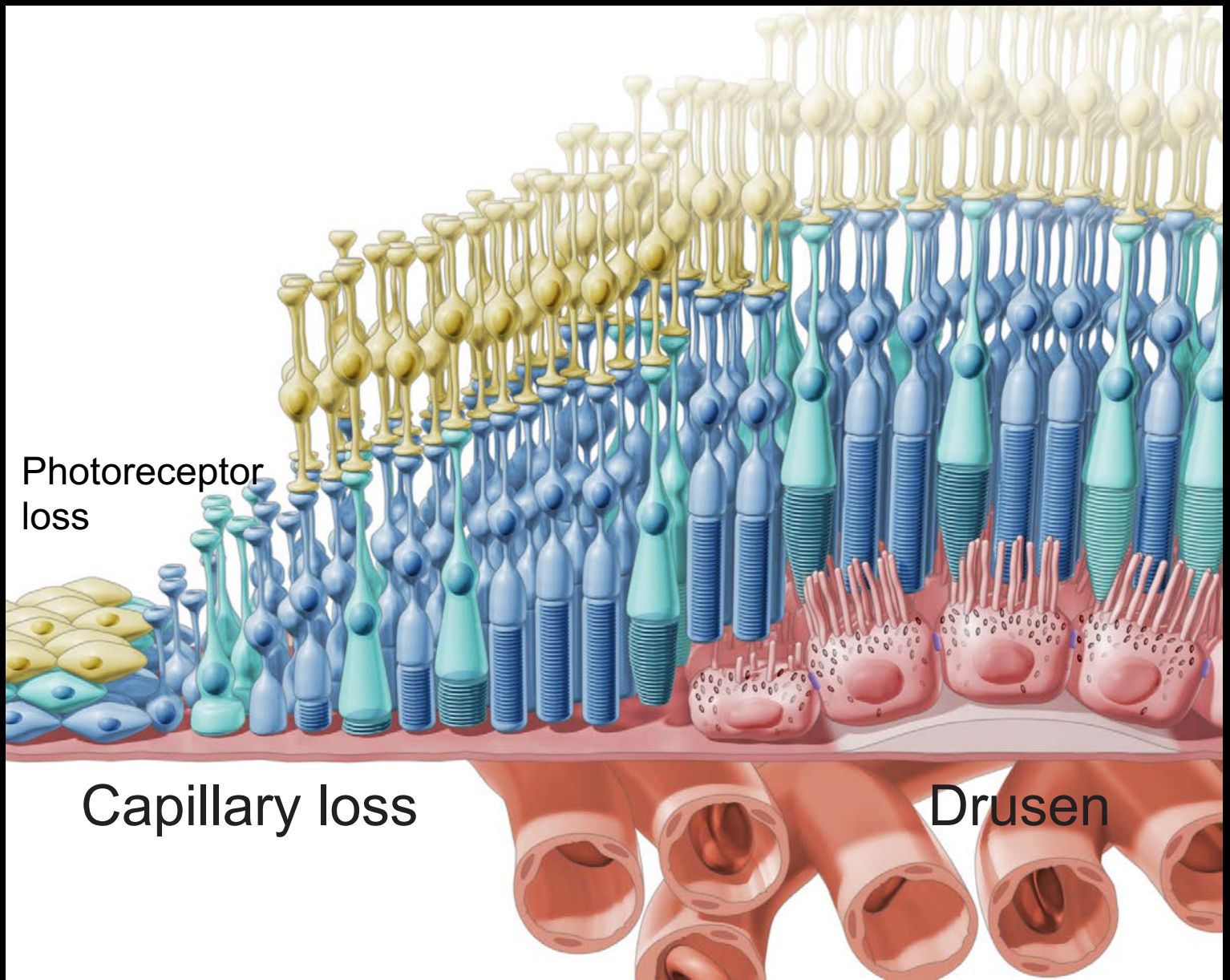
Photoreceptors (PRP)

Retinal pigment epithelium (RPE)

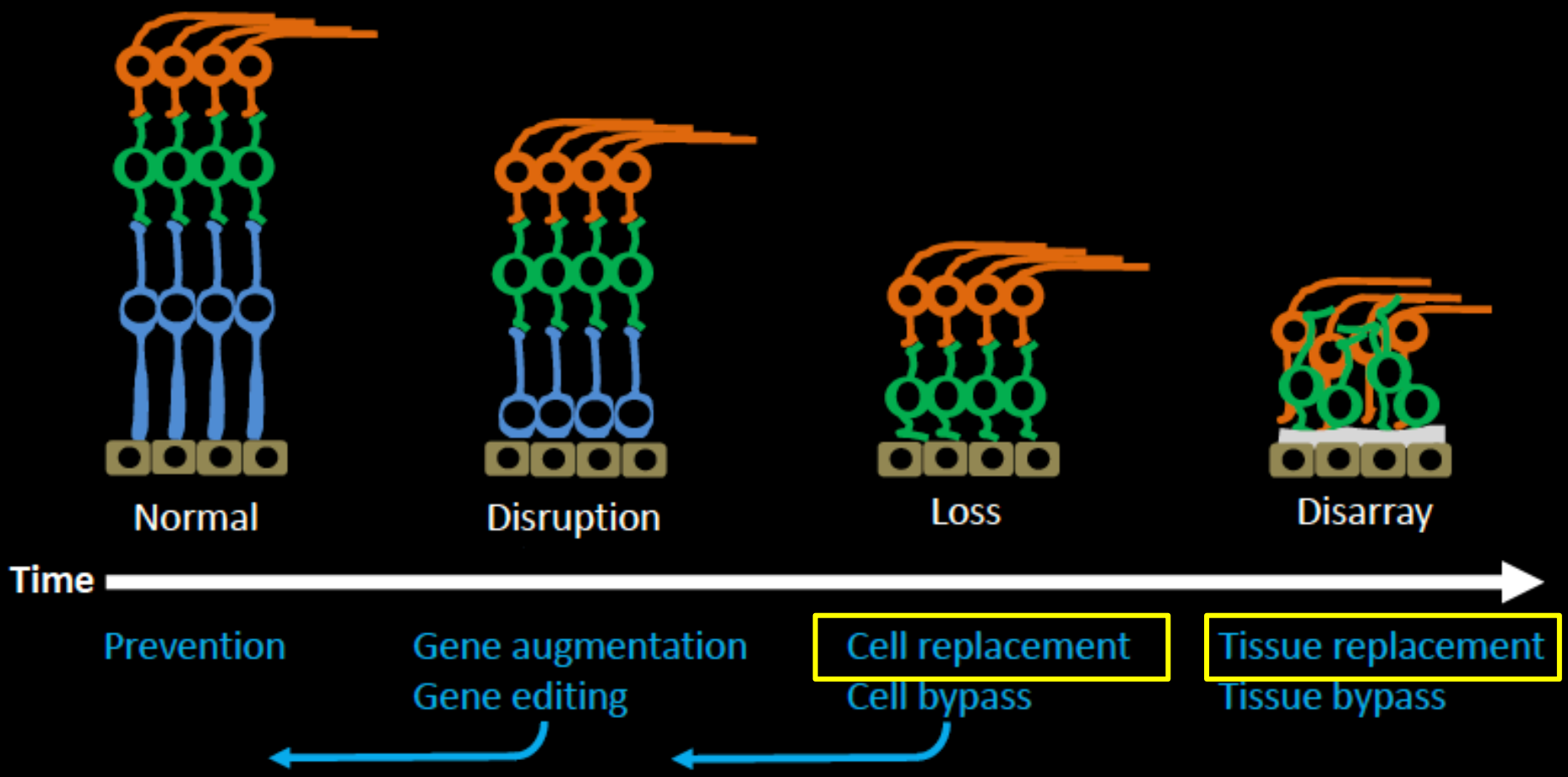
Choroidal capillaries (Choroid)

- RPE is sandwiched between the photoreceptors and the choroidal blood supply

Different Diseases Stages Manifest with the Loss of Different Eye Tissues



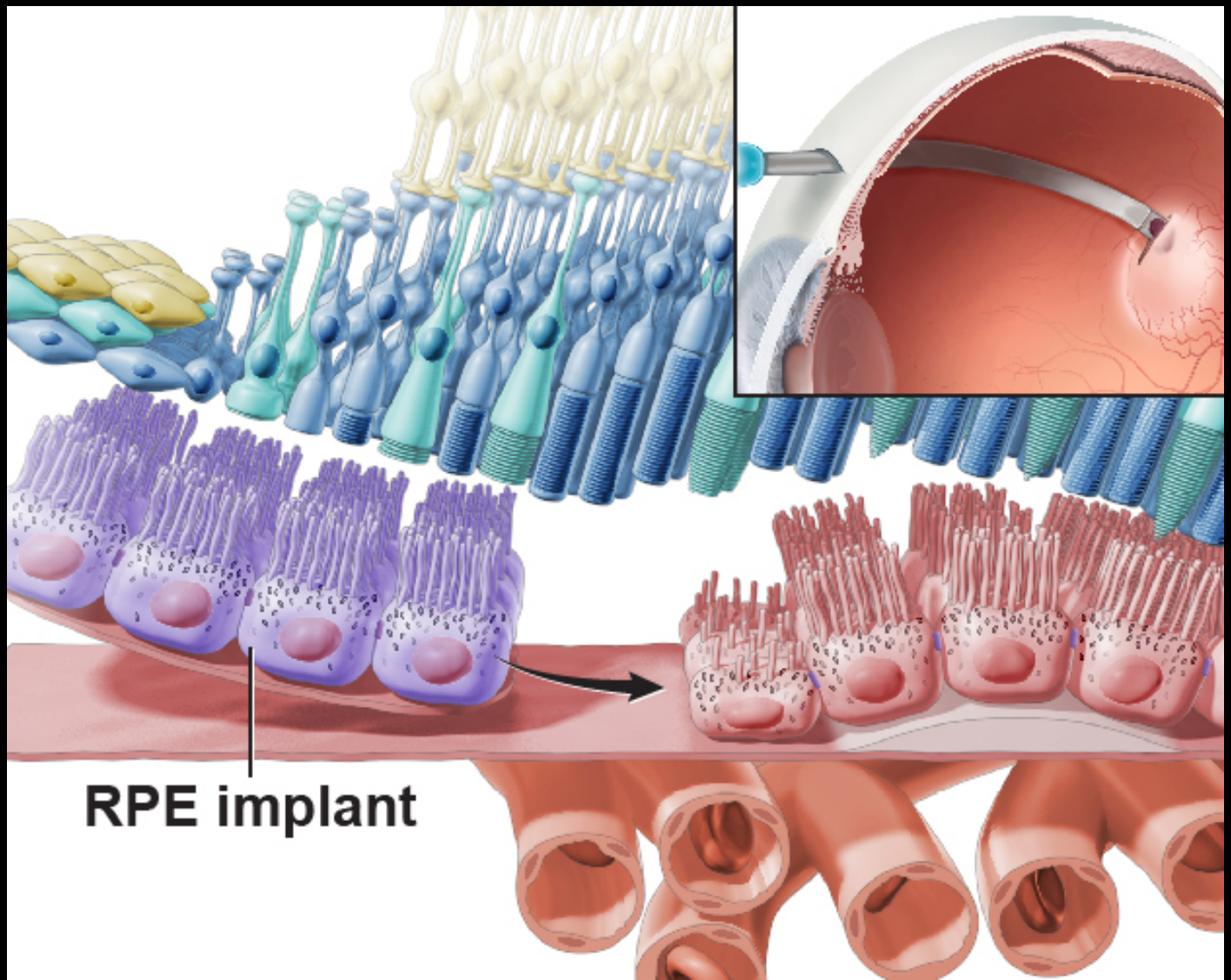
Need for Different Tissues at Different Disease Stages



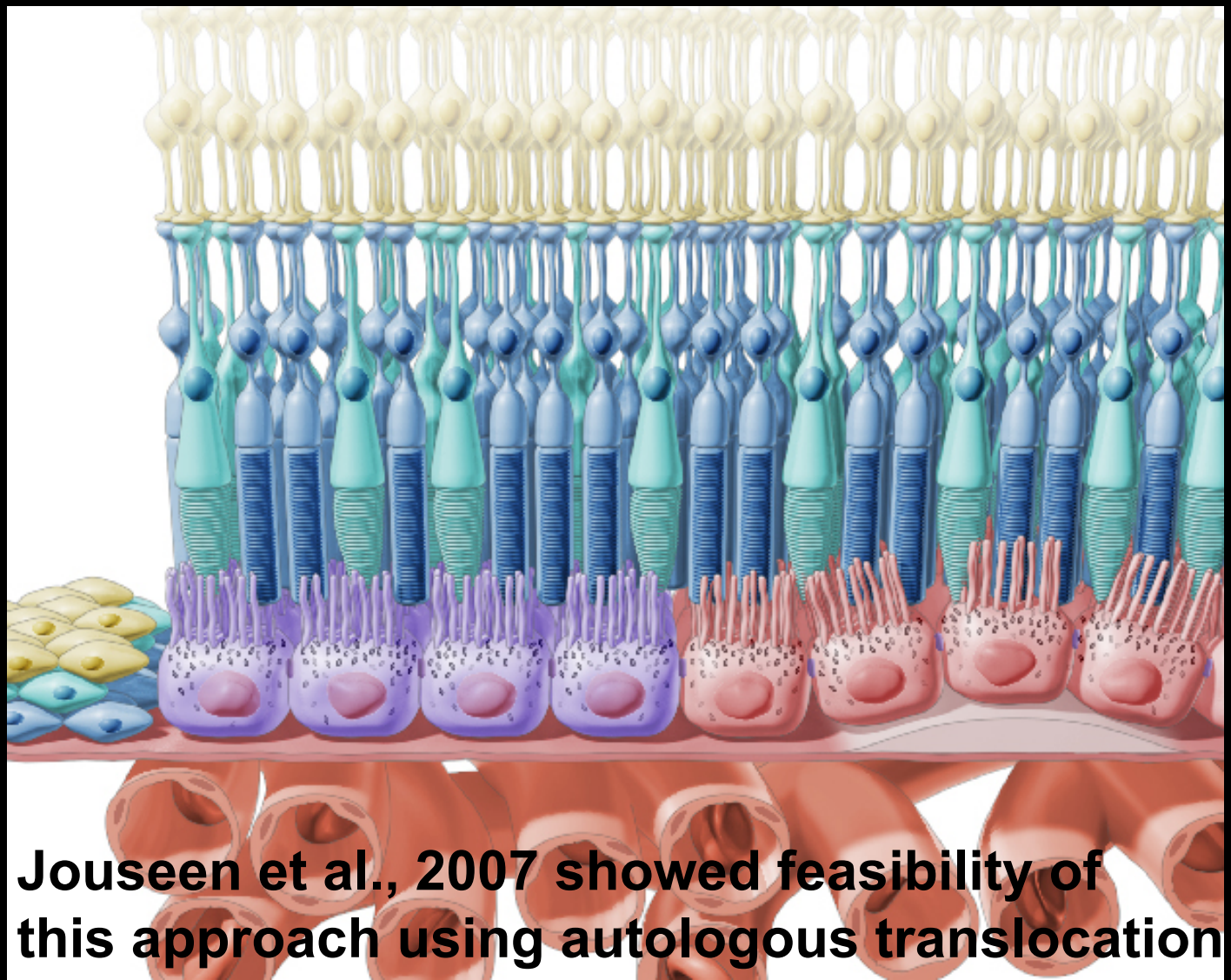
Courtesy: David Gamm (UW, Madison)

Collaboration with: Opsis Therapeutics

RPE Replacement Can Rescue Dying Photoreceptors in Macular Degeneration (Geographic Atrophy)



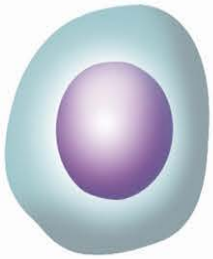
RPE Replacement Can Rescue Dying Photoreceptors in AMD (Geographic Atrophy)

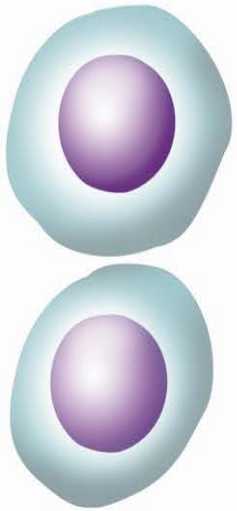


Jouseen et al., 2007 showed feasibility of this approach using autologous translocation

What is a stem cell?

