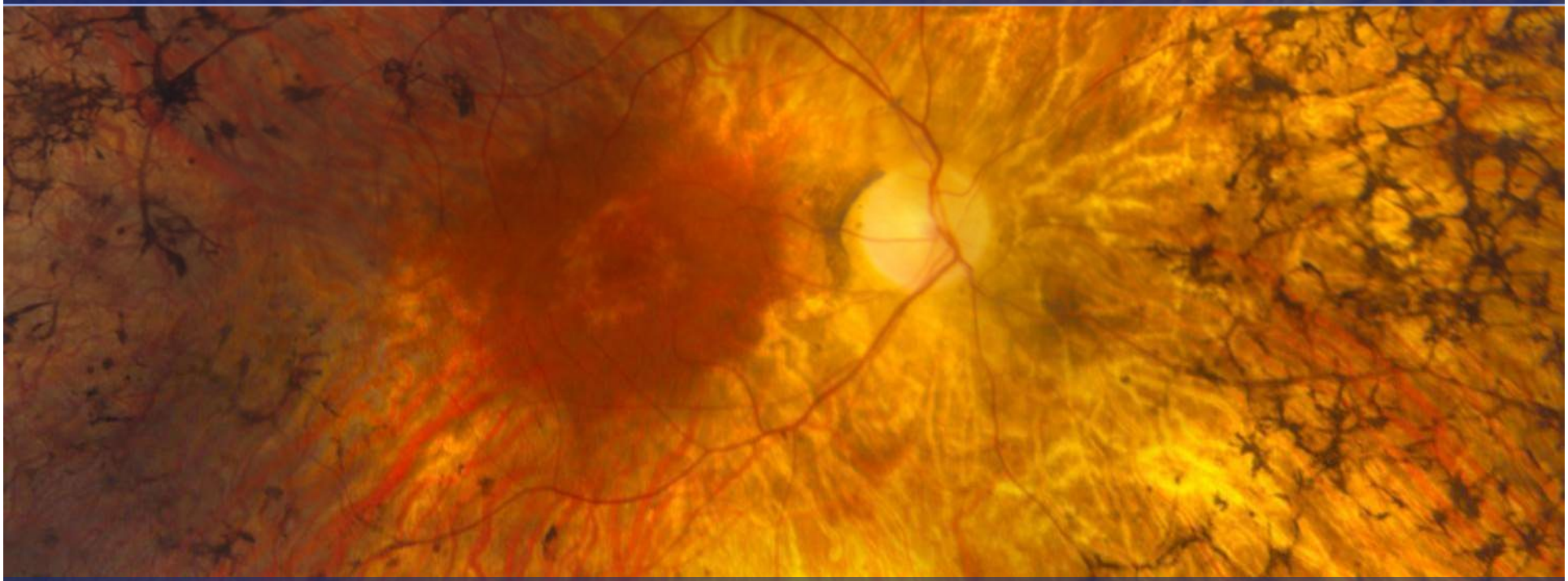
Gene and Stem Cell Therapy for Usher Syndrome



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9th Annual USH Connections Conference | July 15, 2017

Financial Disclosures

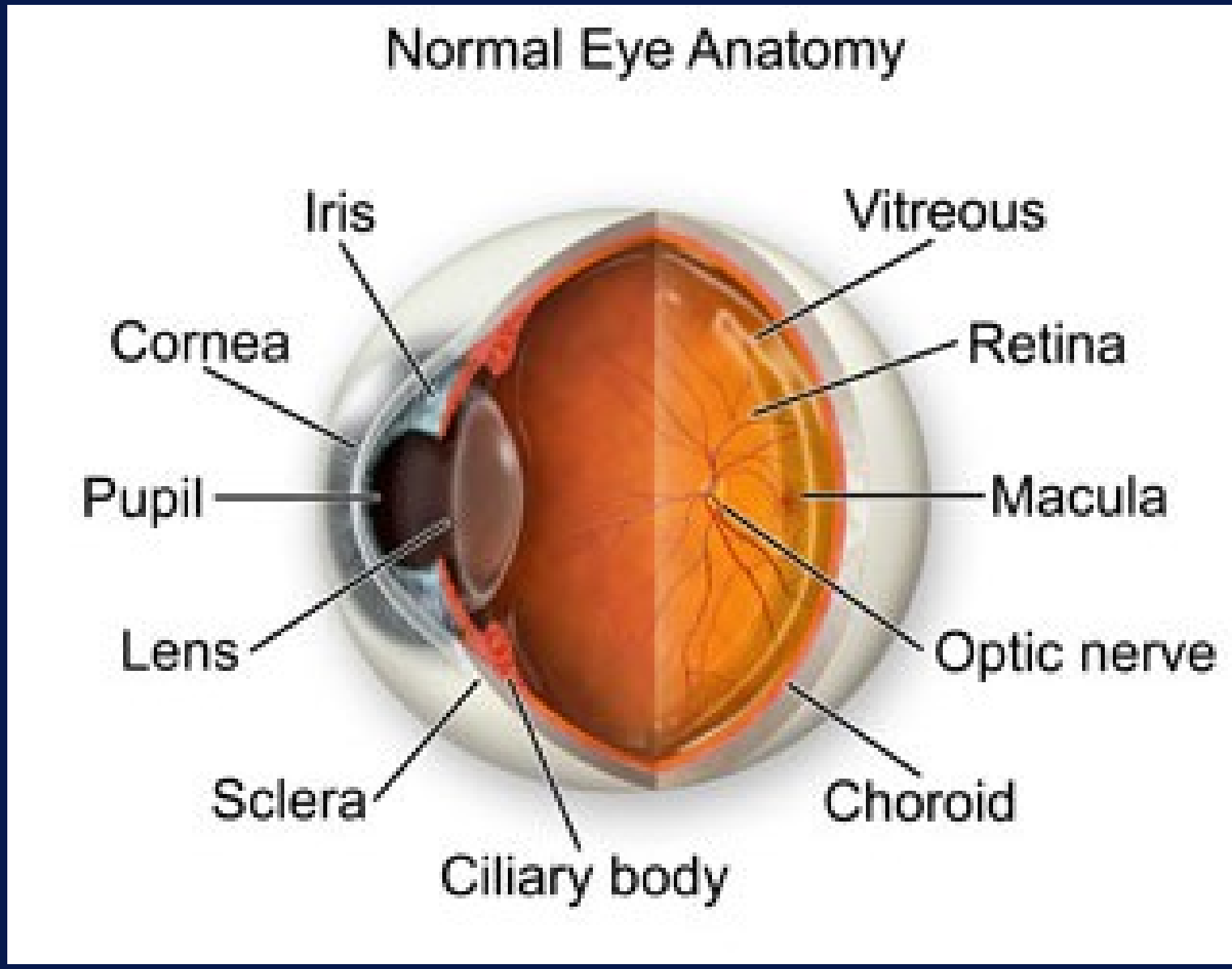
NONE

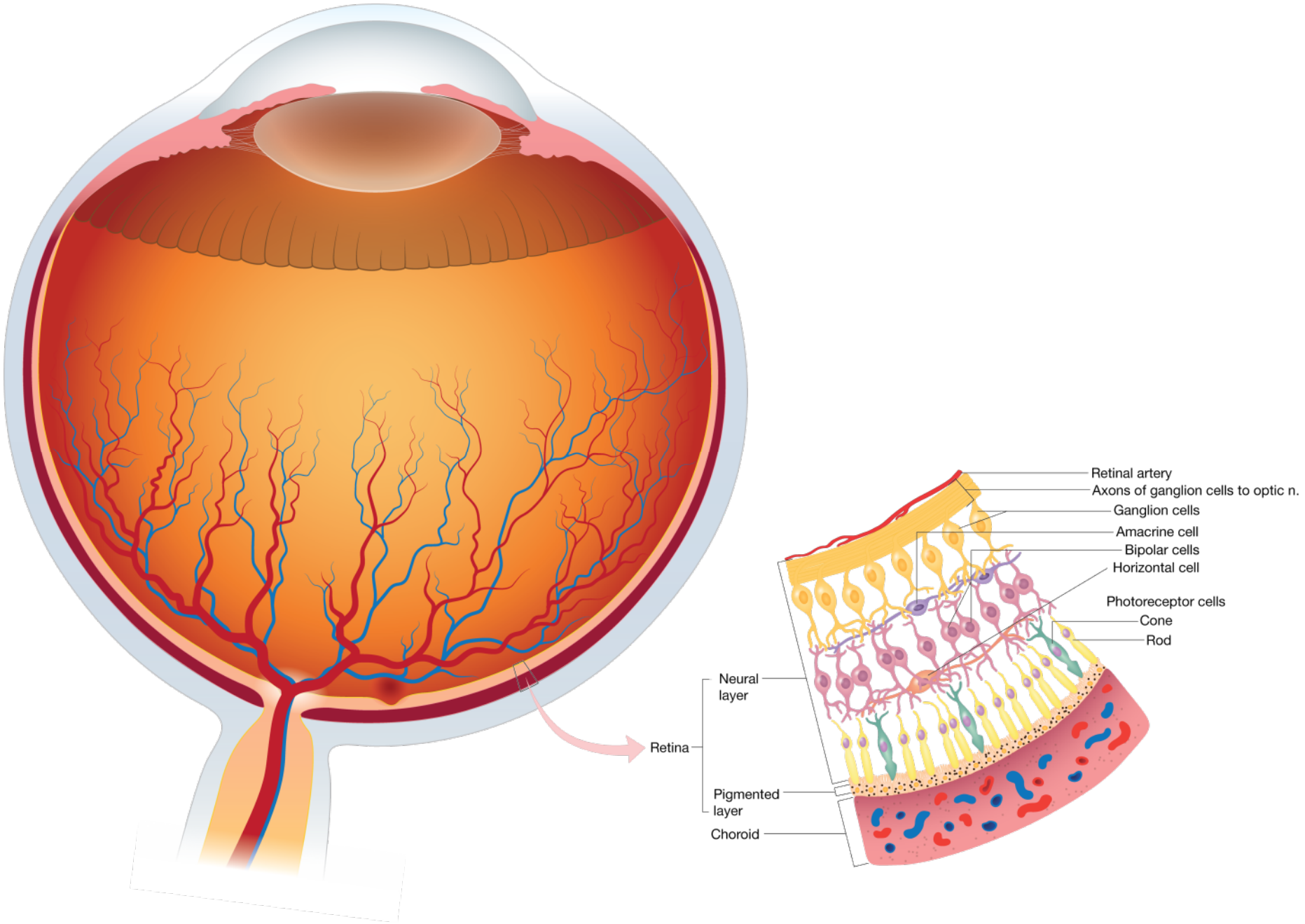
Objective

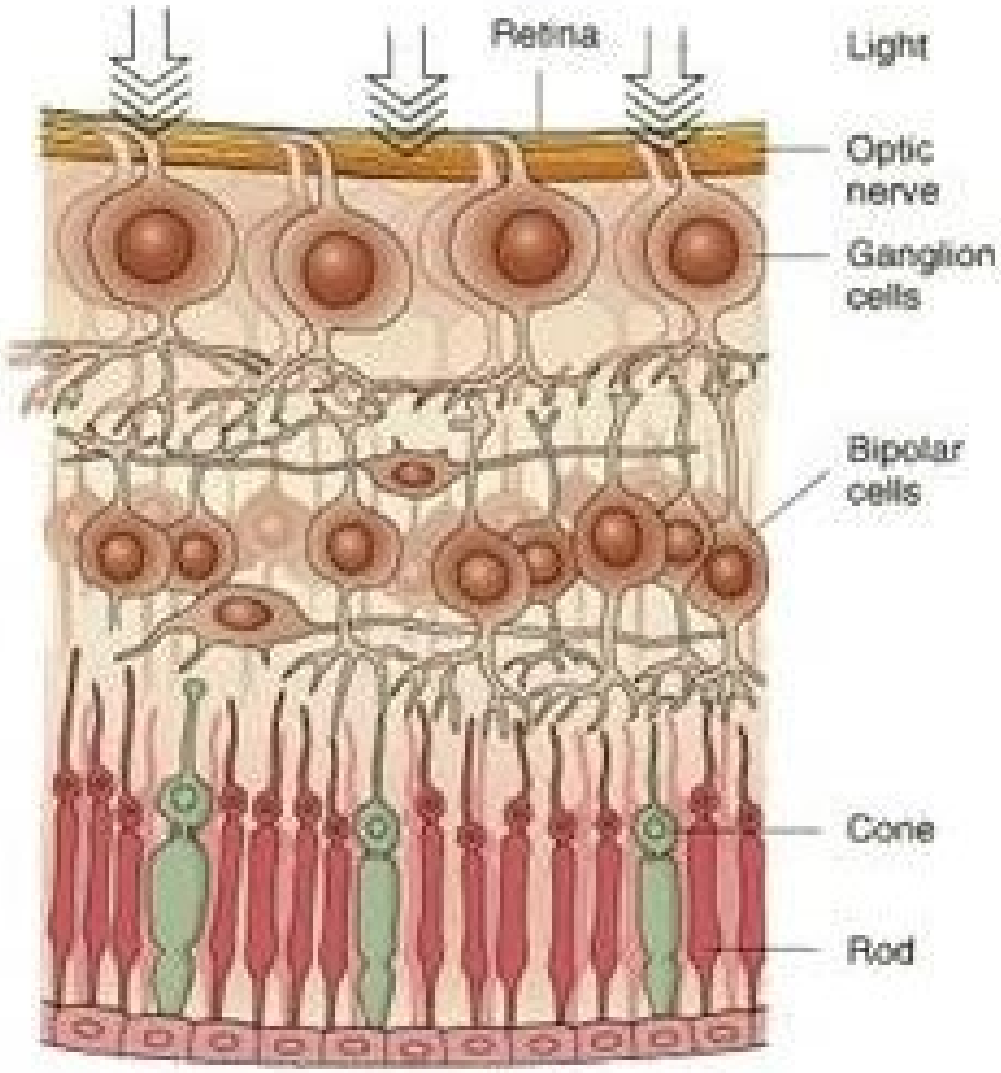
- Describe the treatment strategy for curing vision loss from Usher syndrome