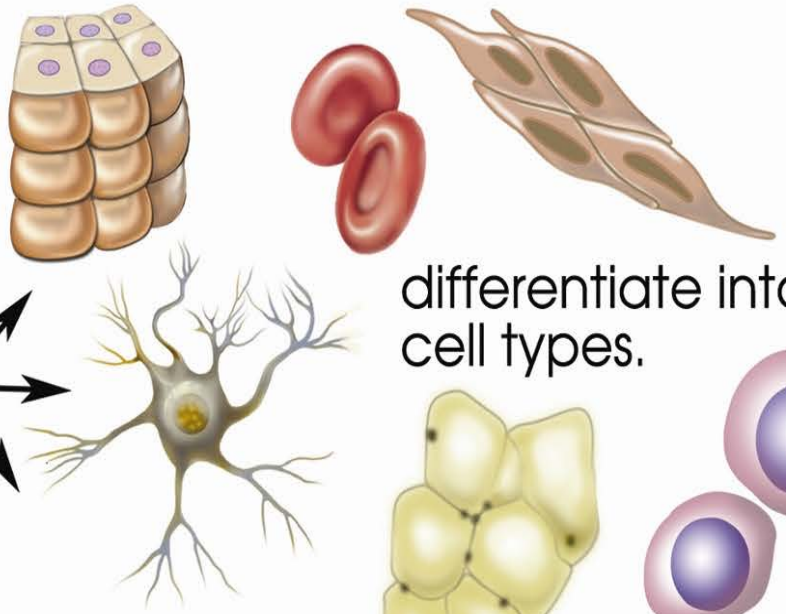
A single cell that can





replicate itself

or



differentiate into many cell types.

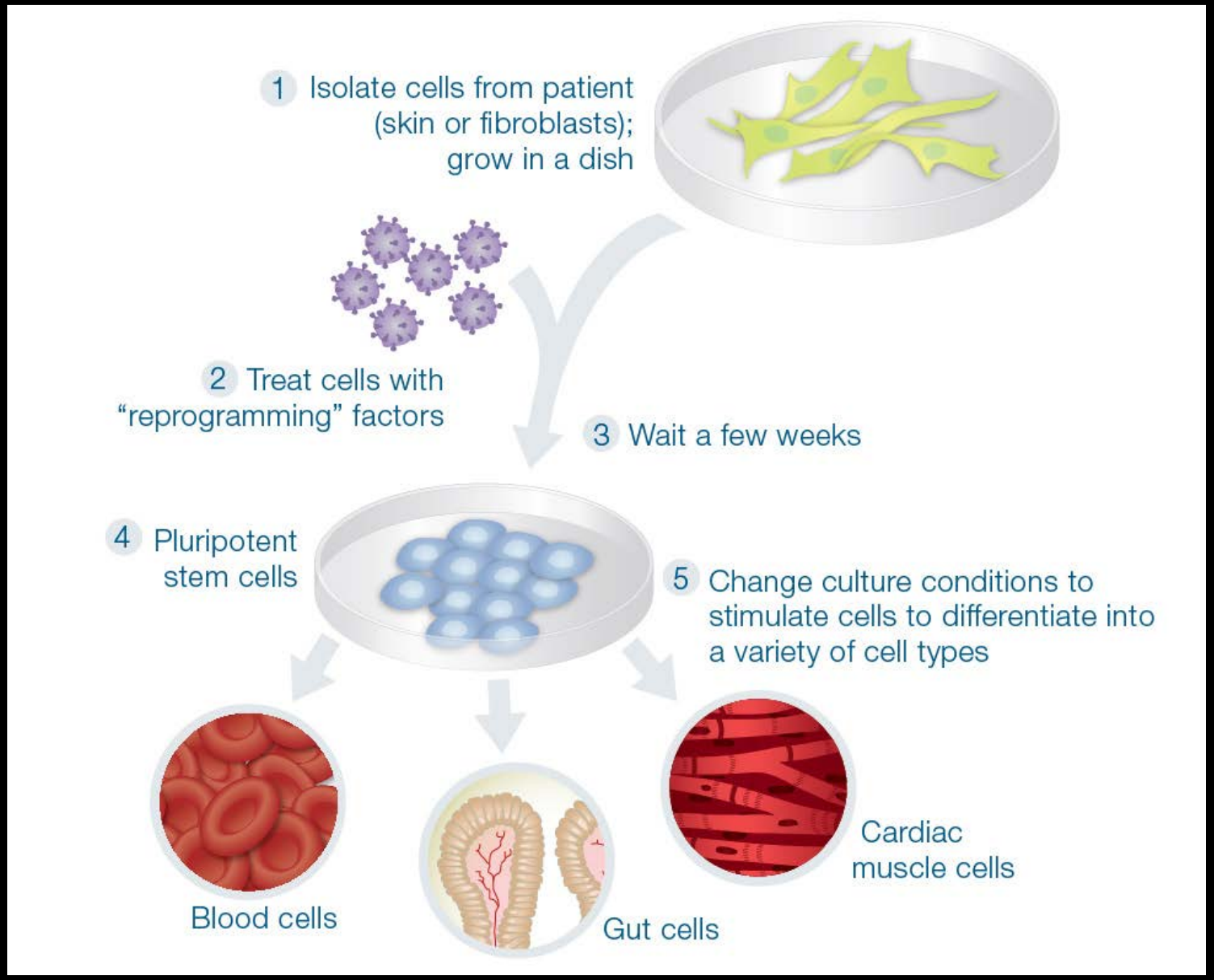
Types of Stem Cells

1. Embryonic Stem (ES) Cells (derived from a preimplantation blastocyst)

2. Induced Pluripotent Stem (iPS) Cells (derived from adult cells & behave similar to ES cells)

3. Tissue-specific Stem Cells (e.g. blood stem cells, umbilical cord stem cells, mesenchymal stem cells/MSCs)

iPS Cells Can be Derived From Any Cell of an Adult Person And Can be Converted into Any Cell



Making Patient-Specific Replacement Cell Therapies

Patients

somatic tissue

+ Reprogramming factors

Derived iPS cells

May need gene editing for monogenic diseases

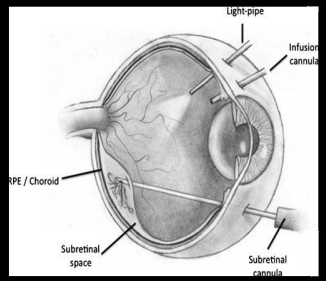
Differentiated 3D RPE/choroid

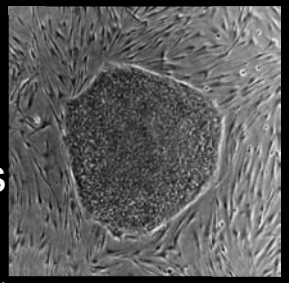
Differentiated RPE cells

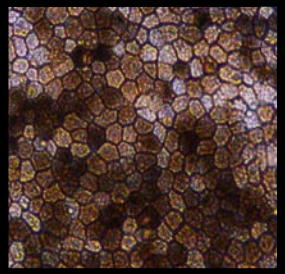
Differentiated PRP cells

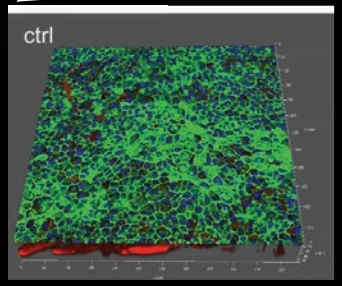
Cell Therapy

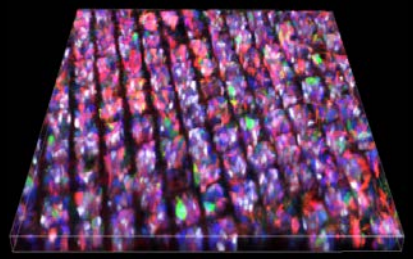
Potential treatment





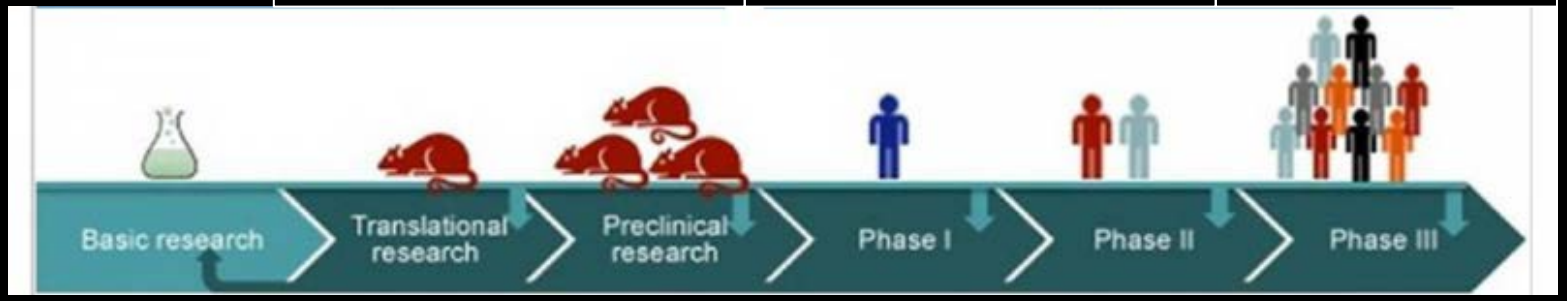






Timeline for Development of an FDA Approved Stem Cell Therapy

5-8 years	4-5 years	3-5 years



Proof-of-concept data in animals

IND-enabling studies

Pre-clinical toxicology and efficacy in animal models

Can be single center and publically funded

FDA approval

Need to be multi-center and often require an industry partner

FDA approval

Commercial Approval

STEM CELL "CLINICS"

(use of unapproved and unproven stem cells in patient treatments)

Vision Loss after Intravitreal Injection of Autologous "Stem Cells" for AMD

Ajay E. Kuriyan, M.D., Thomas A. Albini, M.D., Justin H. Townsend, M.D., Marianeli Rodriguez, M.D., Ph.D., Hemang K. Pandya, M.D., Robert E. Leonard II, M.D., M. Brandon Parrott, M.D., Ph.D., Philip J. Rosenfeld, M.D., Ph.D., Harry W. Flynn, Jr., M.D., and Jeffrey L. Goldberg, M.D., Ph.D.

N Engl J Med. 2017 Mar 16;376(11):1047-1053. doi: 10.1056/NEJMoa1609583.

Source of Information Approved vs Unapproved Stem Cell Therapies

FDA

https://www.fda.gov/consumers/consumer-updates/fda-warns-about-stem-cell-therapies

NIH

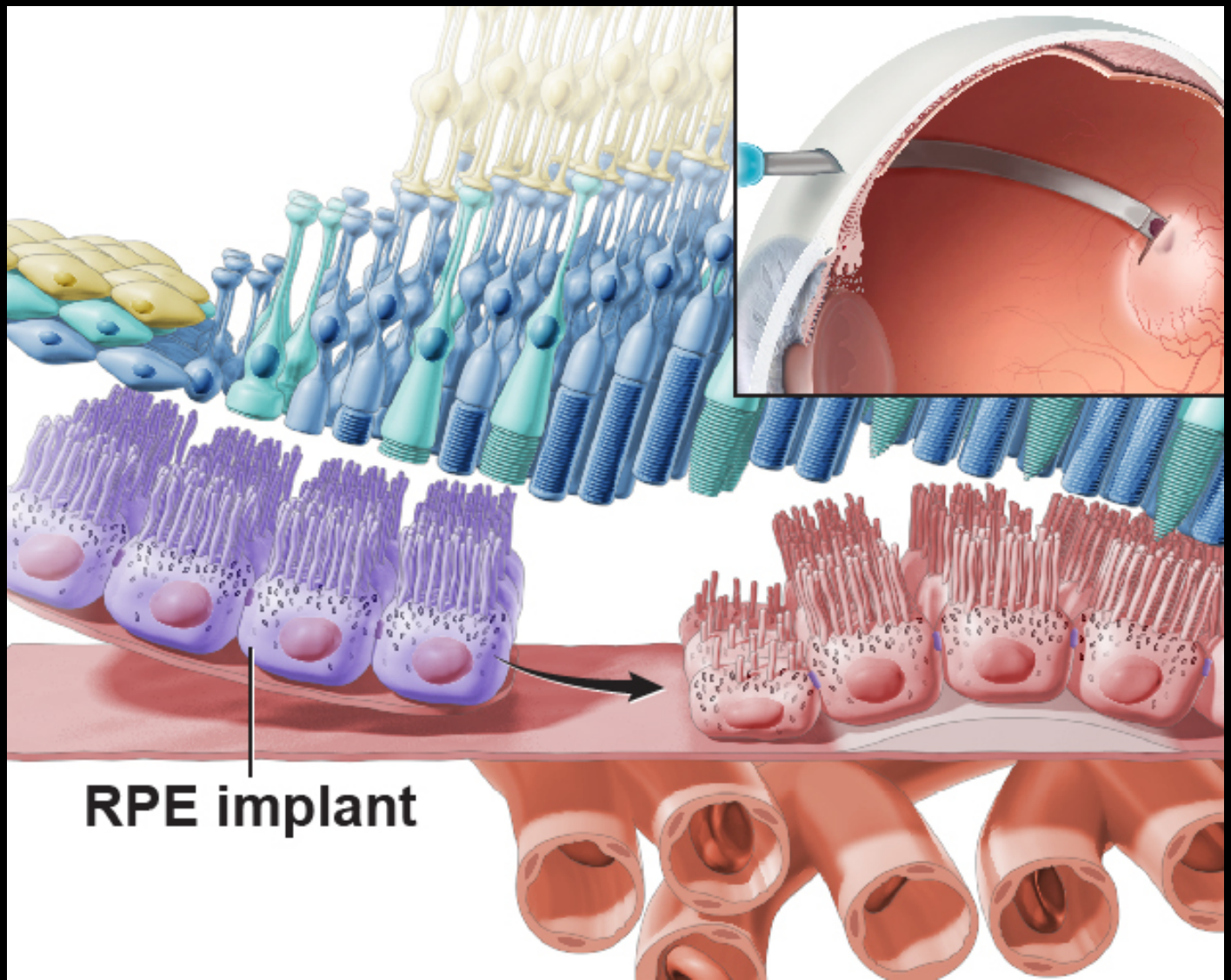
https://stemcells.nih.gov/info/health.htm

International Stem Cell Society (ISSCR)

https://www.closerlookatstemcells.org/patient-resources/

- If someone wants you to pay out of pocket for a stem cell treatment. It is likely not approved.
- Currently, there is no FDA approved commercially available stem cell-based treatment for the eye. All ongoing approaches are at a trial stage

RPE Replacement Can Rescue Dying Photoreceptors in AMD (Geographic Atrophy)



Investigational New Drug (IND) Contents

Manufacturing of a Cell Therapy product

- Develop a clinical-grade manufacturing process
- Validate the manufacturing process and transfer to cleanroom for clinical manufacturing
- Validate the clinical product manufactured in the cleanroom

Pre-clinical Data

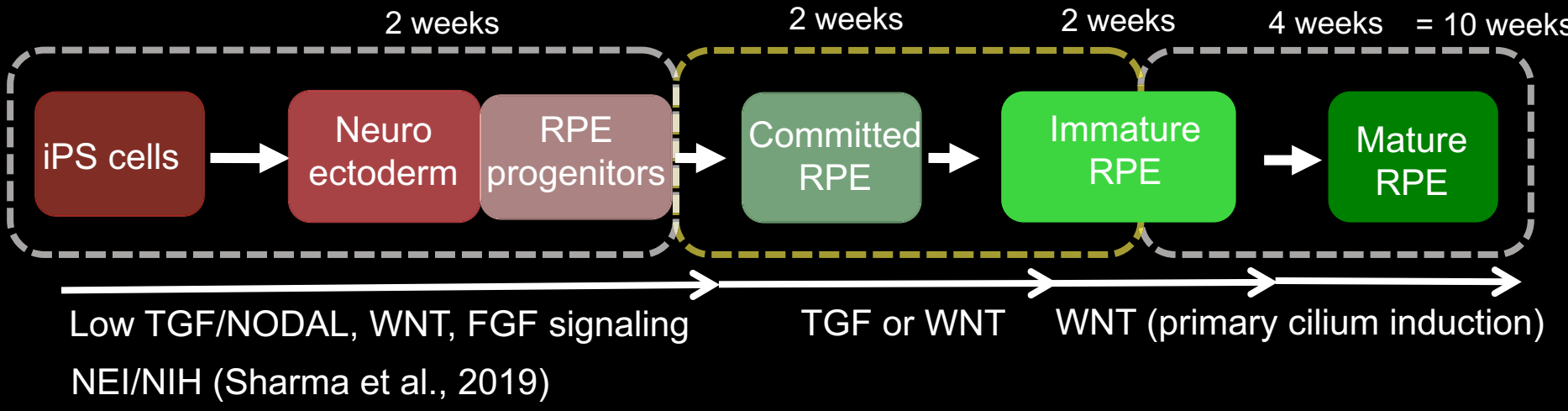
- Confirm lacking Tumorigenicity and toxicity of the product
- Confirm efficacy of the product
- Demonstrate biocompatibility of tools used in the procedure

Clinical Data

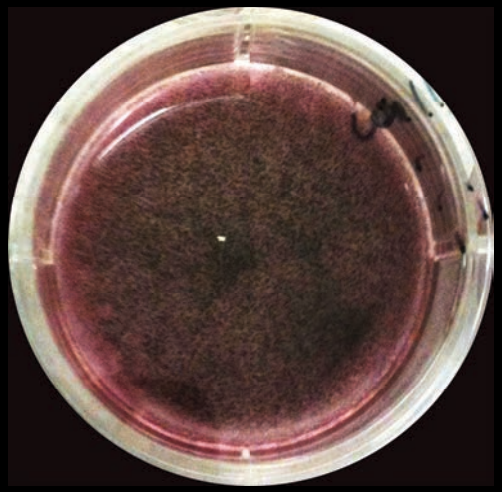
- Clinical protocol, Consent forms

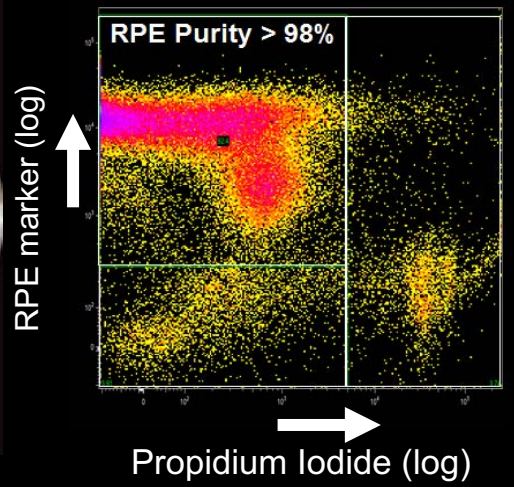
Generating Pure and Mature iPSC-RPE Cells

Guided differentiation of PSCs-RPE (use of growth factors)



NEI/NIH (Sharma et al., 2019)

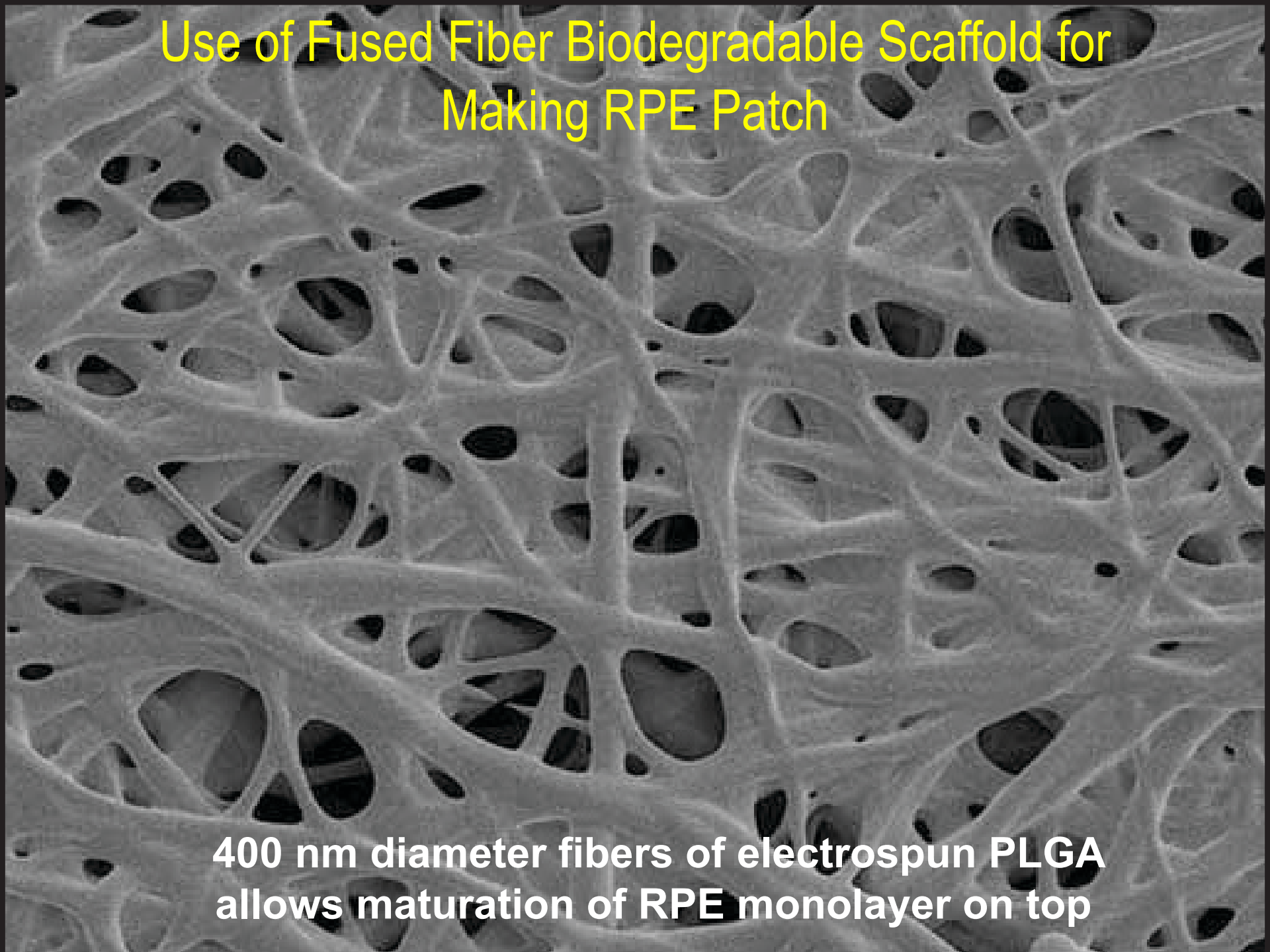




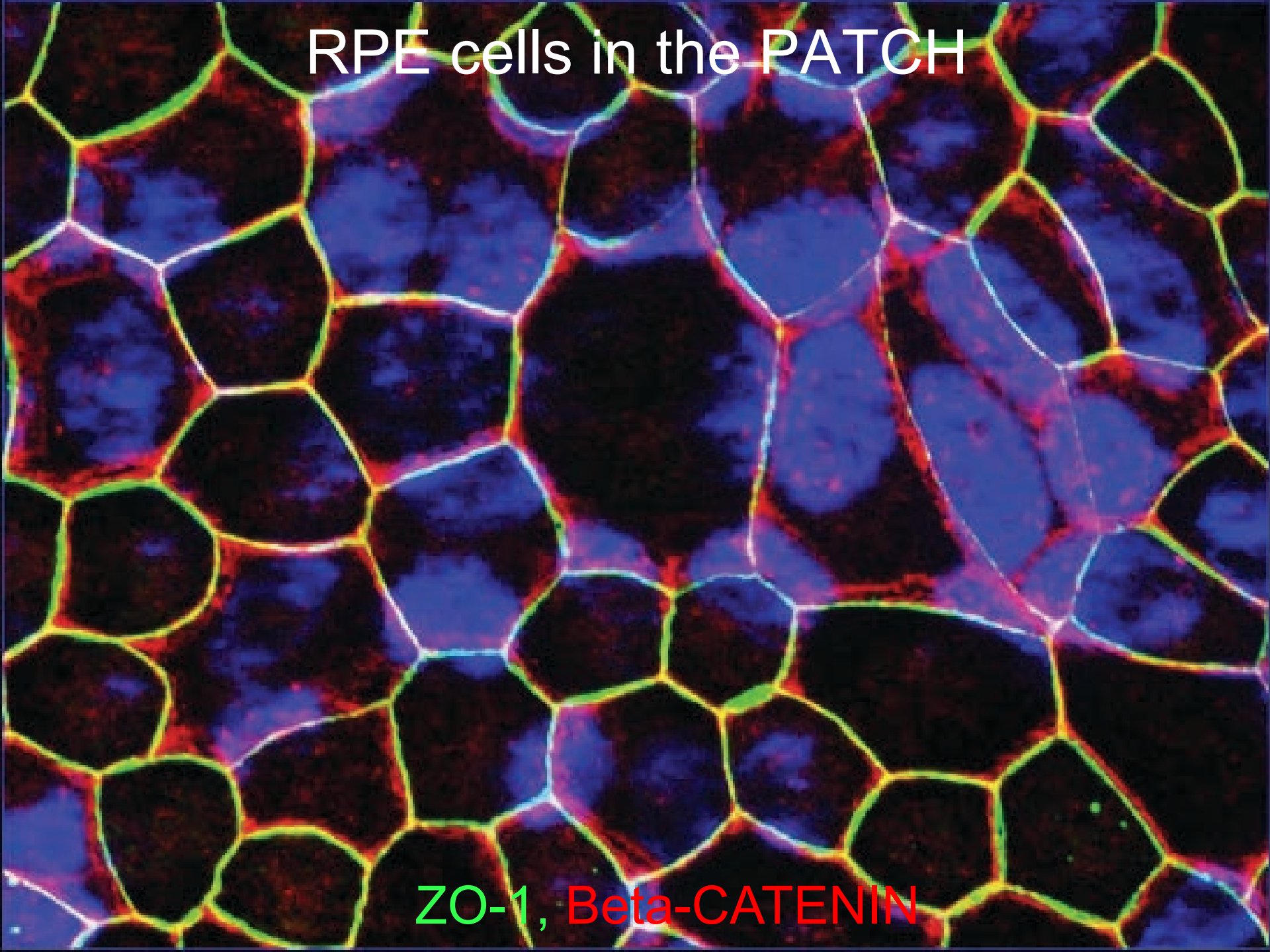
Reproducibility: 34 Donors

- 20 Healthy (6 research-grade, 11 HLA-matched, 3 clinical-grade)
- 14 Diseased (3 AMD research-grade, 4 AMD clinical-grade, 4 albinism, 1 Joubert, 2 STAT3)

Use of Fused Fiber Biodegradable Scaffold for Making RPE Patch



400 nm diameter fibers of electrospun PLGA allows maturation of RPE monolayer on top