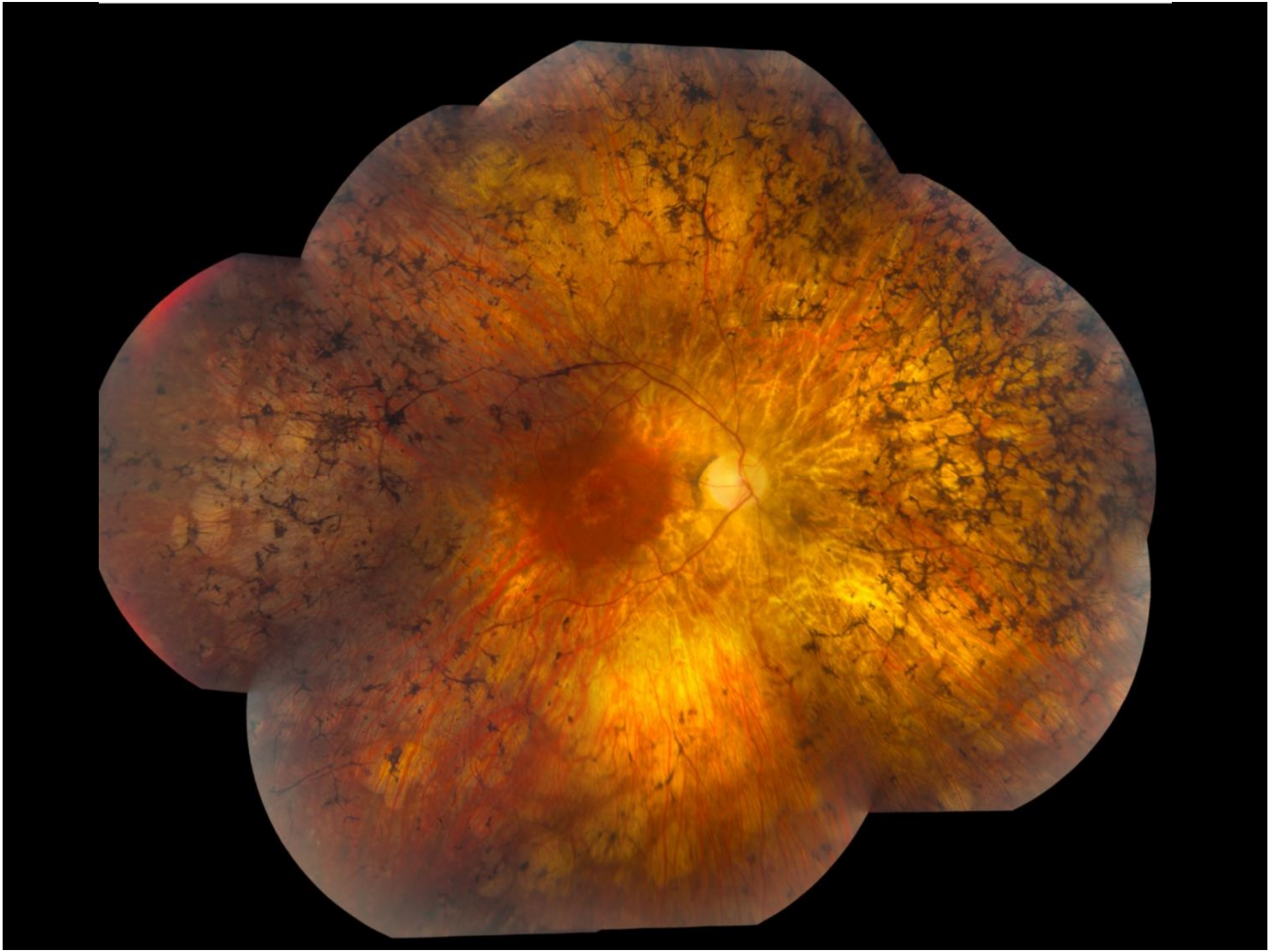
Outline

- Basic eye anatomy
- Basic cell biology
- Treatment based on disease severity
- Gene therapy
- Stem cell therapy
- Surgery to deliver genes and stem cells


Normal Eye Anatomy
Iris
Vitreous
Cornea
Retina
Pupil
Macula
Lens
Optic nerve
Sclera
Choroid
Ciliary body

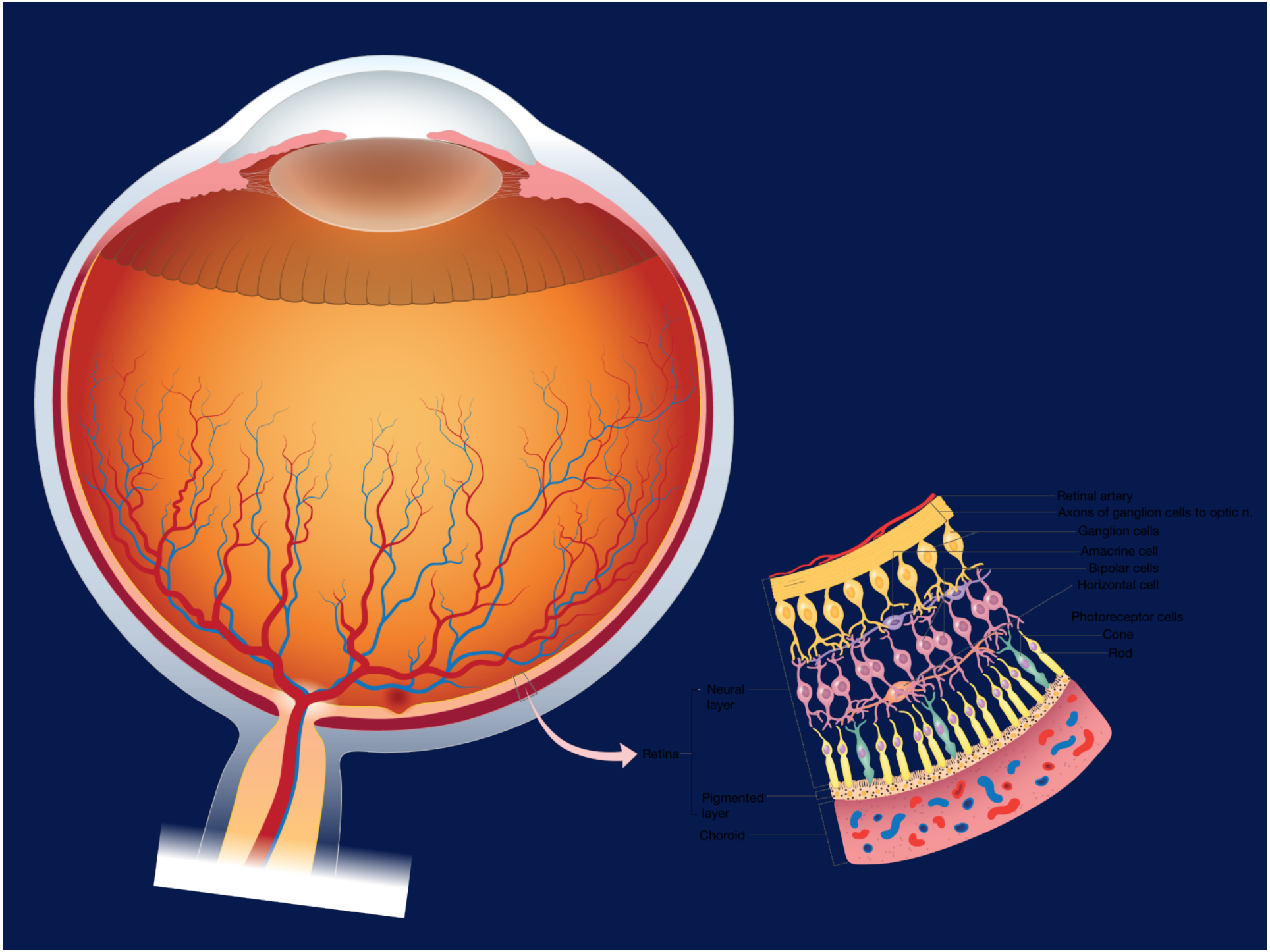

Retinal artery
Axons of ganglion cells to optic n.
Ganglion cells
Amacrine cell
Bipolar cells
Horizontal cell
Photoreceptor cells
Cone
Rod
Neural layer
Retina
Pigmented layer
Choroid


Retina
Light
Optic nerve
Ganglion cells
Bipolar cells
Cone
Rod



Why do photoreceptors degenerate?

- Genetic variants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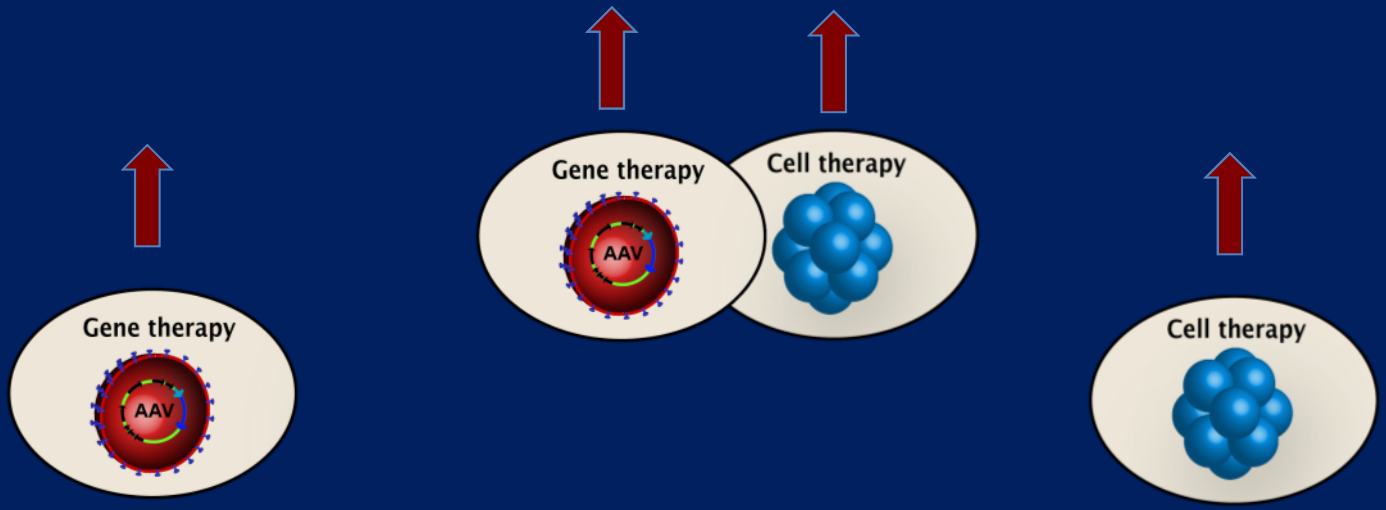
Retina
Pigmented layer
Choroid

The best treatment strategy for Usher syndrome depends on the severity of disease