RPE cells in the PATCH
ZO-1, Beta-CATENIN

Streamlined Clinical-manufacturing Process

In-process quality controls

Cryo steps

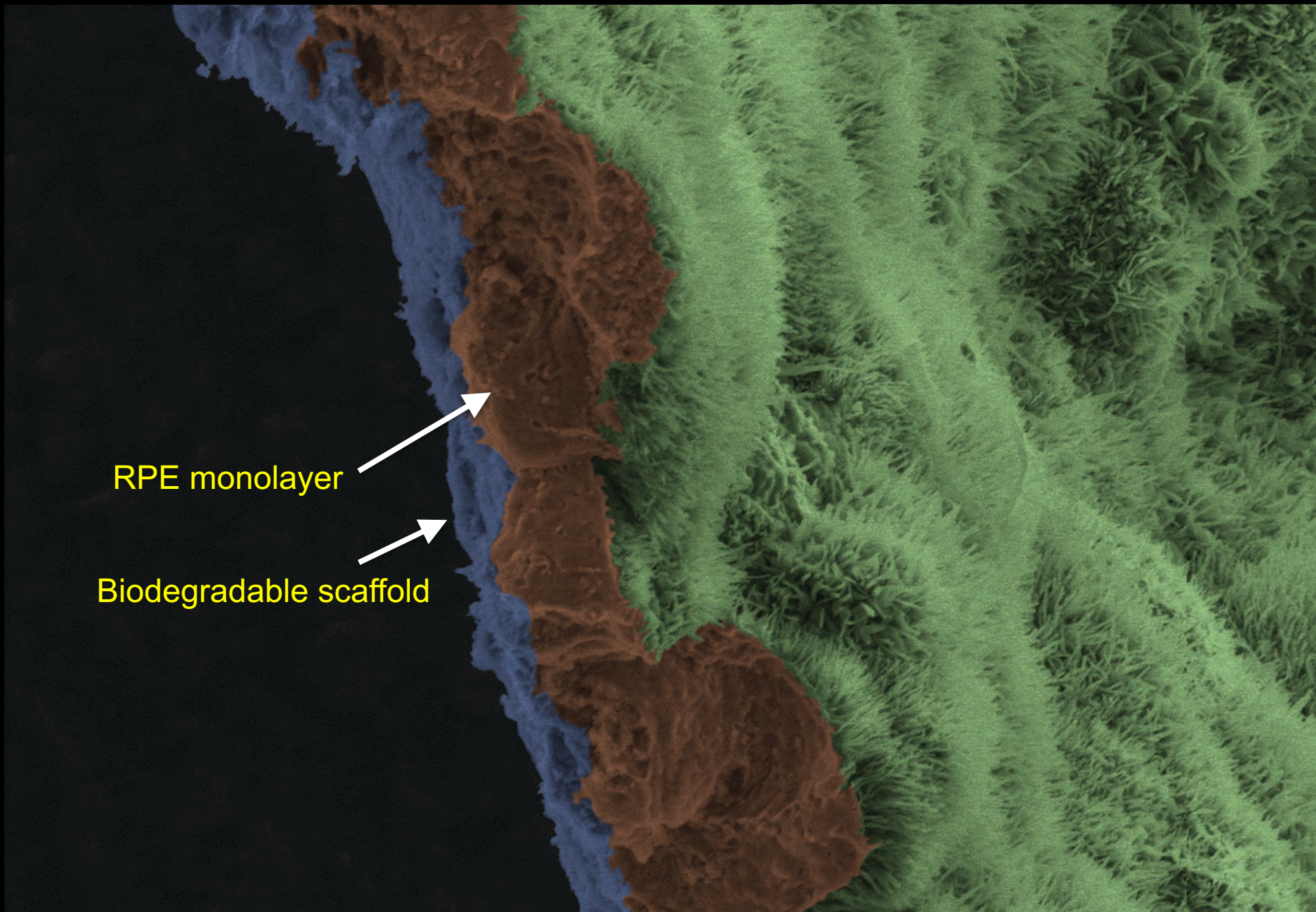
	Blood draw from patient	Day 0
	CD34+ isolation and cryopreservation	Patient material
	Transfection	Day 8
	Passage 1	Day 30
Sterility, karyotyping Orip loss, identity, sequencing	Mini bank p5	Day 55
	Working bank p10	Day 80
	Passage 11 Differentiation set up up to 3 clones	Day 87
Day 99 differentiation efficiency (go/no go)	RPE progenitor stage	Day 114
Purity assay (go/no go)	Committed RPE enrichment	Day 129
	Scaffold seeding only one clone	Day 129
Sterility, purity, Identify, function	Clinical product – RPE patch	Day 164

iPSC Mini bank

iPSC Working bank (~ 8 clones)

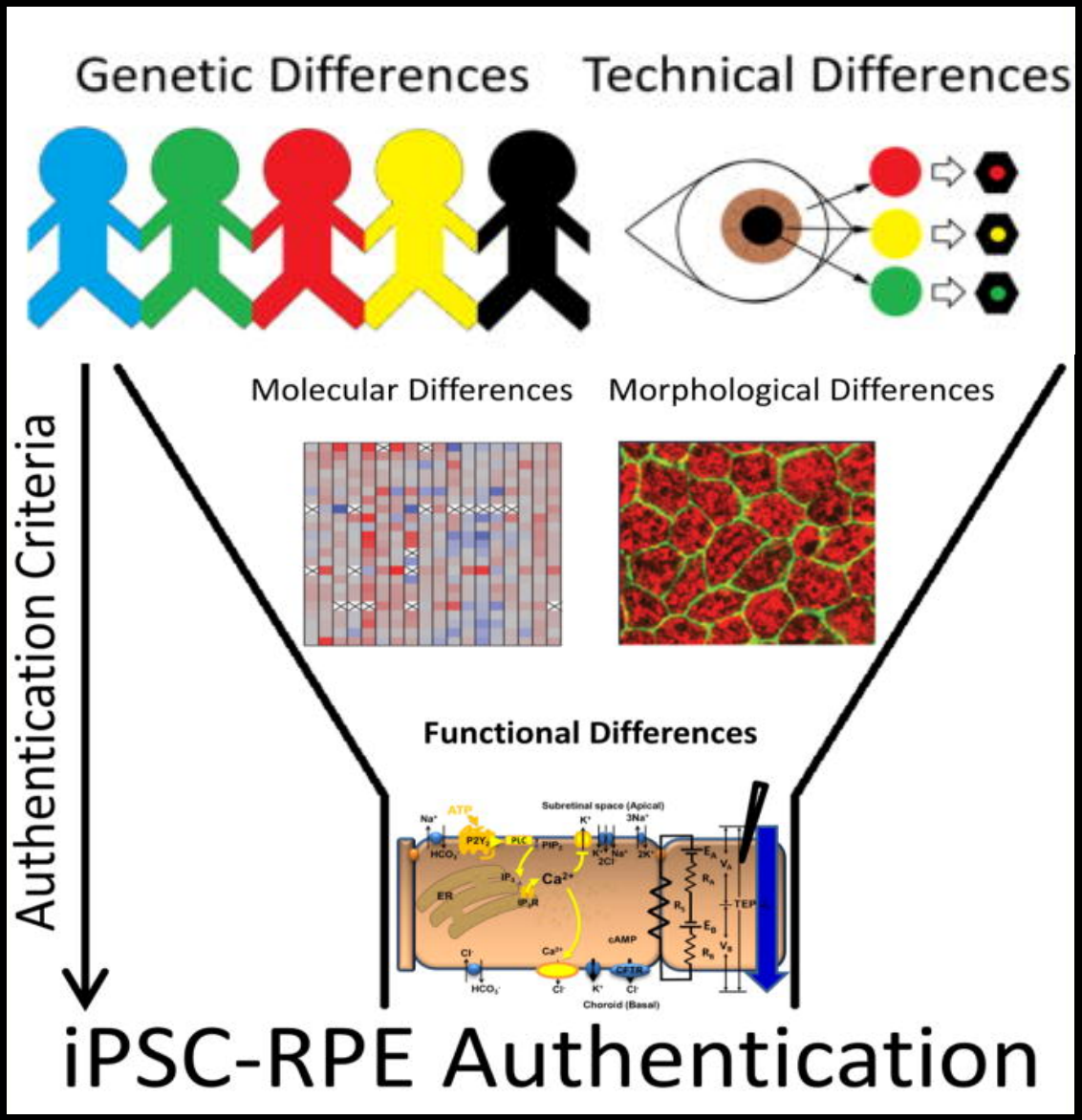
RPE progenitor bank (10 vials/clone)

Functional and Polarized iPSC-RPE Patch on a Scaffold



Validation of Autologous iPSC-RPE Product

- Understanding variability, understanding the allowable limit of variability, controlling variability



- Three AMD donors
- Three clones/donors

1. Purity of cells (flow cytometry)
2. RPE-specific gene expression
3. *Quantitative shape metrics*

4. *Trans epithelial resistance*

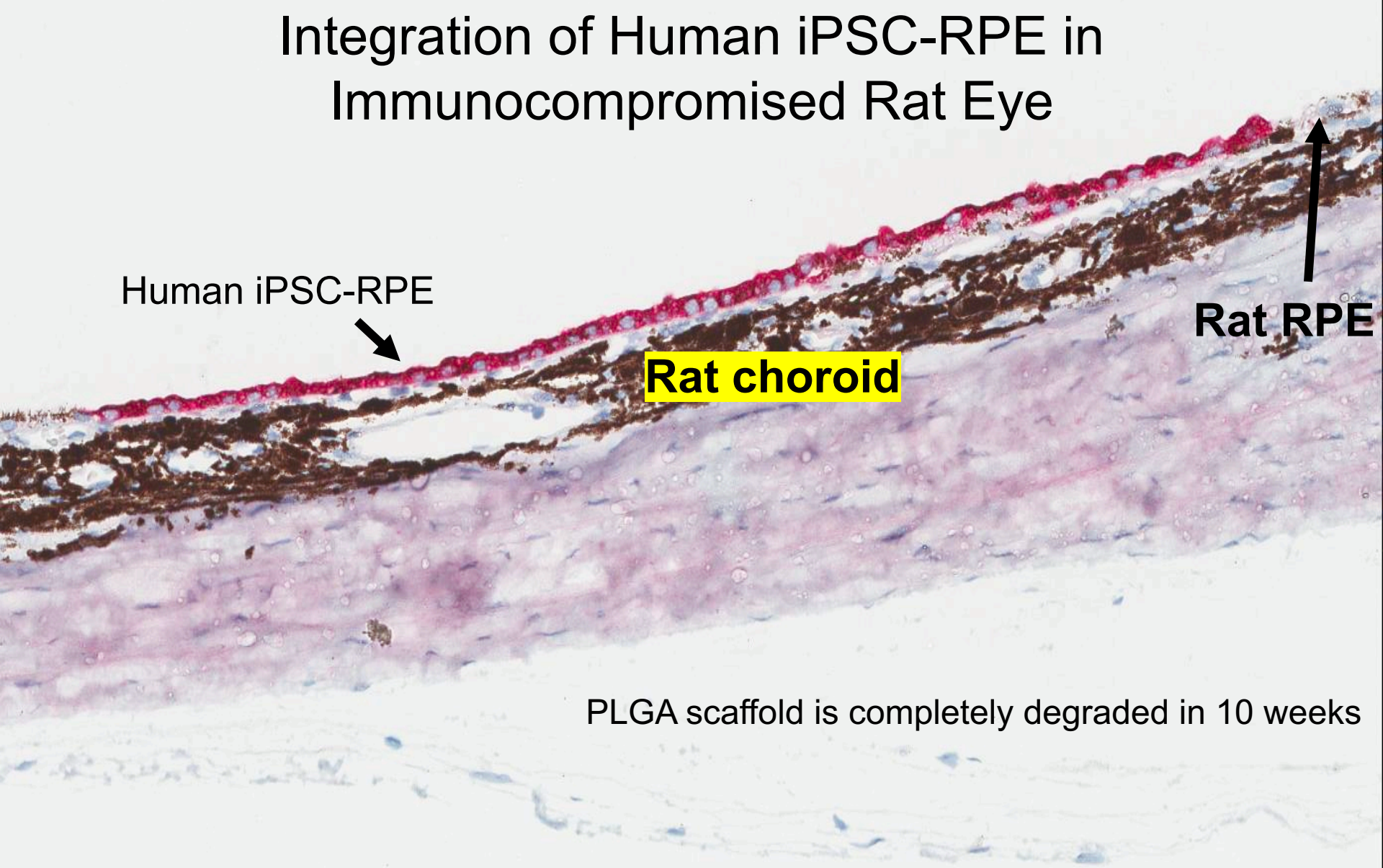
5. Polarized cytokine secretion (VEGF and PEDF)

6. Ability to phagocytose POS

(Sharma et al 2019 STM)

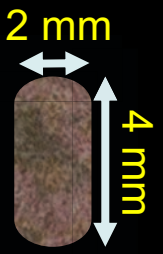
Proof-of-Concept Pre-clinical Efficacy Studies

Integration of Human iPSC-RPE in Immunocompromised Rat Eye

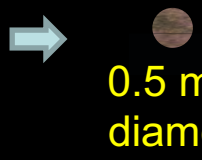


- Transplantation of a 0.5 mm diameter piece in the subretinal space of rats

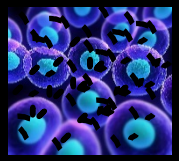
GLP Pre-clinical Toxicity, Biodistribution, and Tumorigenicity Study



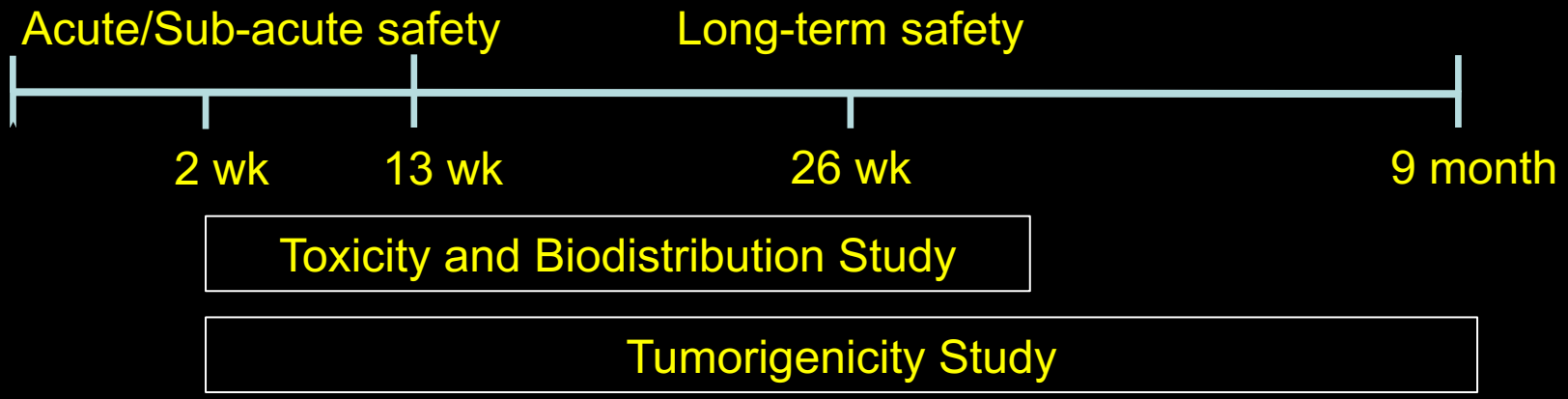
Human dose



Rat equivalent of human dose



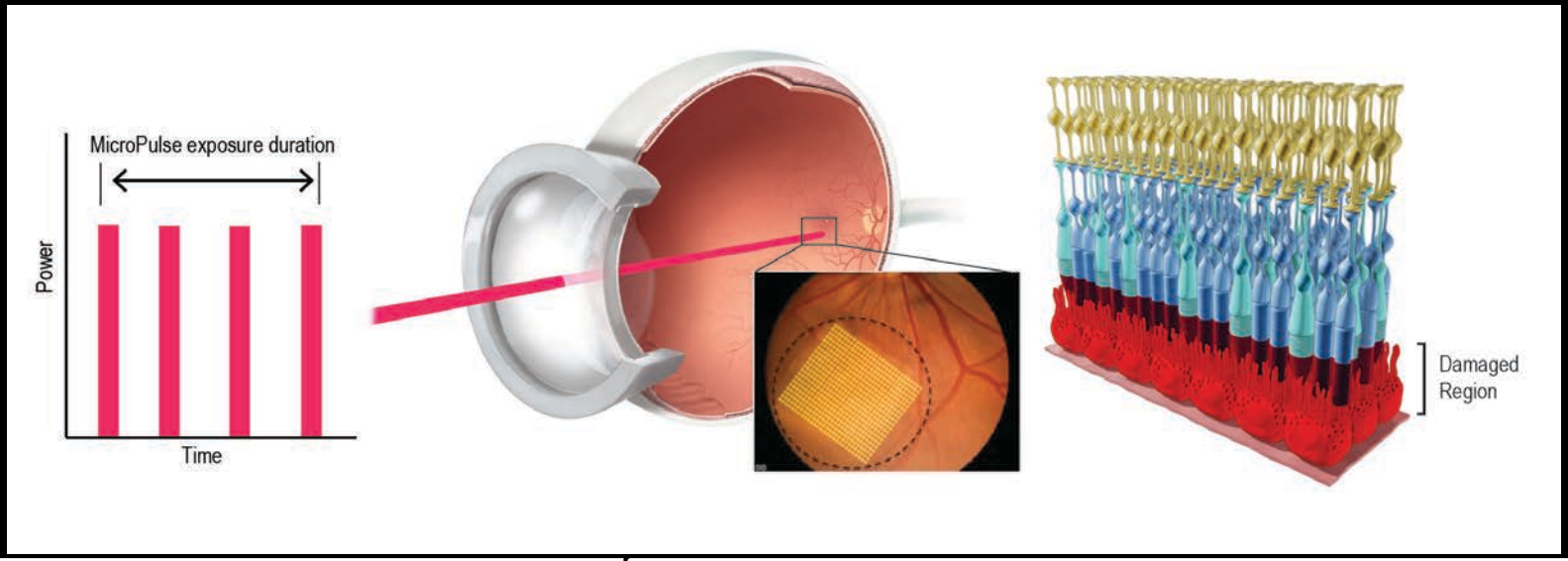
Human dose 70,000 cells

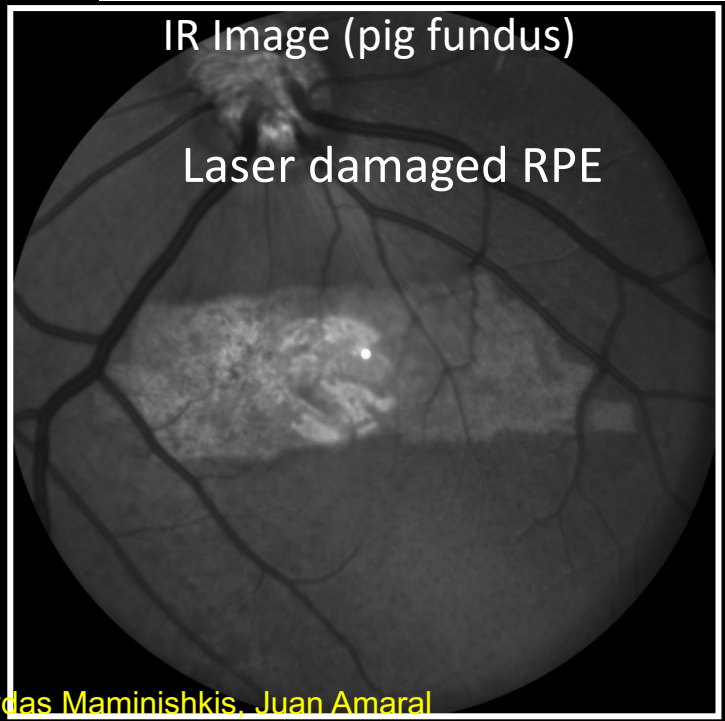


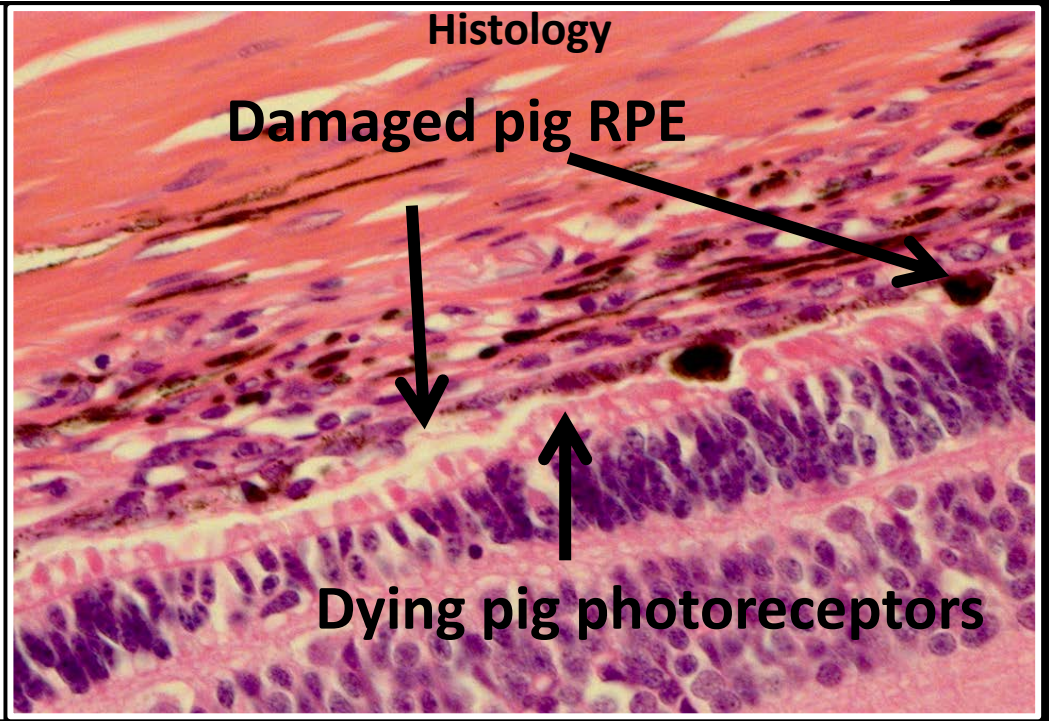
- Cells derived from two different AMD patients were used
- A total of 450 rats were transplanted

Covance/Madison

Laser Induced RPE Ablation in Pigs





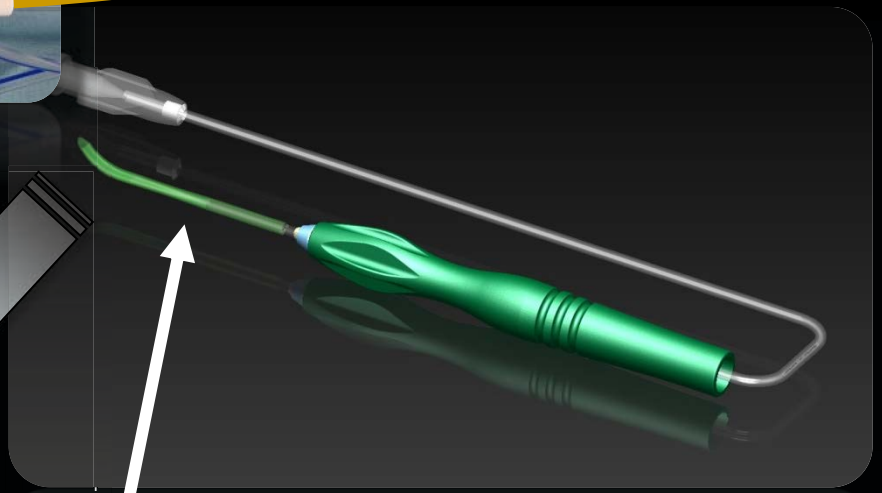


Arvydas Maminishkis, Juan Amaral

Transplantation Tool

VFI adapter

Instrument control Foot pedal

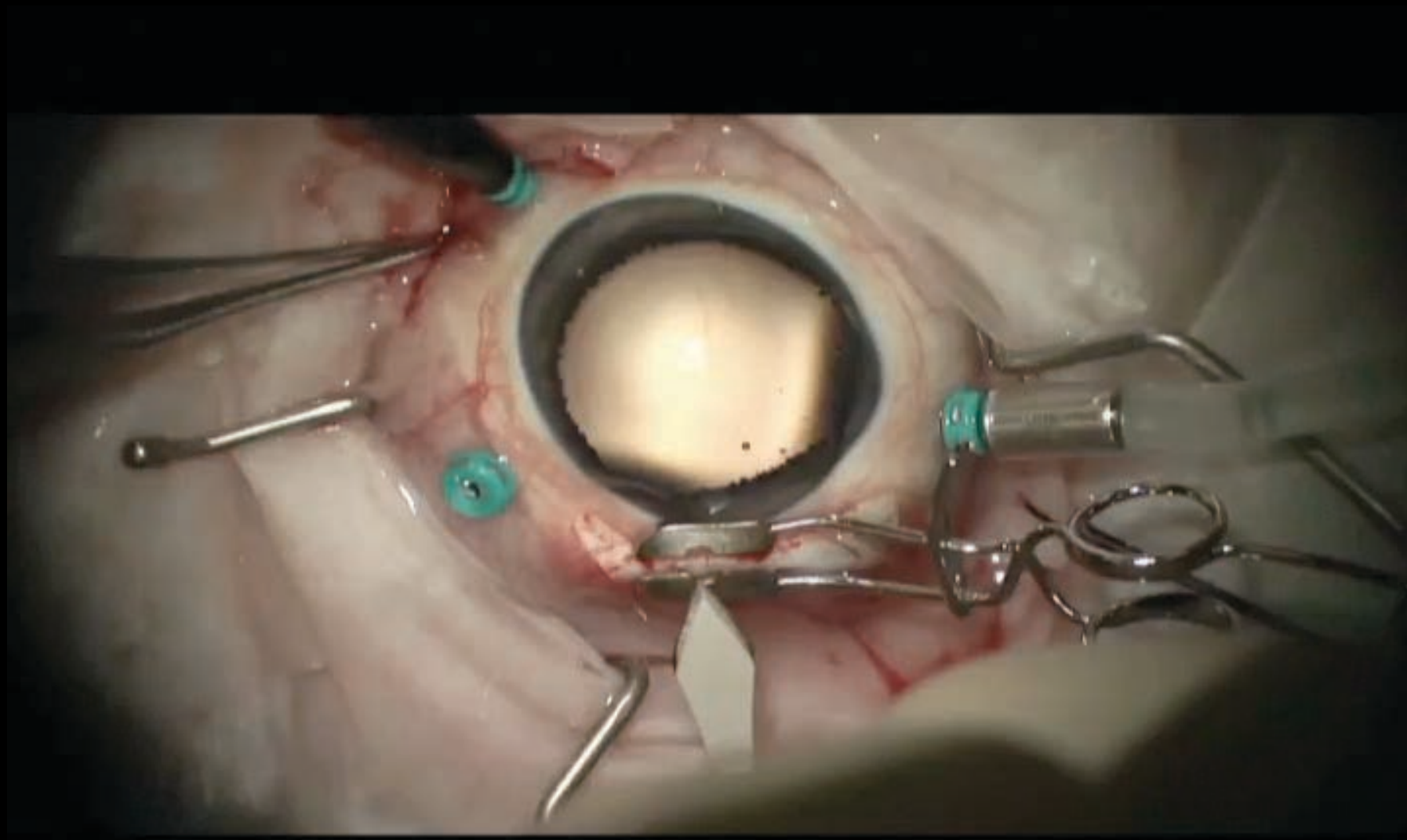


Tool Tip with scaffold inside

Tinted for better visibility, but transparent enough to monitor implant position, disposable, light

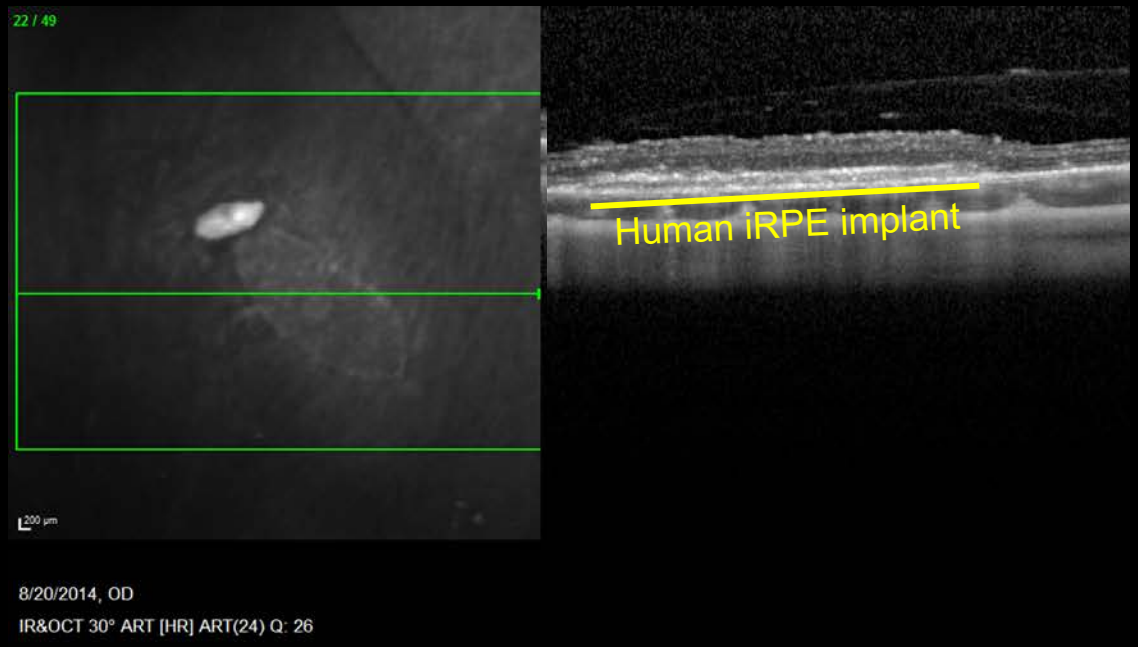
Arvydas Maminishkis, Juan Amaral

Transplantation of Human RPE Patch in Pigs



Juan Amaral & Arvydas Maminishkis (NEI)