Treatment

Disease Course

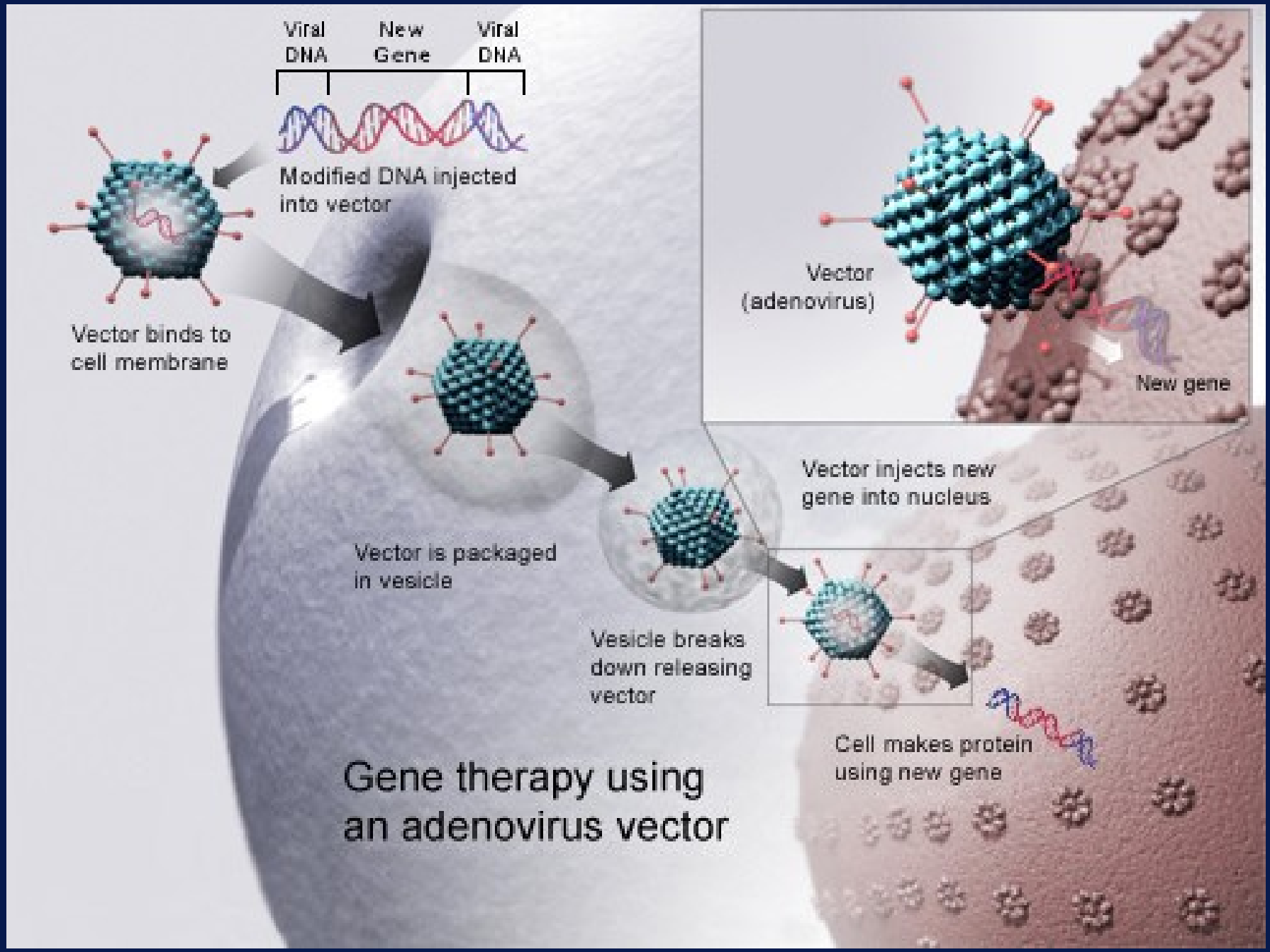
Mild Moderate Severe



Gene Therapy

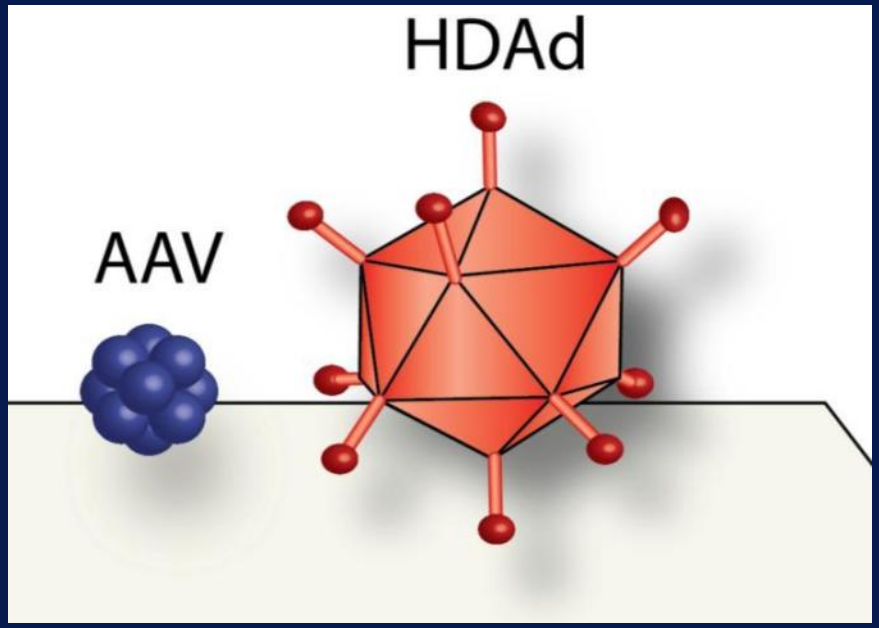
Gene Therapy

- Replace or correct the gene variant
- Requires viable cells to make the gene product (proteins)
- Several ways to deliver gene therapy


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector (adenovirus)
New gene
Vector injects new gene into nucleus
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Cell makes protein using new gene
Gene therapy using an adenovirus vector

104 Genes

- 75% have cDNAs that will fit into AAV (less than ~5Kb)
- The remainder will fit into HDAd (~35Kb)



Usher Genes

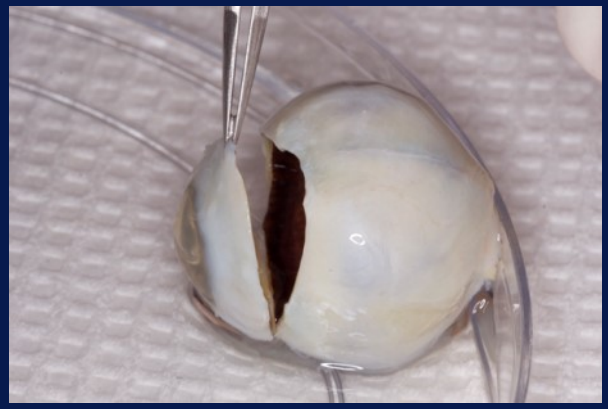
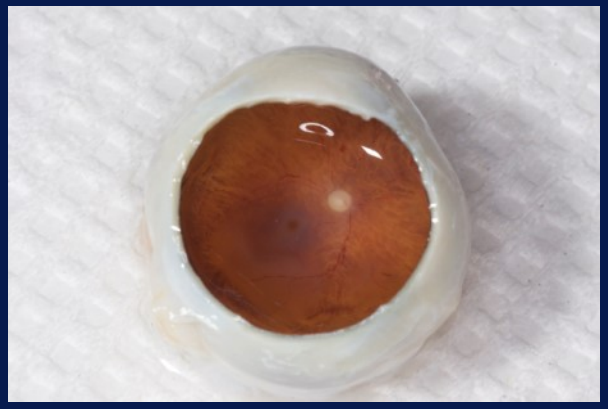
USH# Nomenclature	Gene	Clinical Type	Protein	cDNA Size
Usher Type 1				
USH1B	MYO7A	USH Type 1	Myocin 7A	6,645
USH1C	USH1C	USH Type 1	Harmonin	2,697
USH1D	CDH23	USH Type 1	Cadherin-Like 23	10,062
USH1F	PCDH15	USH Type 1	Protocadherin 15	5,871
USH1G	USH1G	USH Type 1	Scaffold Protein Containing Ankyrin Repeats and SAM Domain	1,683
USH1J	CIB2	USH Type 1	Calcium and Iintegrin Binding Family Member 2	561
Usher Type 2				
USH2A	USH2A	USH Type 2	Usherin	15,606
USH2C	GPR98	USH Type 2	Monogenic Audiogenic Seizure Susceptibility 1 Homolog	18,918
USH2D	DFNB31	USH Type 2	Whirlin	2,721
Usher Type 3				
USH3A	CLRN1	USH Type 3	Clarin-1	735
USH3B	HARS	USH Type 3	Histidyl-tRNA Synthetase	1,527
N/A	ABHD12	USH Type 3-Like	Abhydrolase Domain Containing Protein 12	1,212

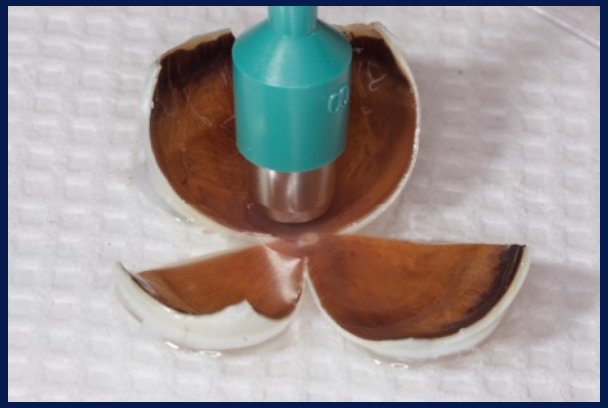
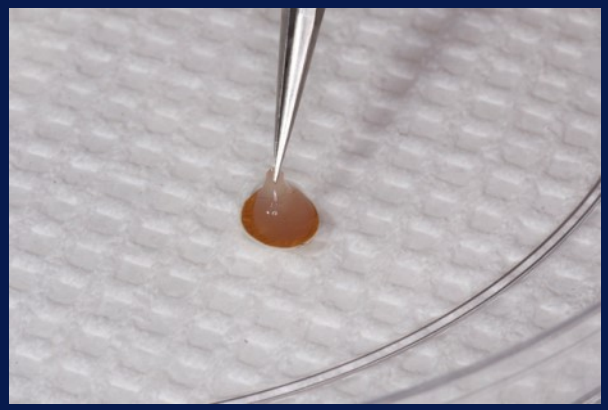
HDAd can solve the large gene problem

- USH2A is the second most common inherited retinal disease gene
- 92% of Usher is caused by genes that won’t fit into AAV (e.g. MYO7A, USH2A, PCDH15, CDH23)

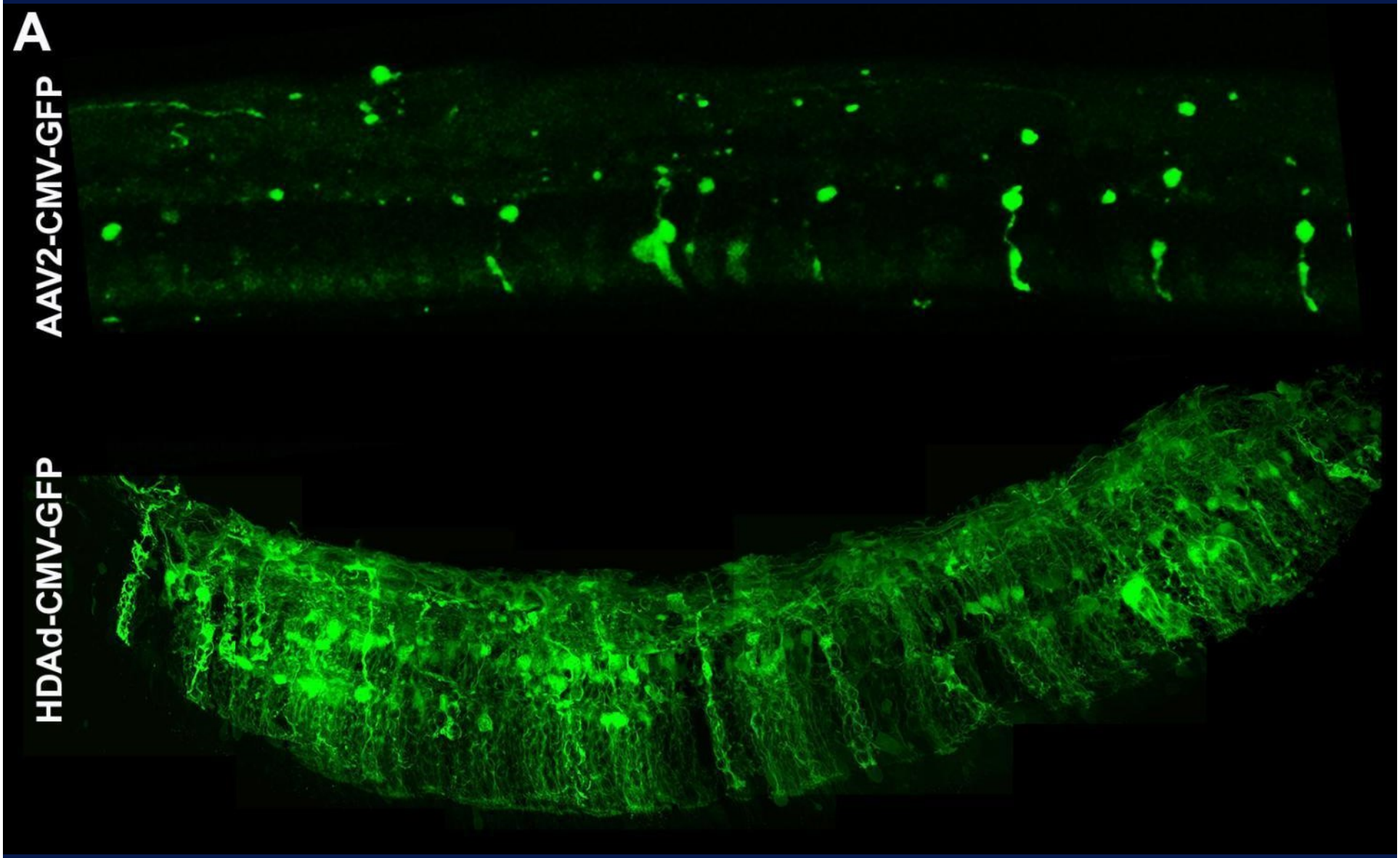
Progress in Gene Therapy for Usher syndrome at the WIVR

- We have manufactured every known Usher gene
- We are currently testing these gene products in cell, tissue, and animal models
- We are testing HDAd gene delivery


Human Retinal Organ Culture



What is involved in testing a gene before human clinical studies?