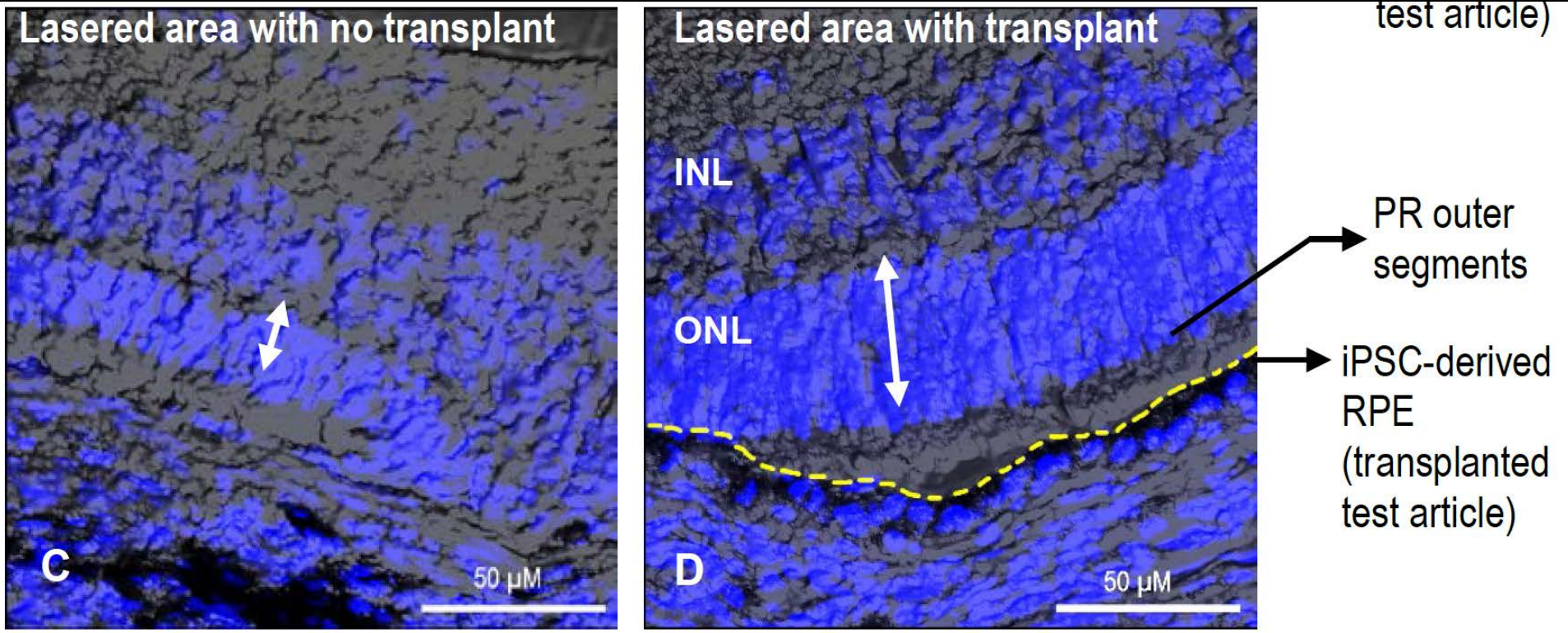
Structural and Functional Assessment of the Transplant and RPE Injury



- Multifocal ERG combined with OCT imaging of laser injury model in pigs
- Use of focal ERG and adaptive optics for patients in iRPE-patch phase I trial (collaboration with Brett Jeffery and Johnny Tam, NEI clinic)

Aaron Rising, Yichao Li, and Haohua Qian (NEI)

Survival and Efficacy of iPSC-RPE Monolayers On Biodegradable Scaffolds In Laser-injured Pig Eyes



Aaron Rising and Mercedes Campos (NEI)

Sharma et al., Macular Degeneration Patient Specific Clinical-Grade iPS Cell-Derived RPE Patch Rescues Retinal Degeneration in Rodents and Pigs (STM 2019)

Phase I/IIa Clinical Trial Initiated at NEI

Protocol Title: A Phase I/IIa Trial for Autologous Transplantation of Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium for Geographic Atrophy Associated with Age-Related Macular Degeneration

Abbreviated Title: STEM-RPE

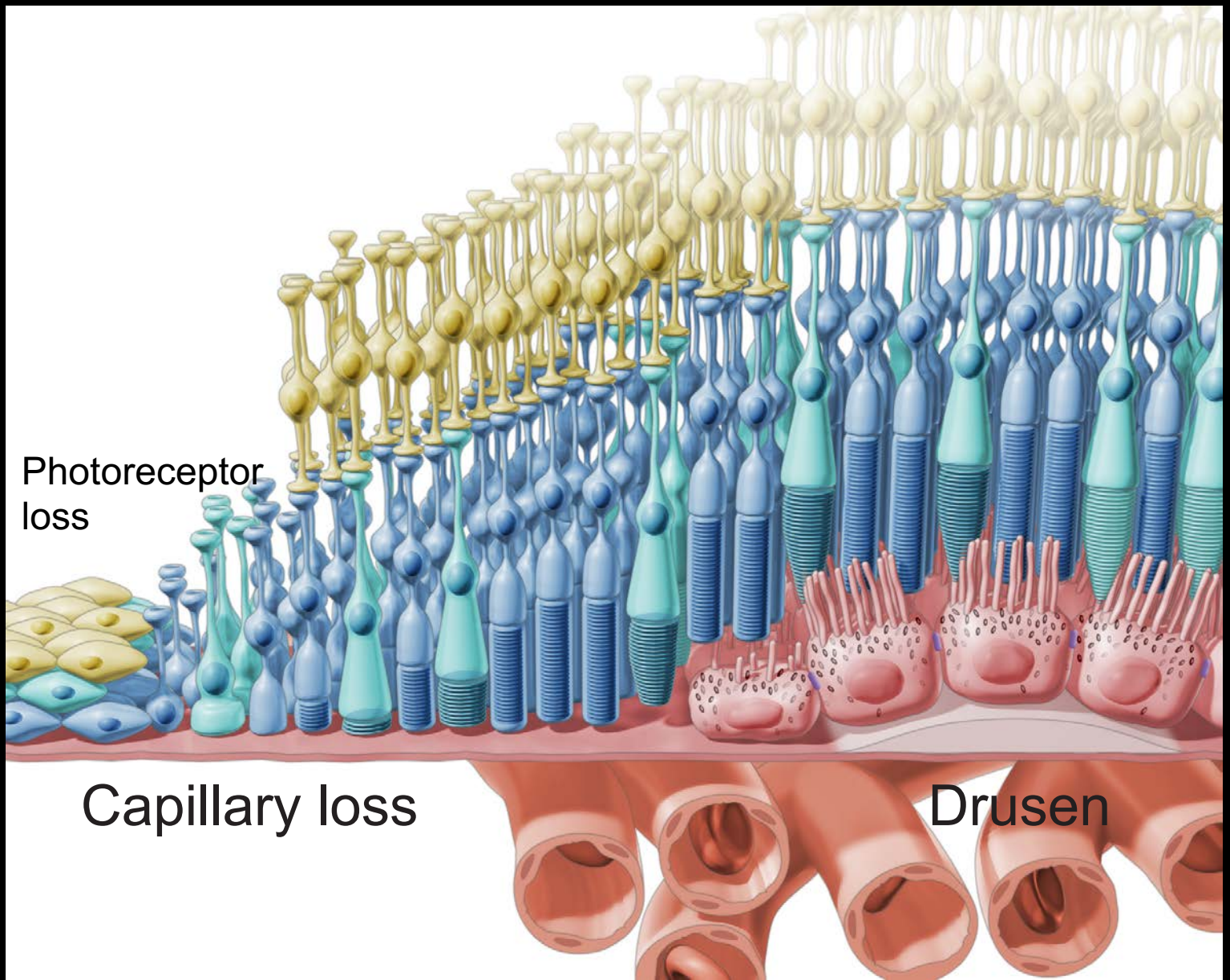
Protocol Number: T-EI-1678

Two Cohorts of GA patients:

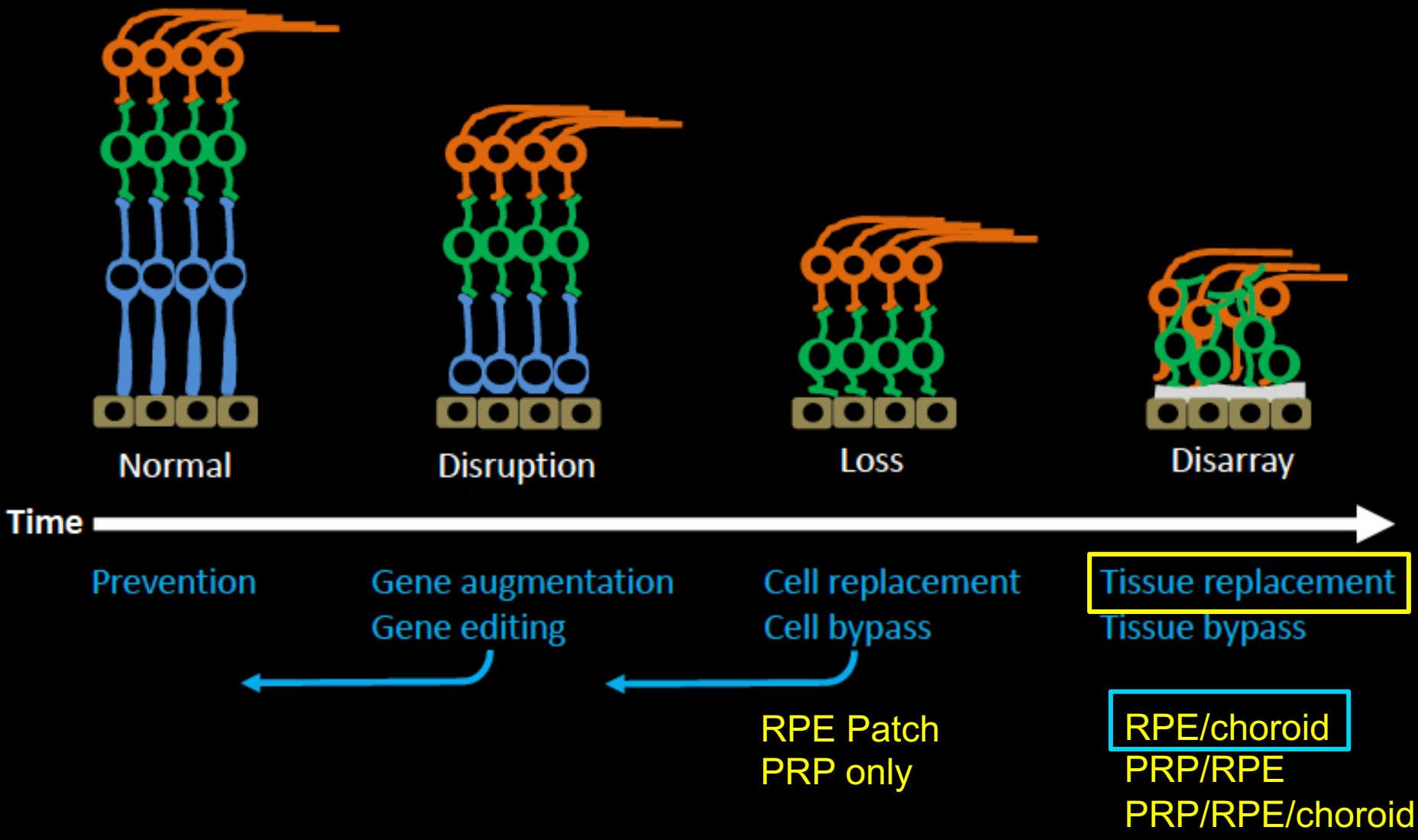
First five patients with BCVA between 20/100-20/500

Next seven patients with BCVA between 20/80-20/500

Different Diseases Stages Manifest with the Loss of Different Eye Tissues

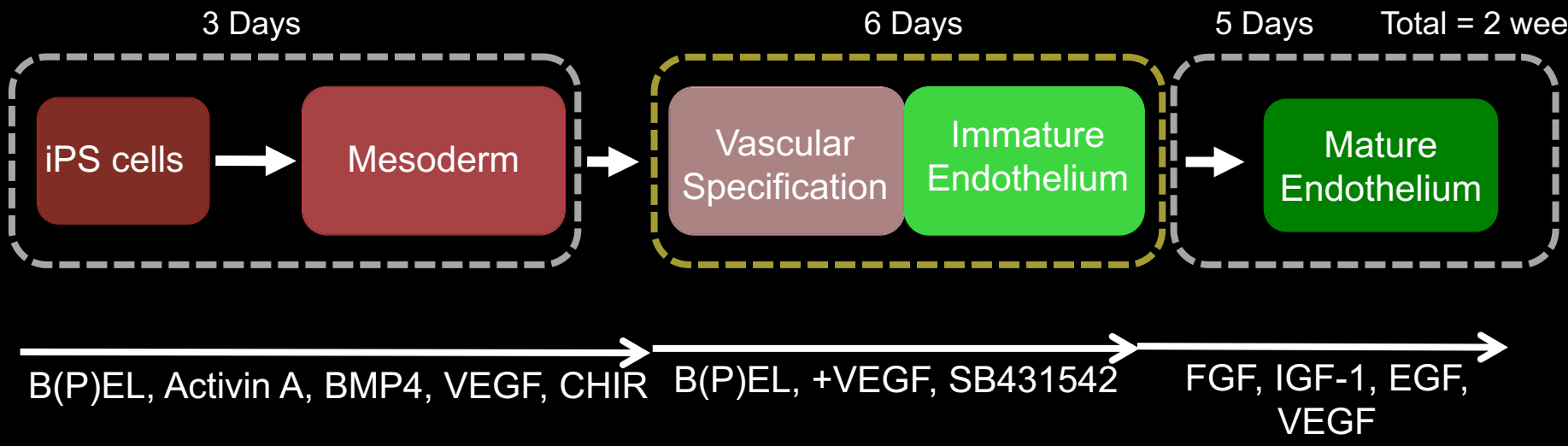


Need for Different Tissues at Different Disease Stages

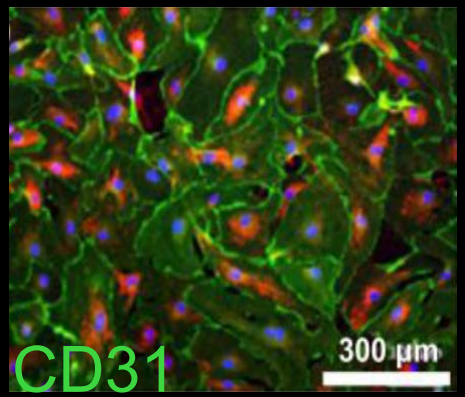


Courtesy: David Gamm (UW, Madison)