What is involved in testing a gene before human clinical studies?

- FDA regulations to ensure that genes are safe before it reaches a human eye

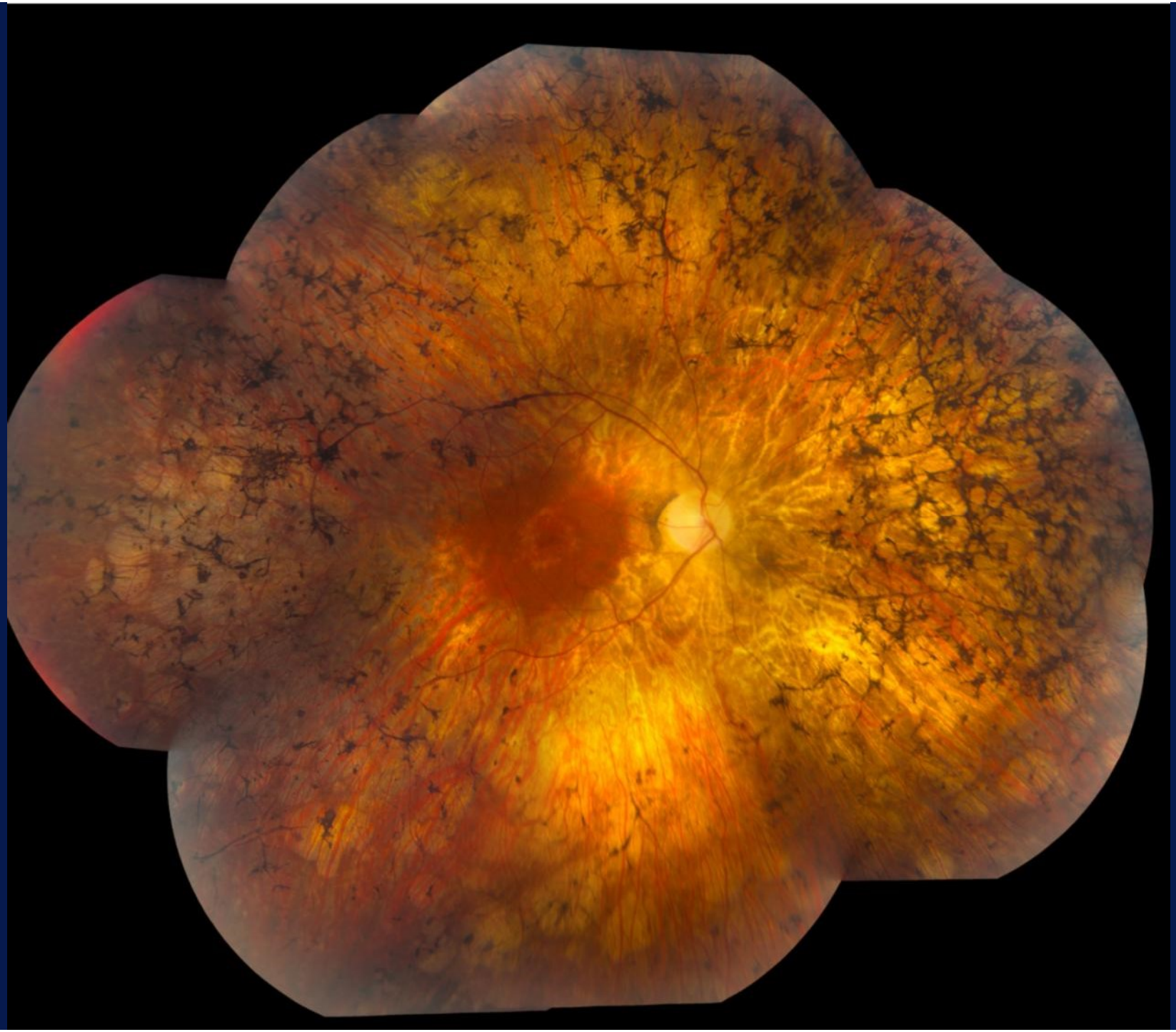
Multiple New Studies at the WIVR Per Year

- We can package one product per month in the cGMP facility
- We can re-use the FDA IND documents (each trial is very similar in rationale and design)
- The rate limiting steps are now generating pre-clinical data and conducting the clinical trials themselves

Steven W. Dezii Research Facility

Good Manufacturing Practices, Open-source, Non-profit, FDA-registered

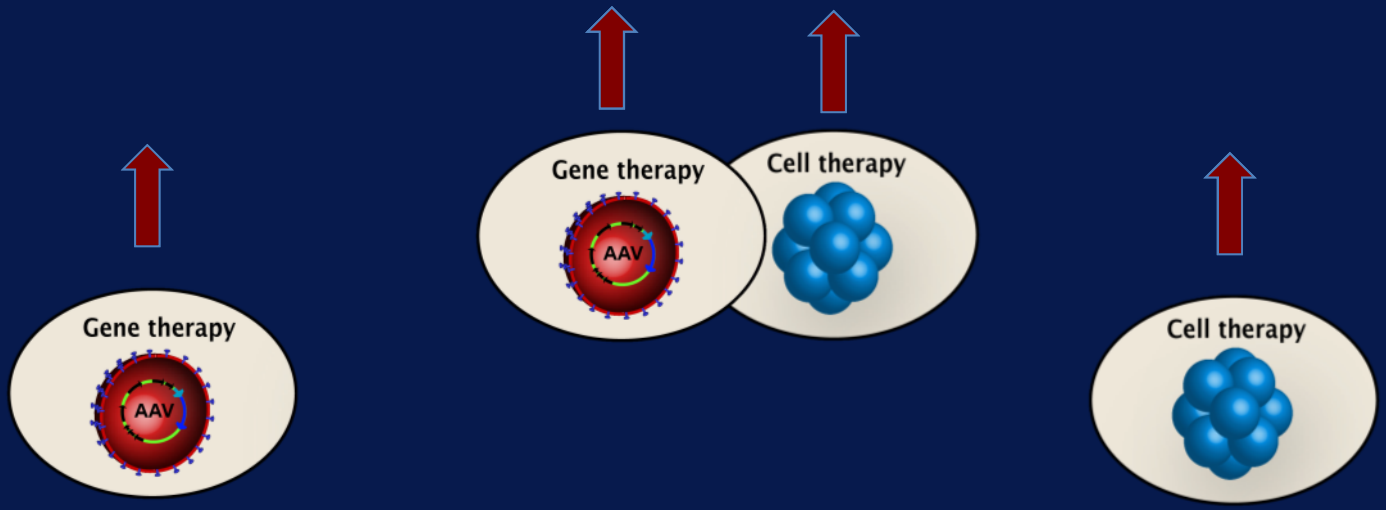
What about treatment for more severe disease?

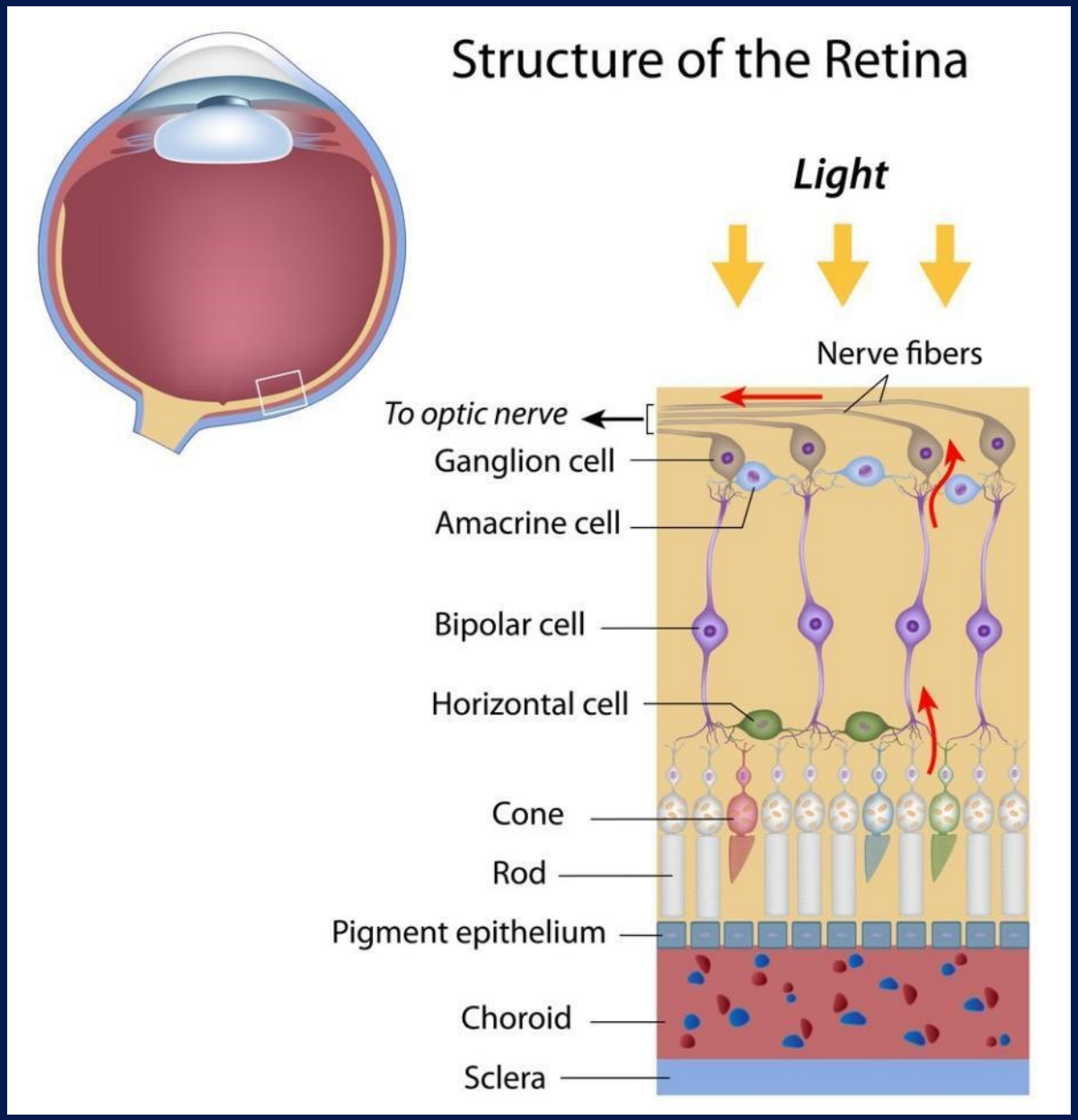


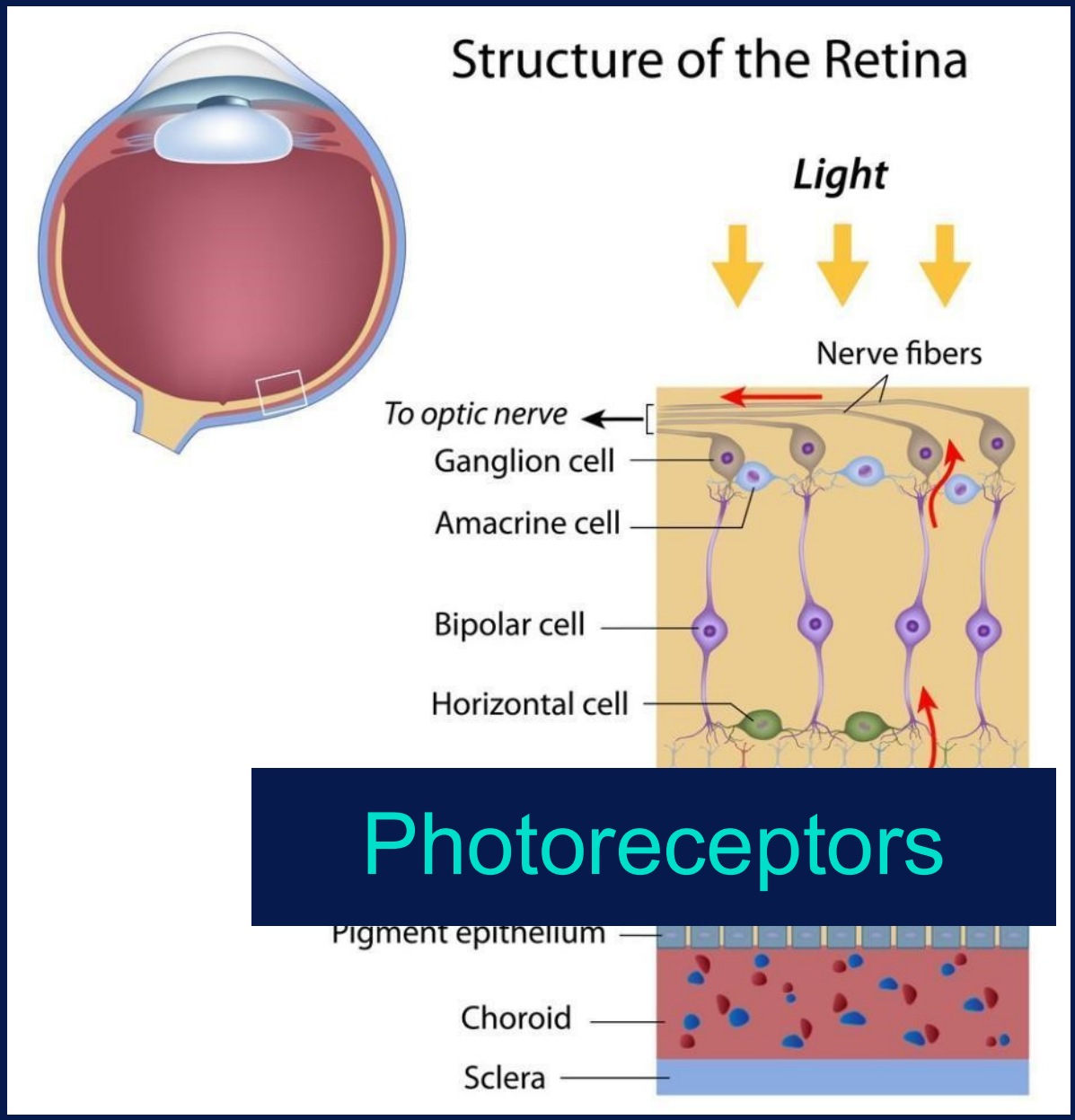
Treatment

Disease Course

Mild Moderate Severe




Structure of the Retina
Light
Nerve fibers
To optic nerve
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Cone
Rod
Pigment epithelium
Choroid
Sclera


Structure of the Retina
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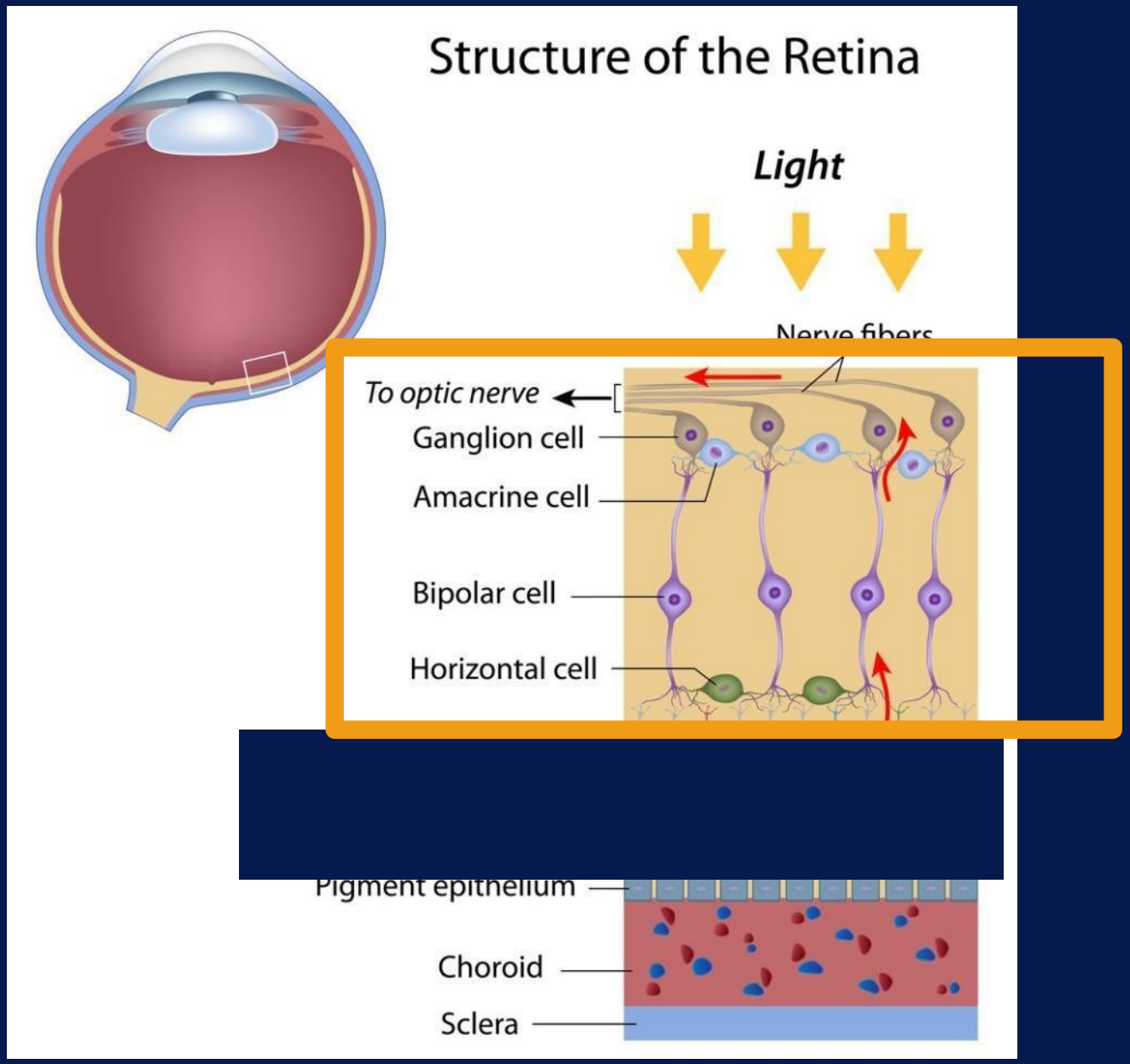
To optic nerve
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Photoreceptors
Pigment epithelium
Choroid
Sclera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Horizontal cell
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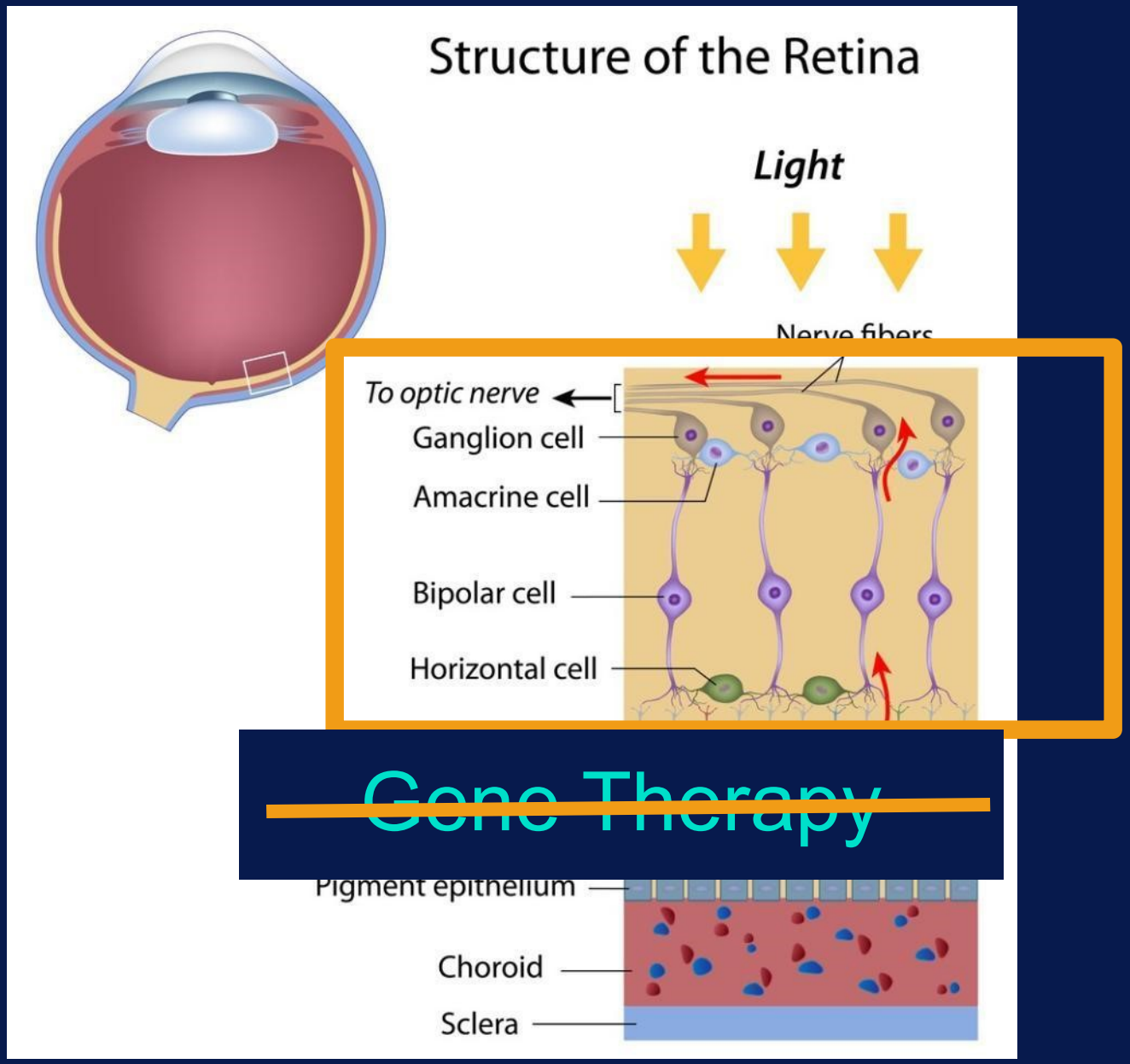
Choroid
Sclera