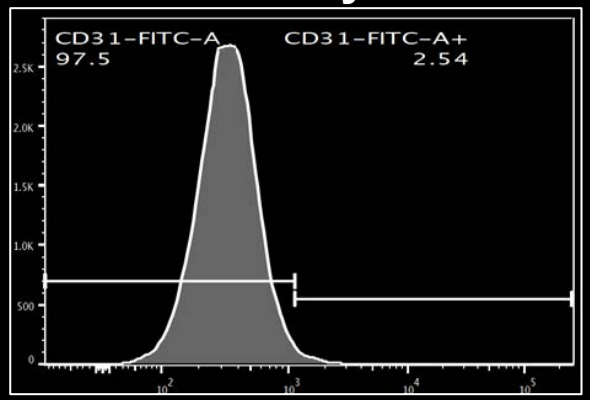
Differentiation Pure and Mature iPSC-Endothelial Cells



iPSC-endothelial cells



Endo Purity >97%

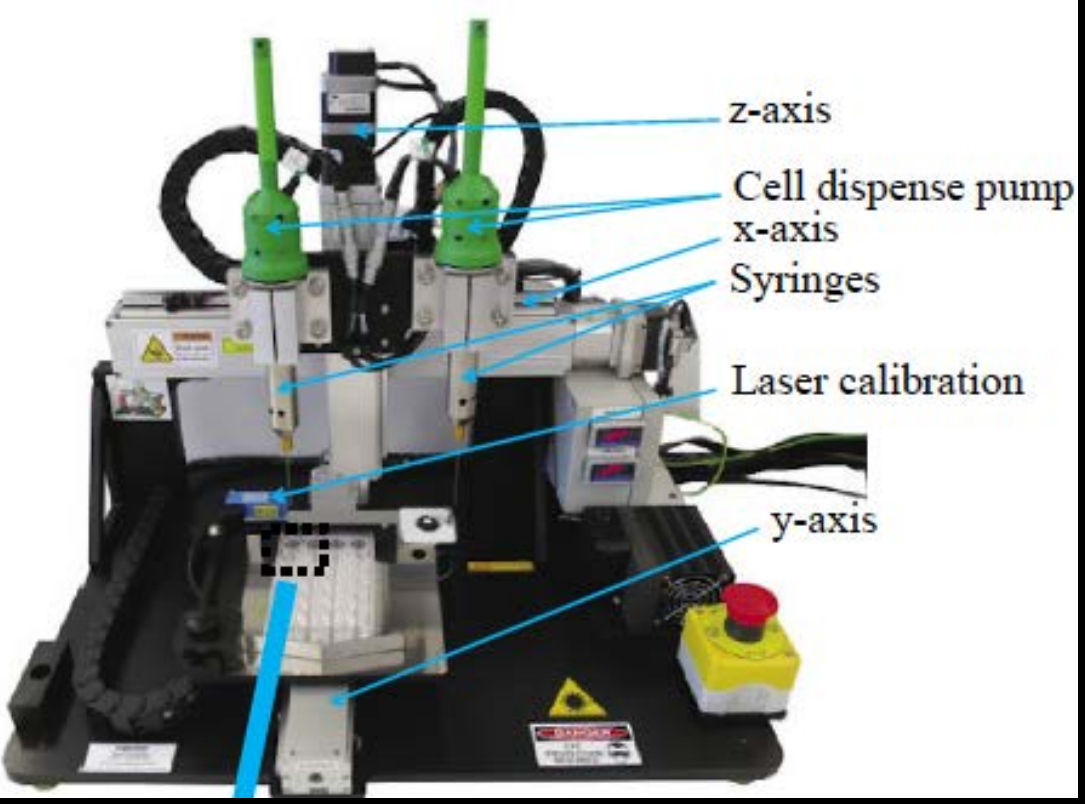


Orlova et al., 2014

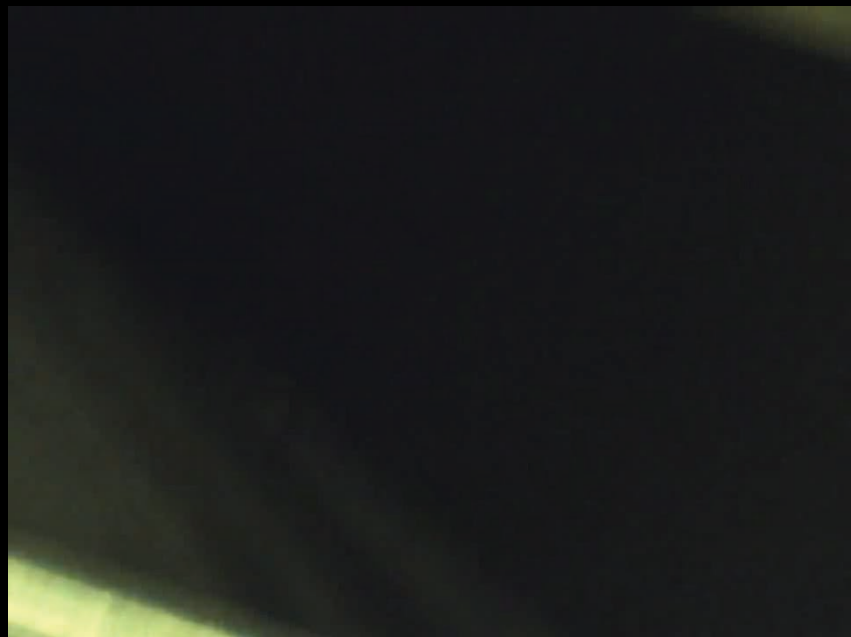
8 Donors
- 6 Healthy
- 2 Diseased (AMD, STAT3)

Roba Dejene, Tea Soon Park

3D Bioprinter

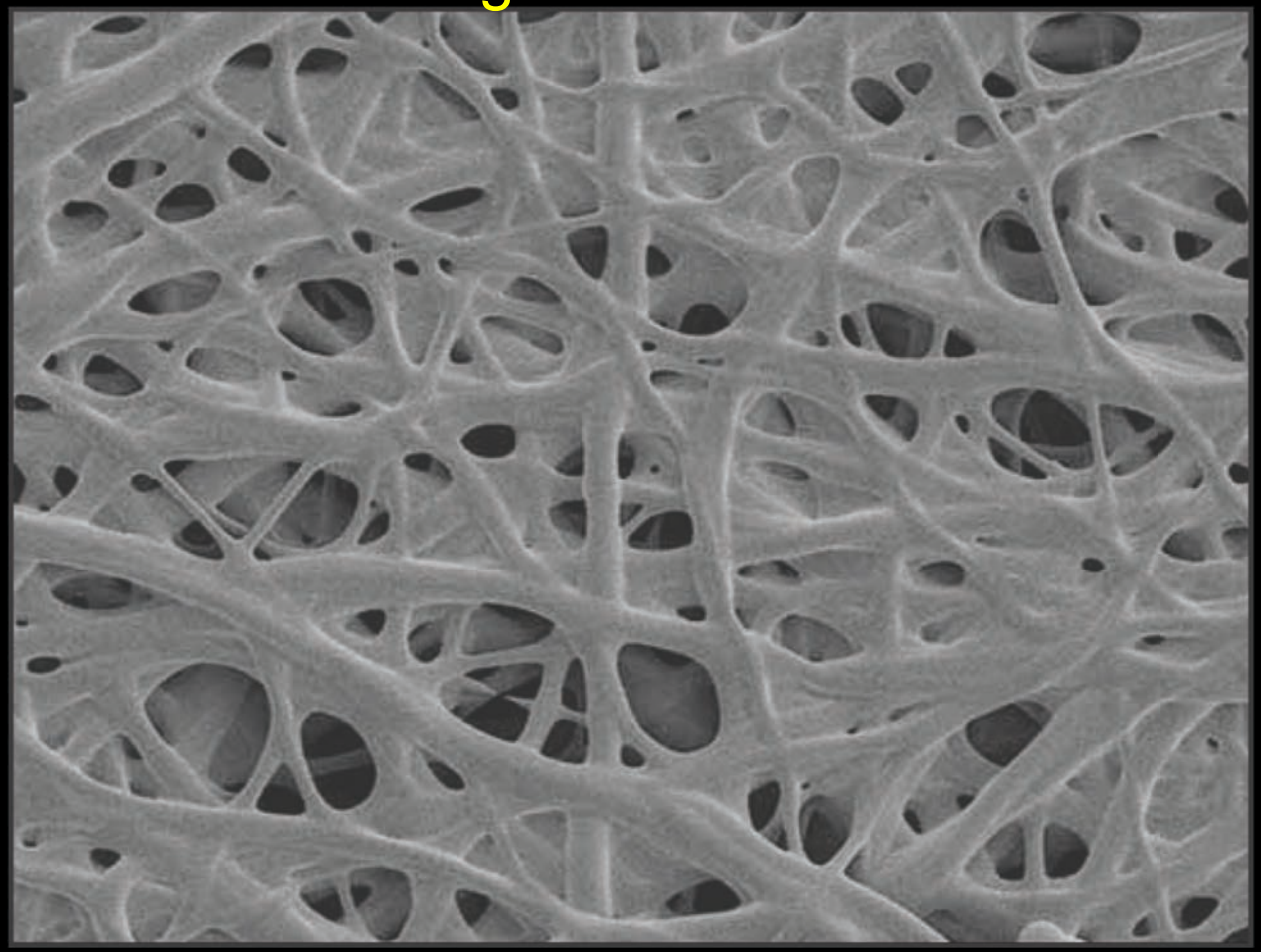


"Bioink" contains endothelial cells, pericytes, fibroblasts and hydrogel



Bioprinting to Develop a 3D RPE/ “Choroid” Tissue

Biodegradable scaffold



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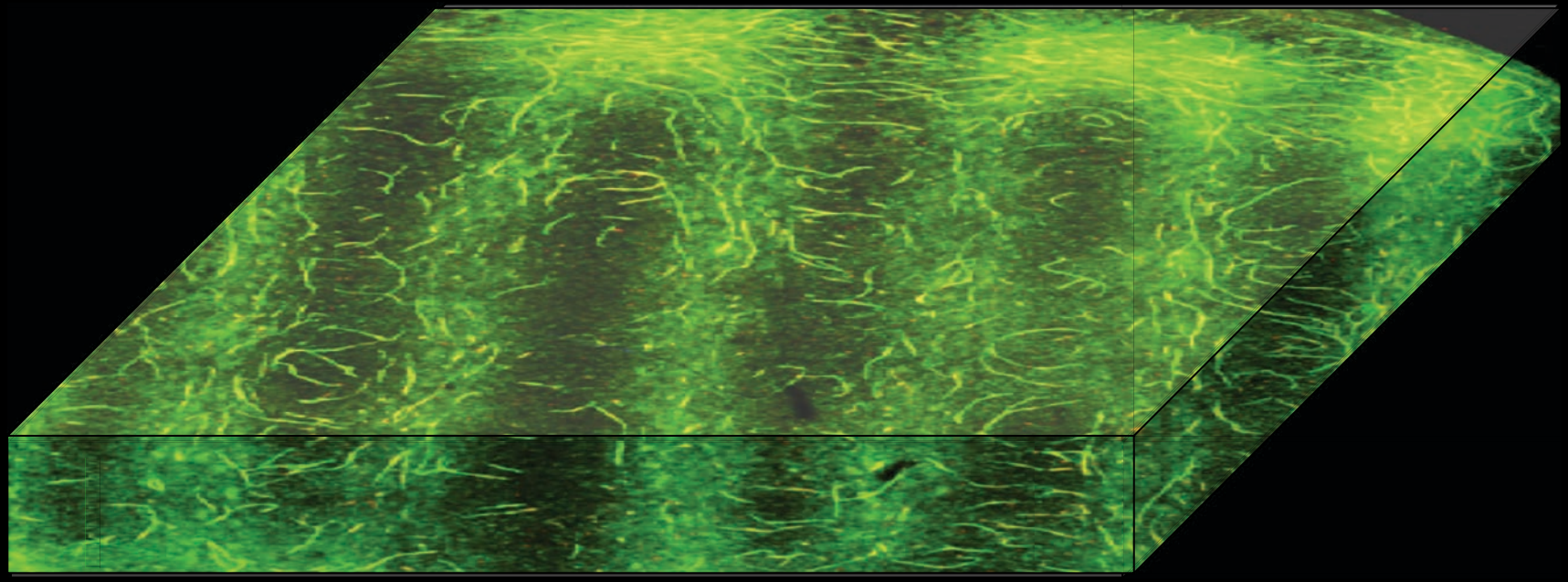
Bioprinting to Develop a 3D RPE/ “Choroid” Tissue

Bioprint “bioink” (hydrogel, endothelials cells, fibroblasts, pericytes)



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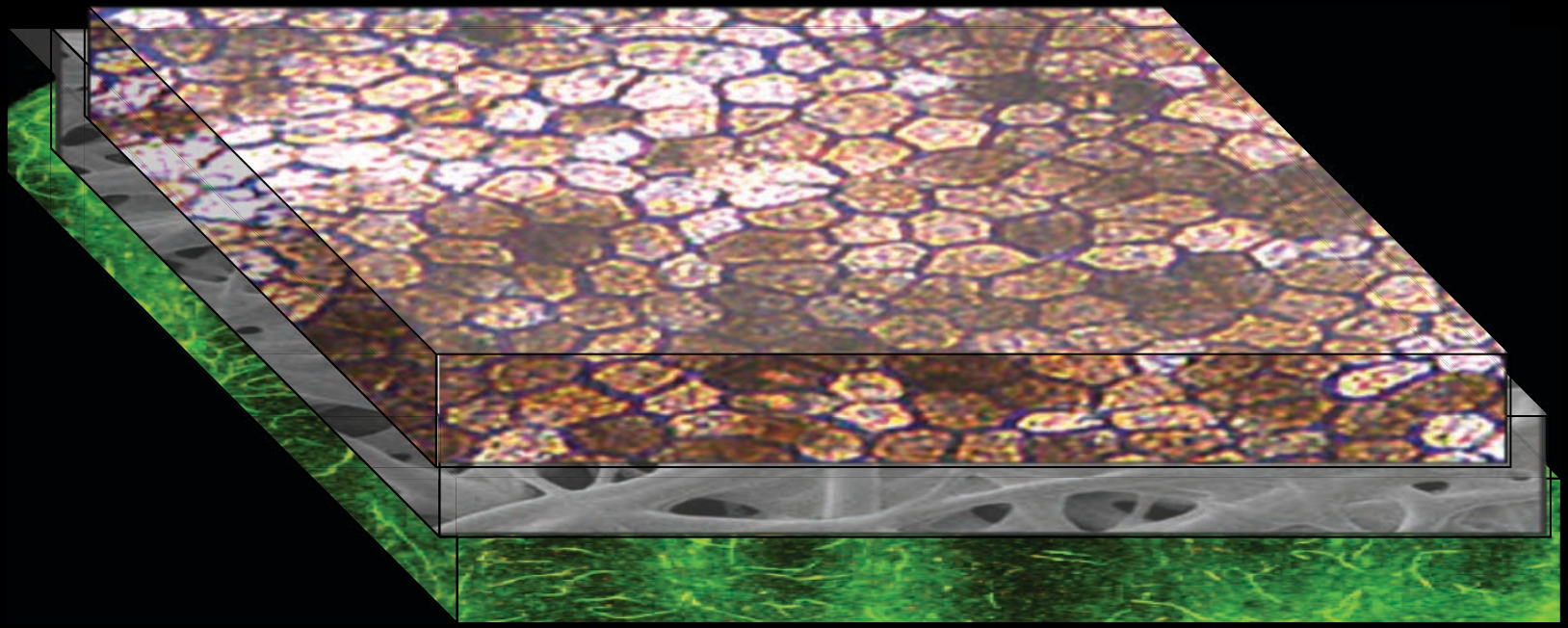
Bioprinting to Develop a 3D RPE/ “Choroid” Tissue