Structure of the Retina
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Nerve fibers
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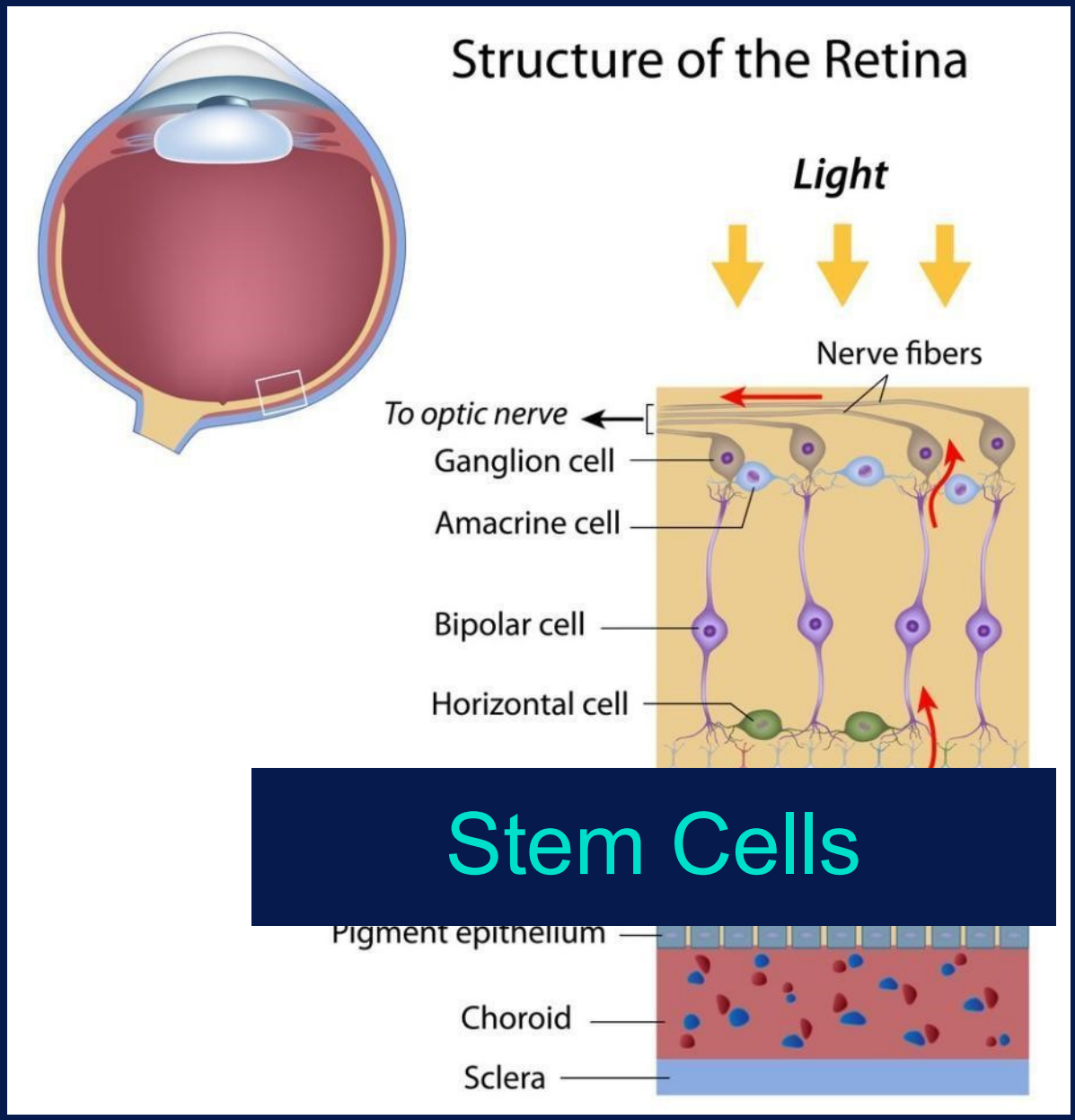
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Gene Therapy
Pigment epithelium
Choroid
Sclera


Structure of the Retina
Light
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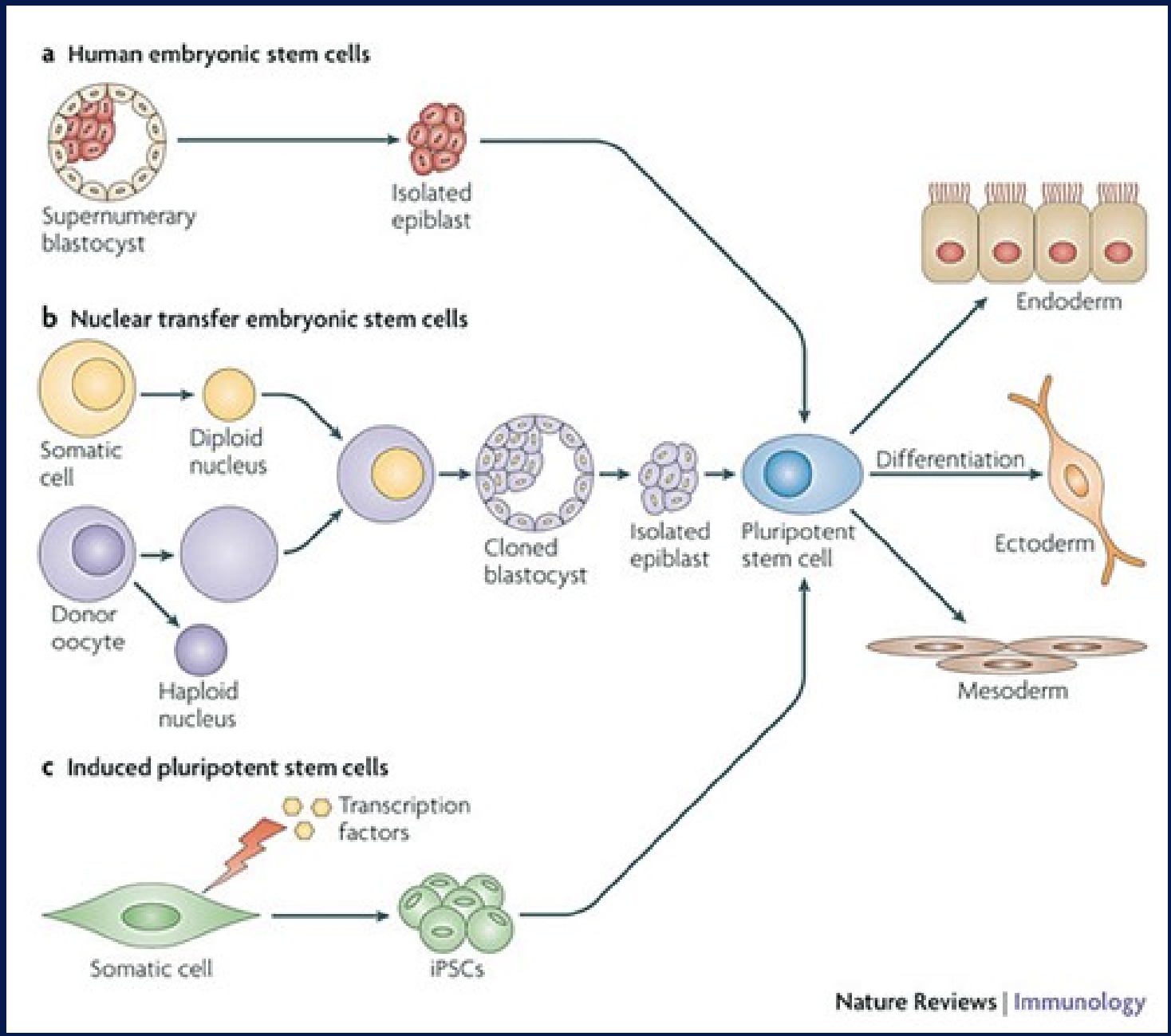
To optic nerve
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Pigment epithelium
Choroid
Sclera

Stem Cells

Stem Cell Therapy

What are stem cells?

- Multipotent, undifferentiated cells
- Can be directed to change into specific cells in the body


a Human embryonic stem cells
Supernumerary blastocyst
Isolated epiblast
b Nuclear transfer embryonic stem cells
Somatic cell
Diploid nucleus
Donor oocyte
Haploid nucleus
Cloned blastocyst
Isolated epiblast
Pluripotent stem cell
Differentiation
Endoderm
Ectoderm
Mesoderm
c Induced pluripotent stem cells
Transcription factors
Somatic cell
iPSCs
Nature Reviews | Immunology

Three Key Strategic Decisions

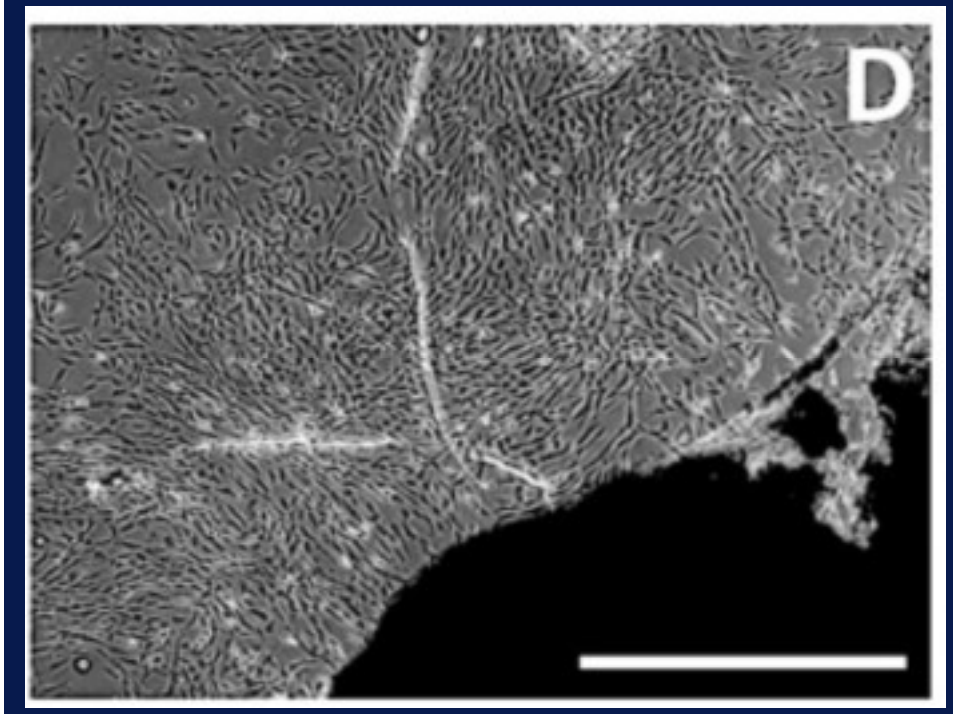
- Autologous cells or not
- Polymer supported or not
- Blind or sighted eyes

Our Strategic Decisions

- Autologous cells
- Polymer supported
- Blind eyes

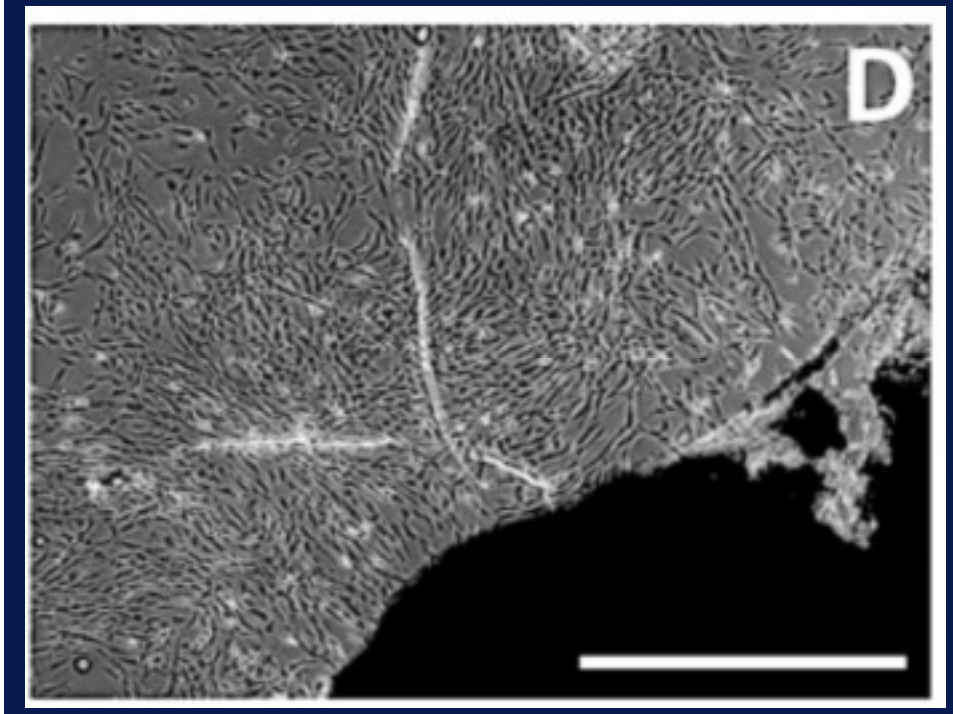
Induced Pluripotent Stem Cells

- iPSCs can be used to create very authentic photoreceptor precursor cells suitable for autologous transplantation, BUT . . .
- They still harbor the mutation(s) that caused the disease in the first place
- Fortunately, CRISPR/CAS9 genome editing can be used to correct the mutations in the iPSCs before differentiating them into retinal cells



Skin fibroblasts

(from 3mm punch biopsy)

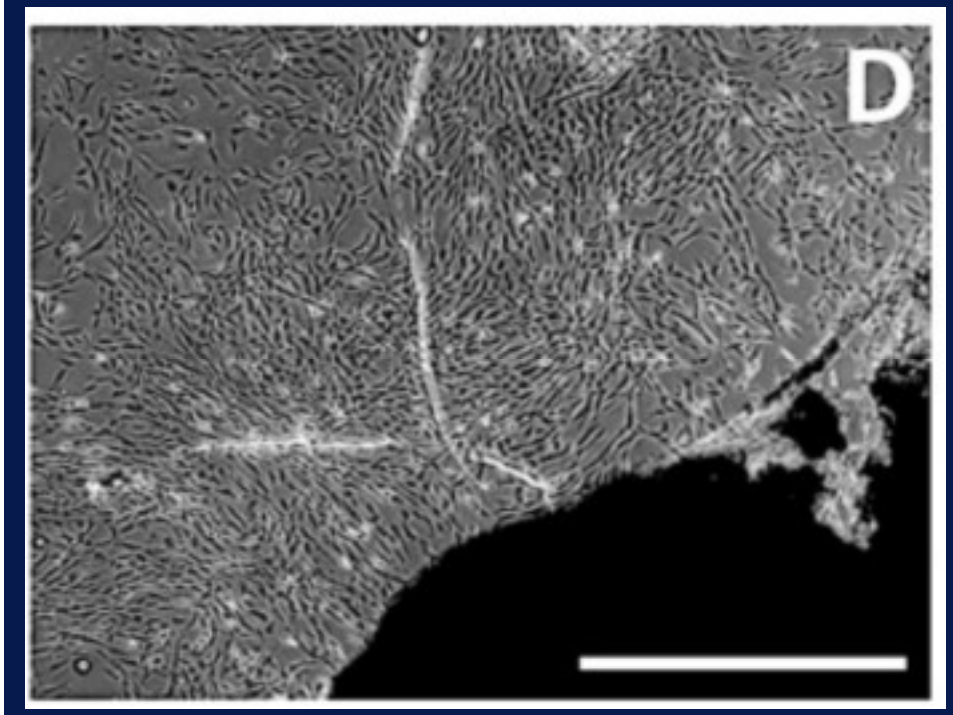


Skin fibroblasts

(from 3mm punch biopsy)

GMP cell lines have been generated from 35 patients with severe visual loss

Pluripotency Factors



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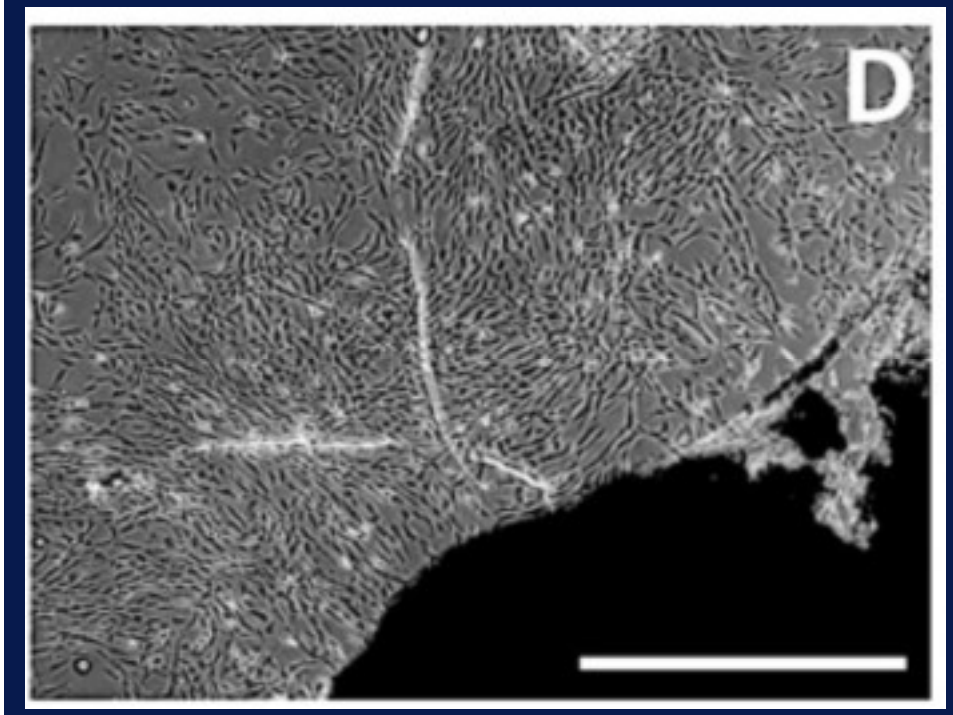
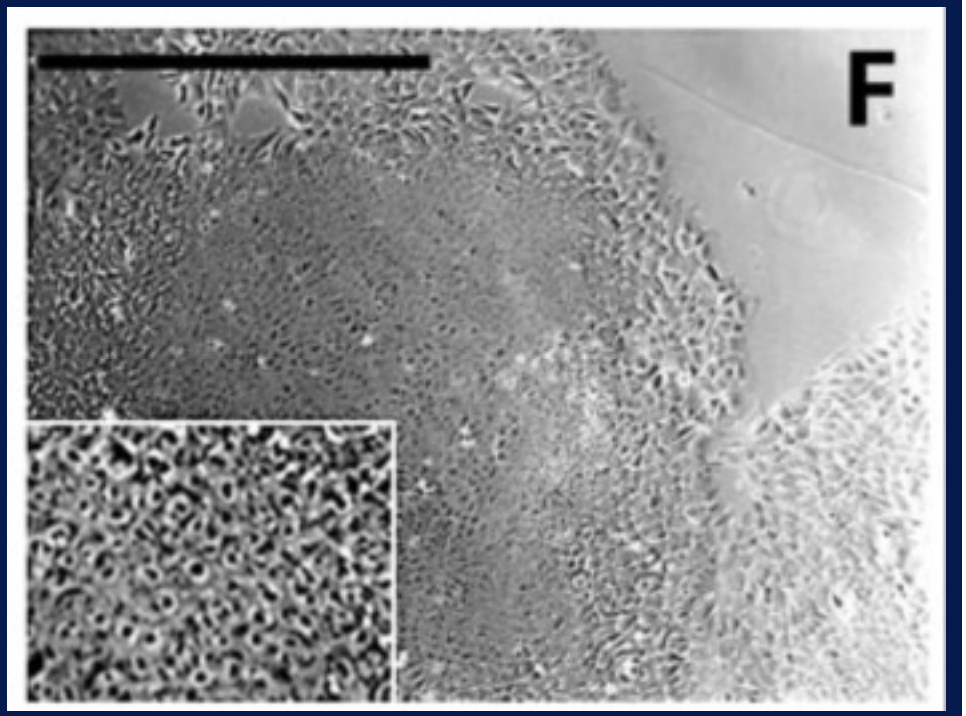
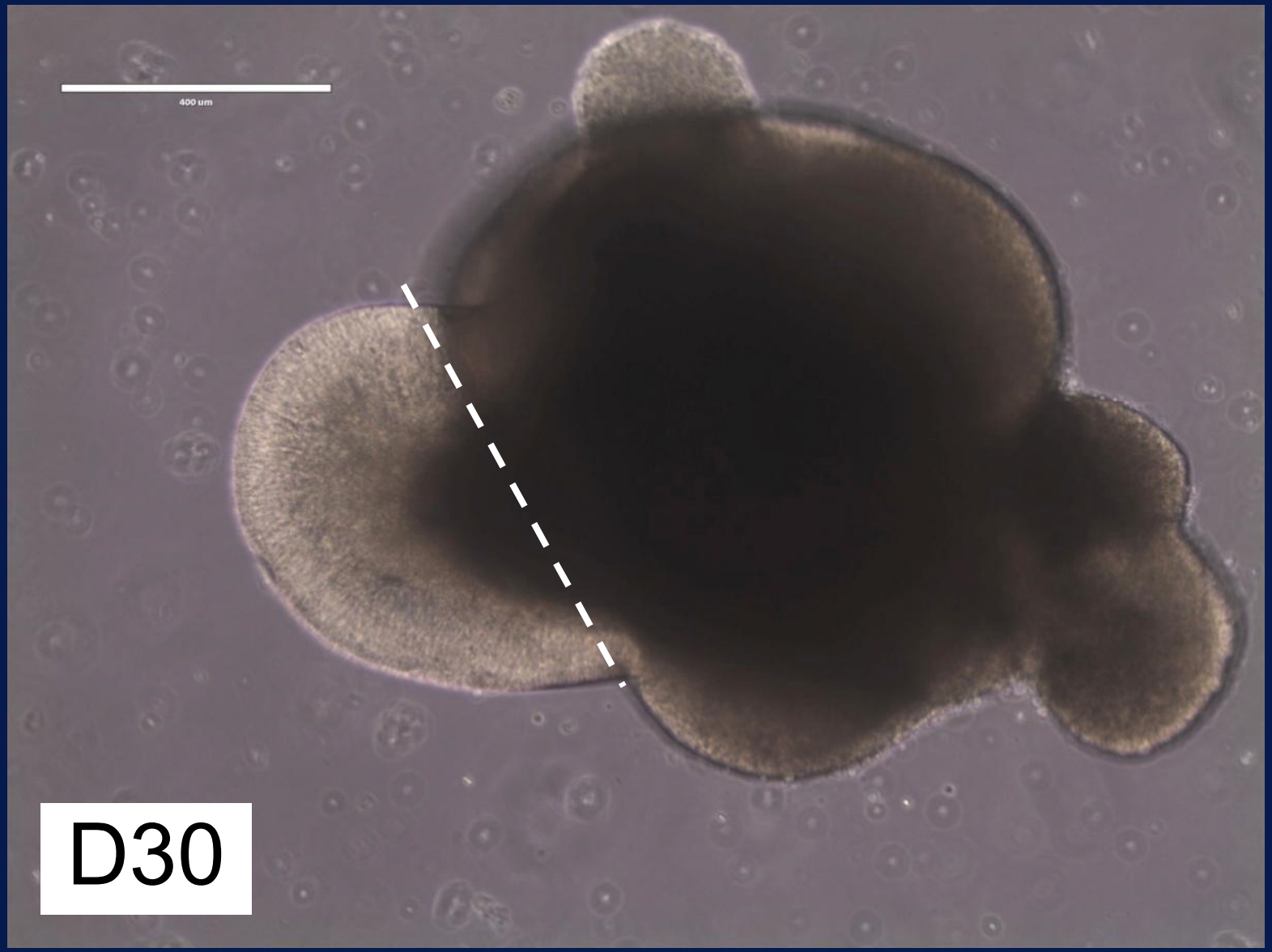


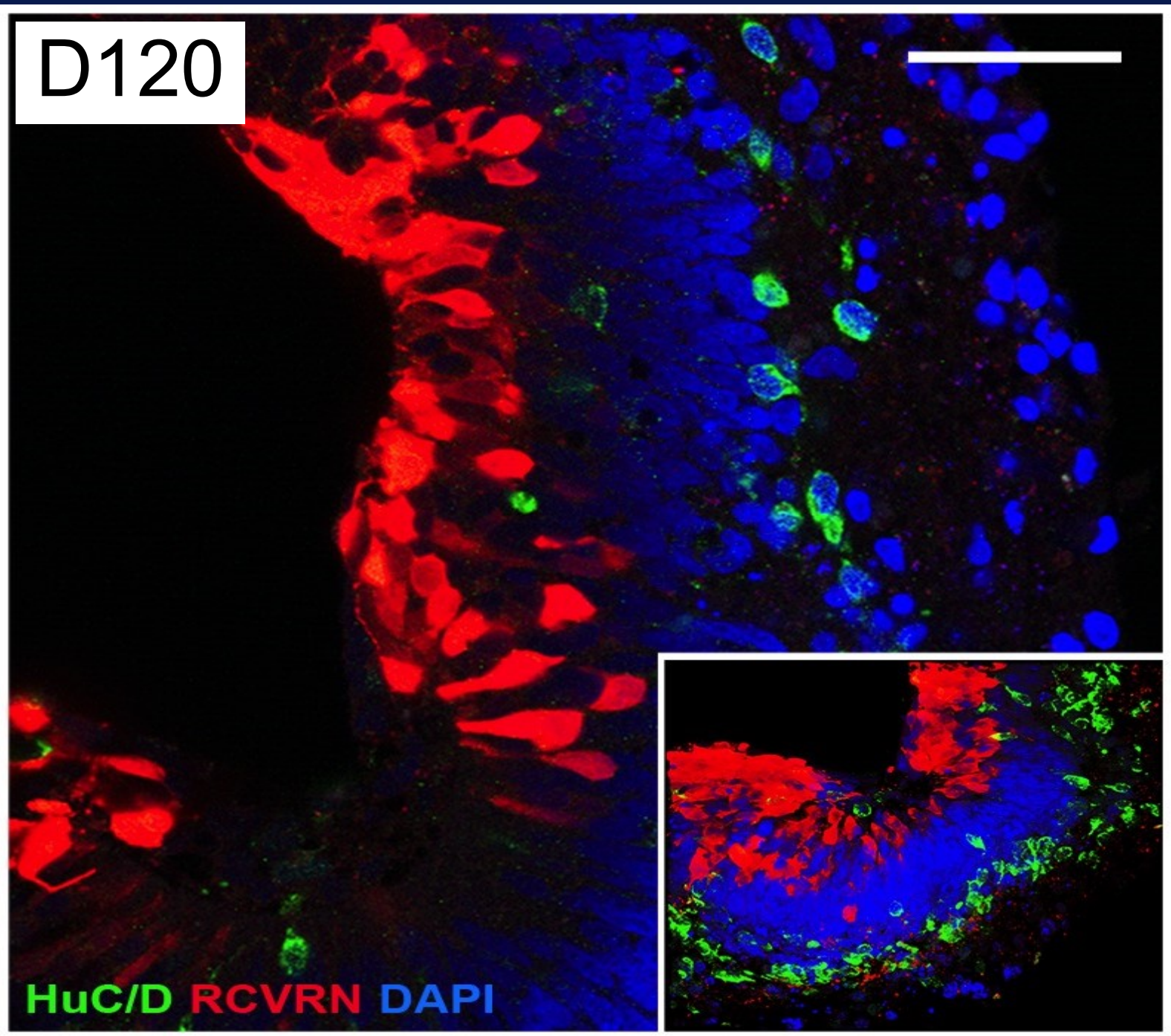


Skin fibroblasts
(from 3mm punch biopsy)