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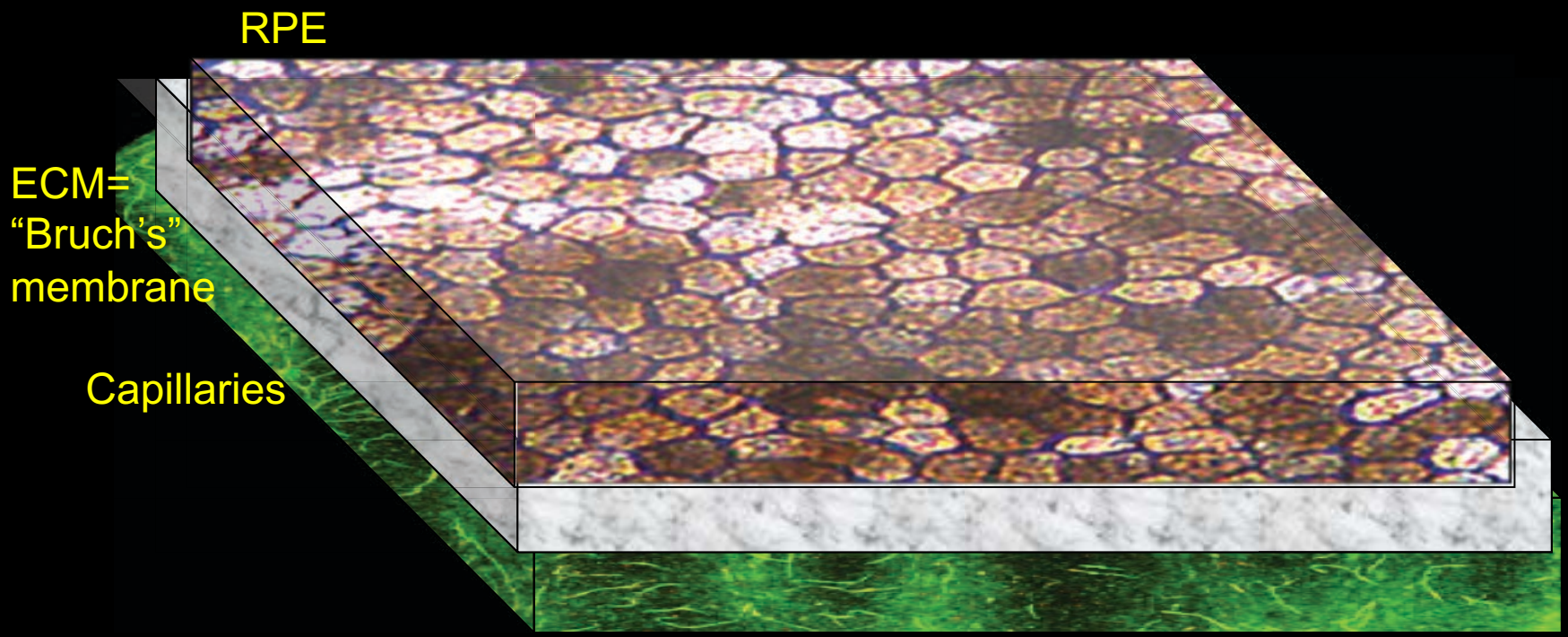
Bioprinting to Develop a 3D RPE/ “Choroid” Tissue



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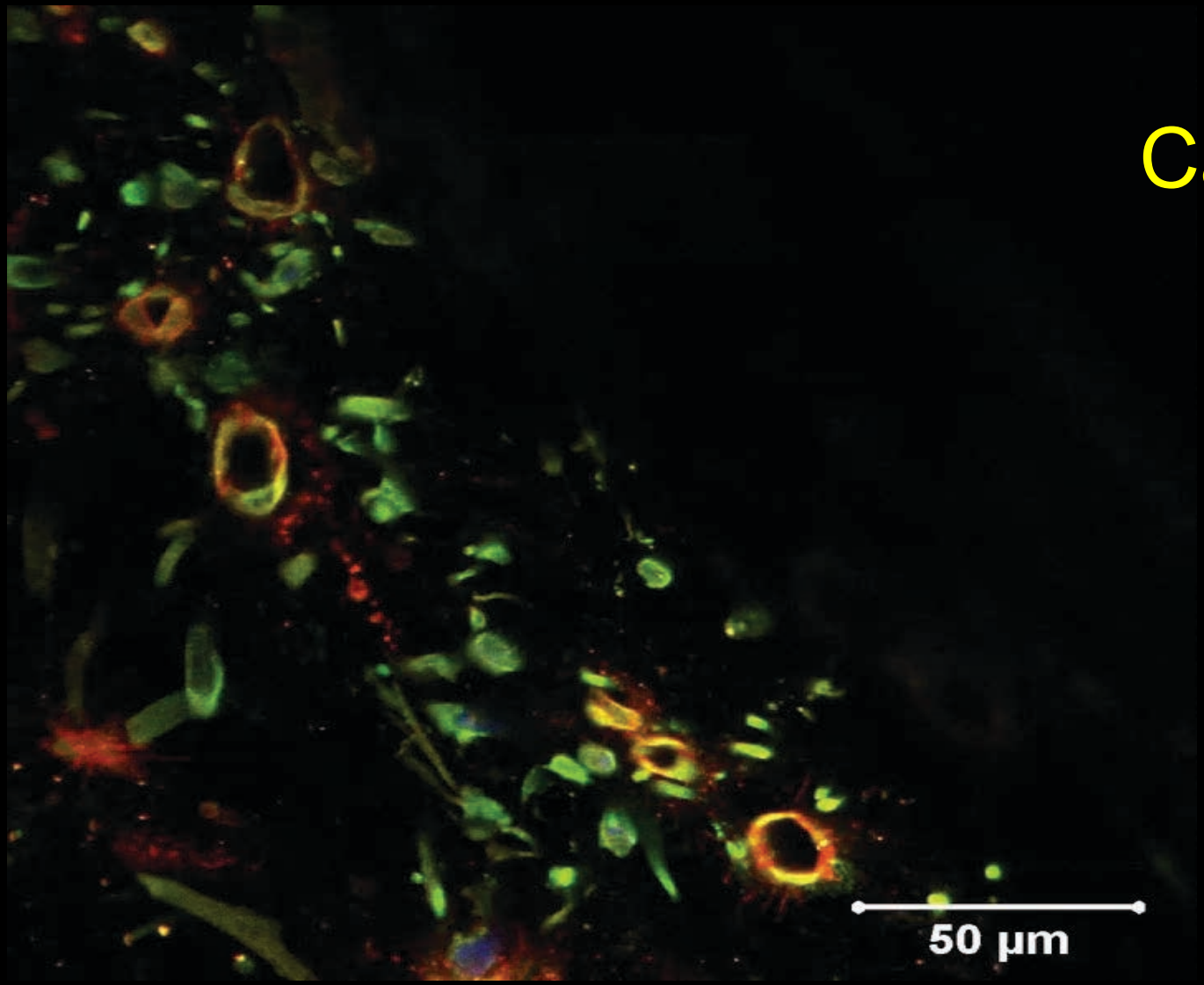
3D Bioprinted “Choroid”



Capillaries

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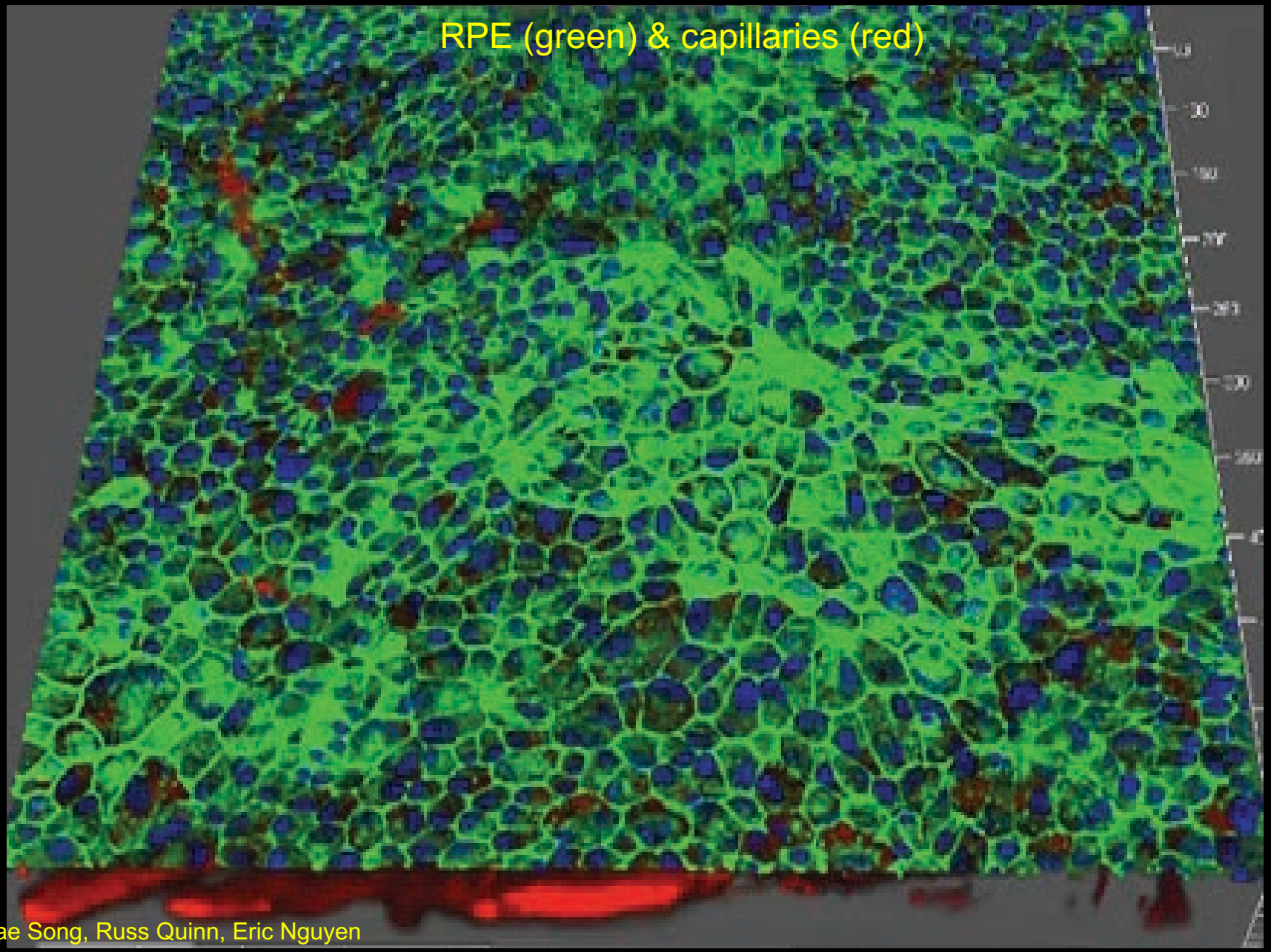
3D Choroid/RPE Tissue



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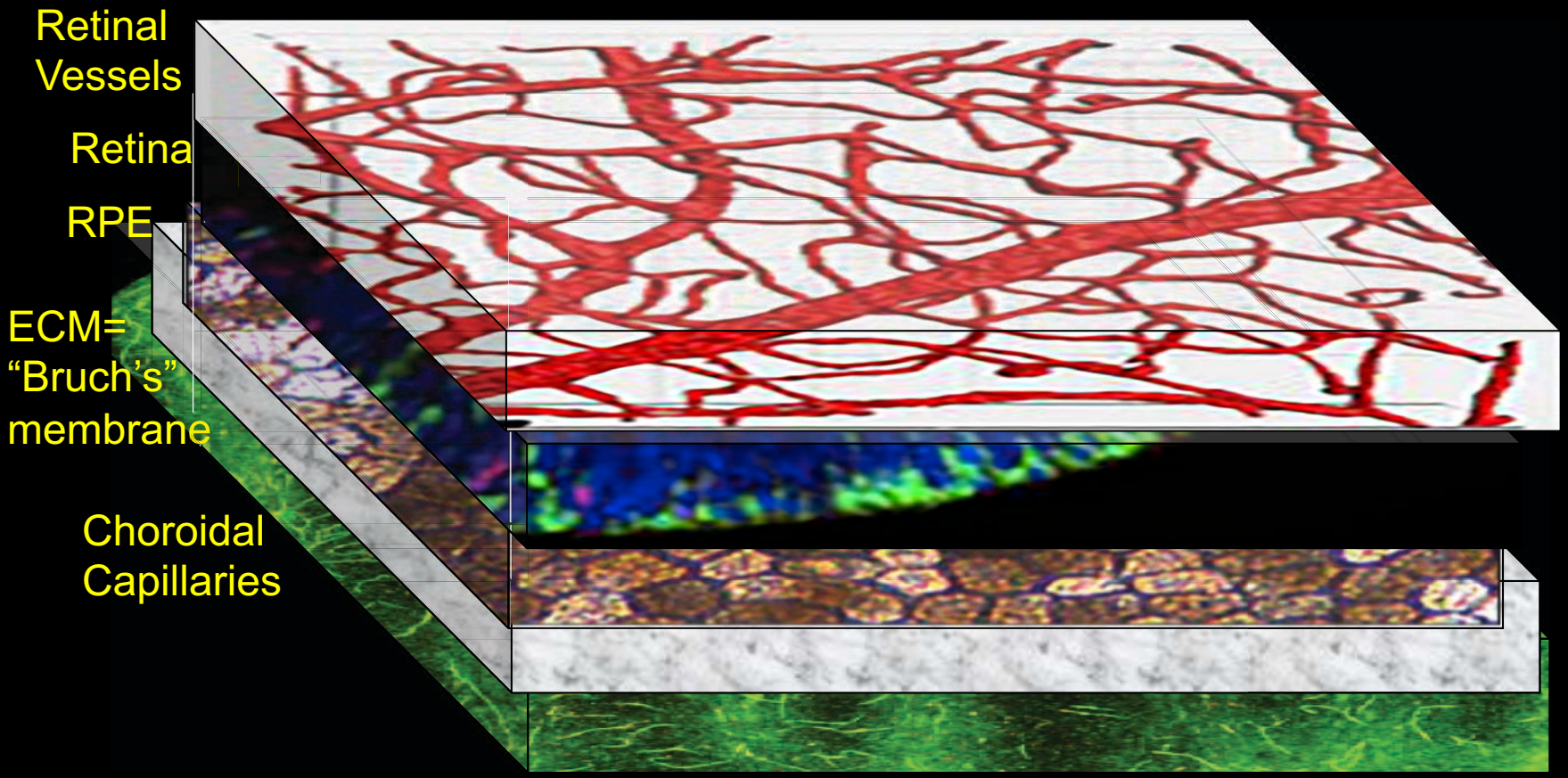
TIME FOR A 3D BACK of the EYE (Retina/RPE/Choroid Tissue)



Min Jae Song, Chris Hampton, Russ Quinn, Eric Nguyen, Tea Soon Park


3D bioprinting team

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