IPSCs

3D Differentiation




D120
HuC/D RCVRN DAPI

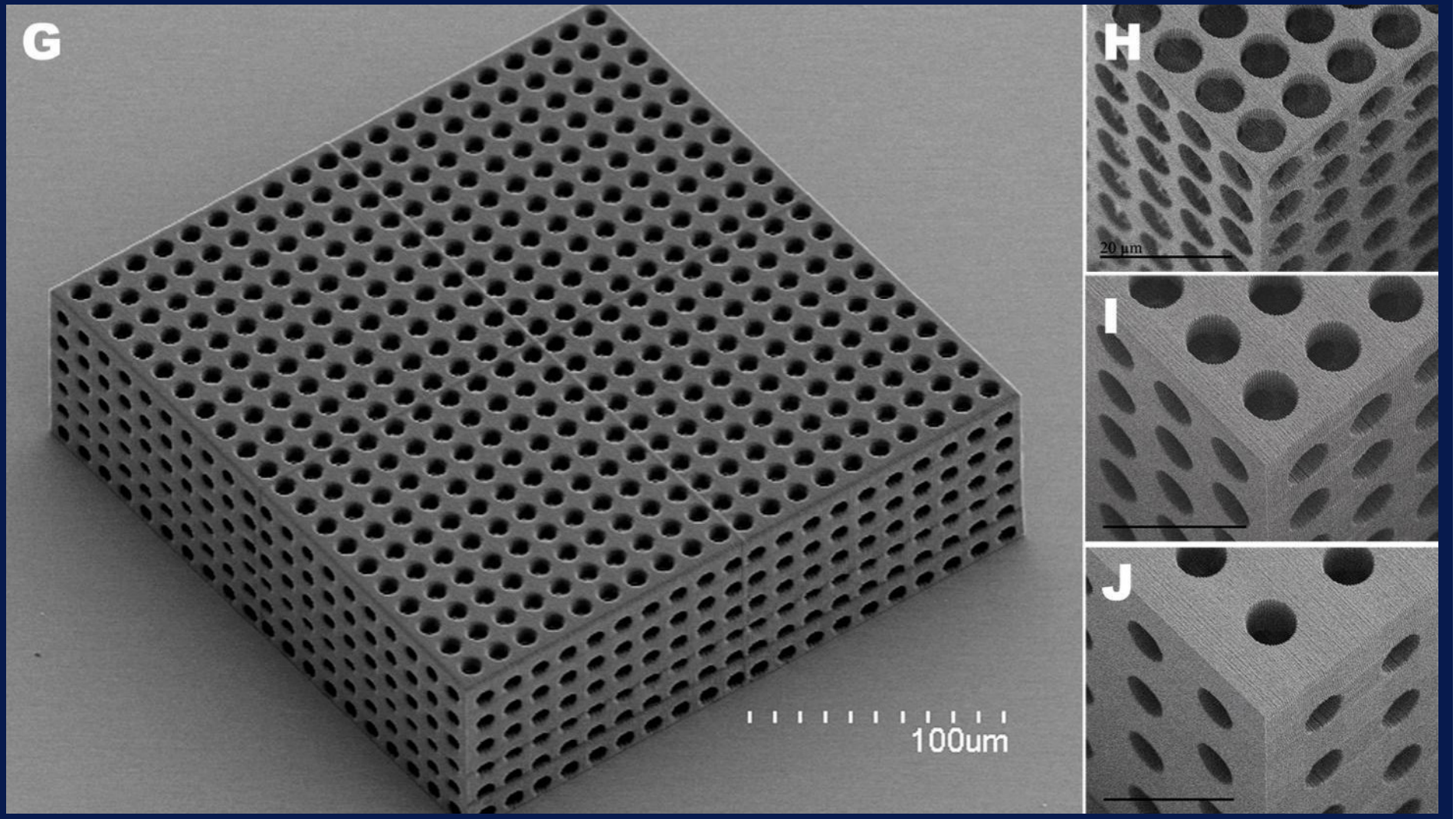
Our Strategic Decisions

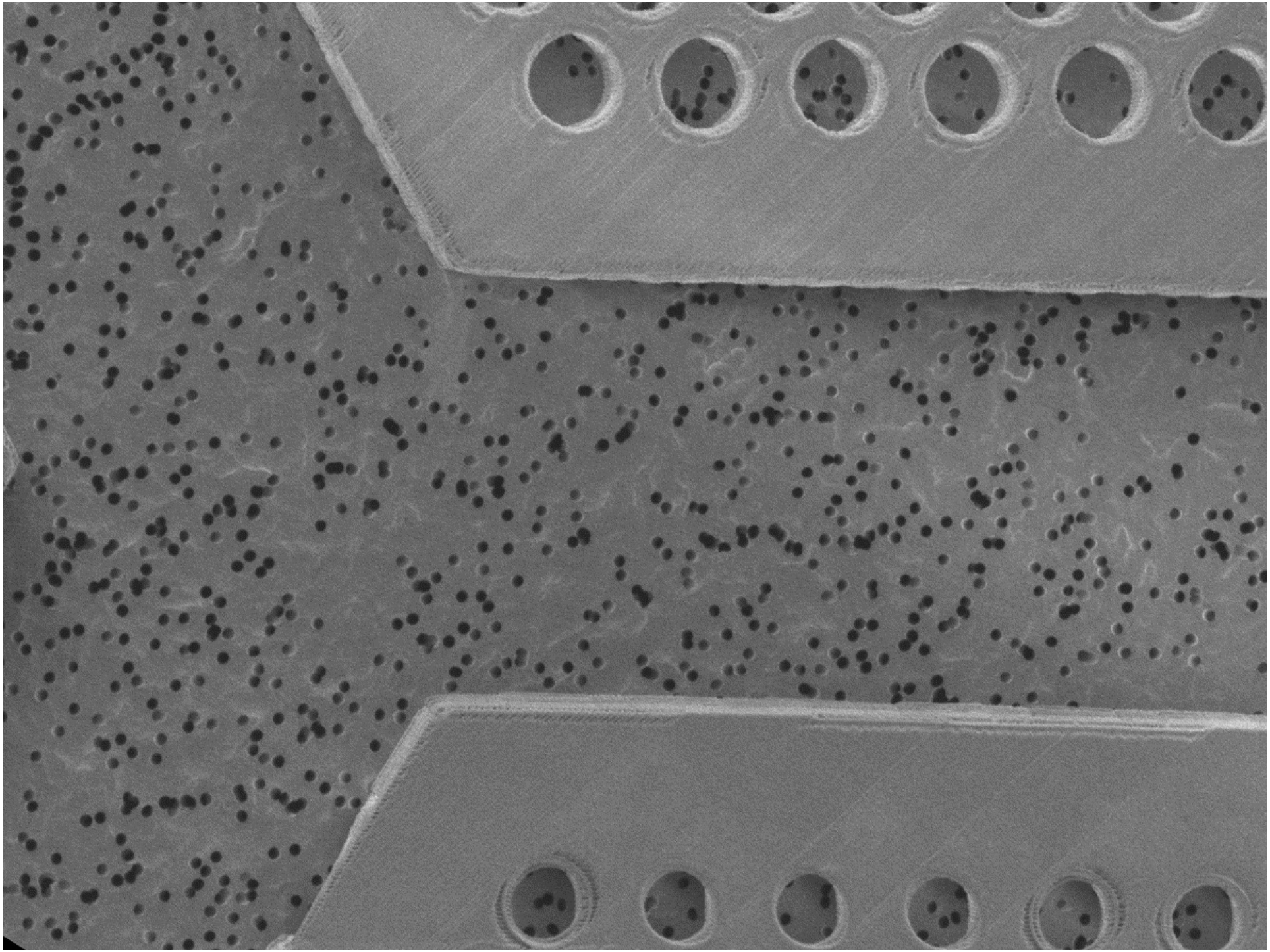
- Autologous cells
- Polymer supported
- Blind eyes

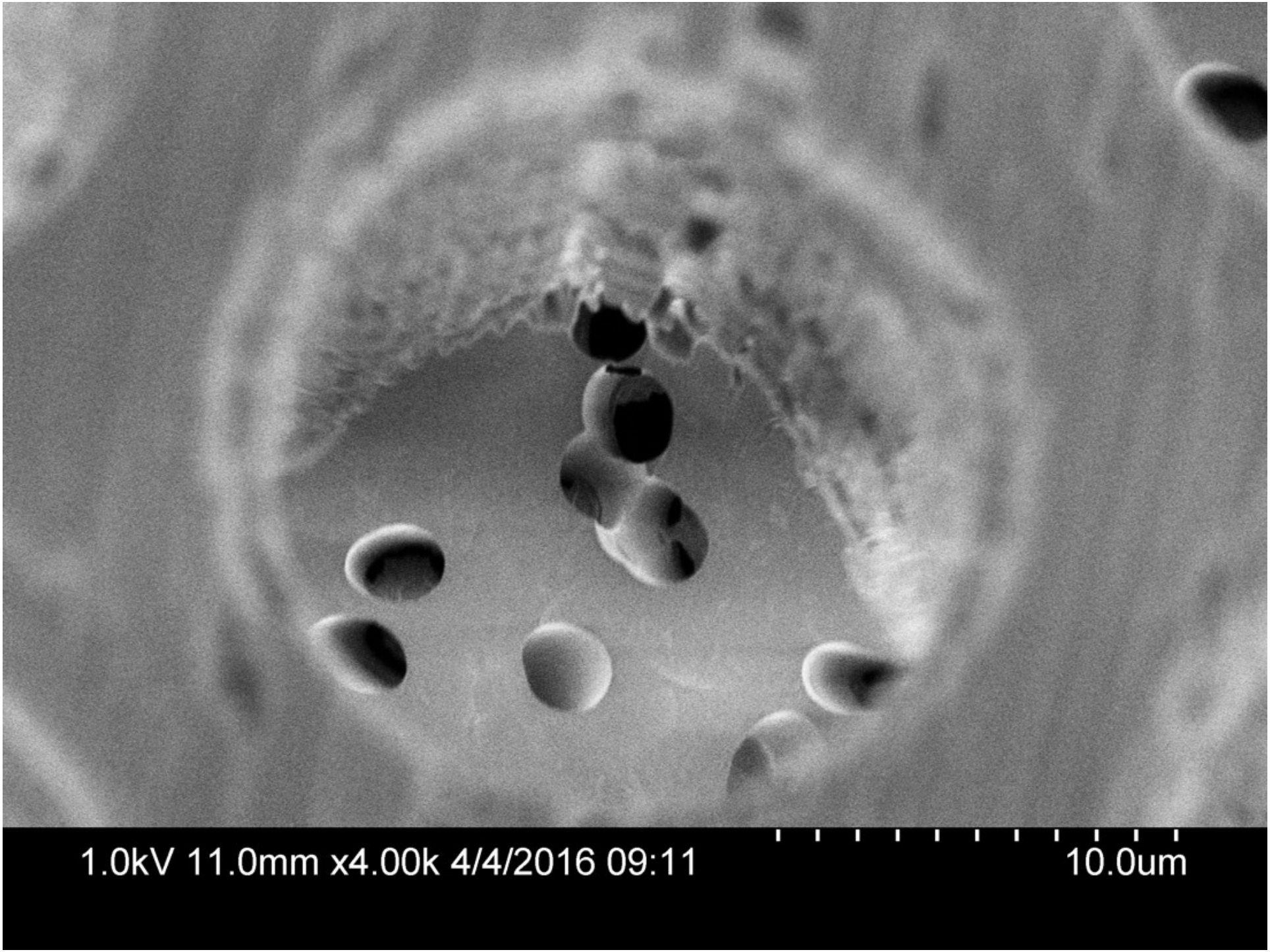
Why Polymer Supported?

- Cells with a scaffold of support have upwards of 50 fold higher rate of survival than non-supported cells

3D-Printed Cell Delivery Scaffolds



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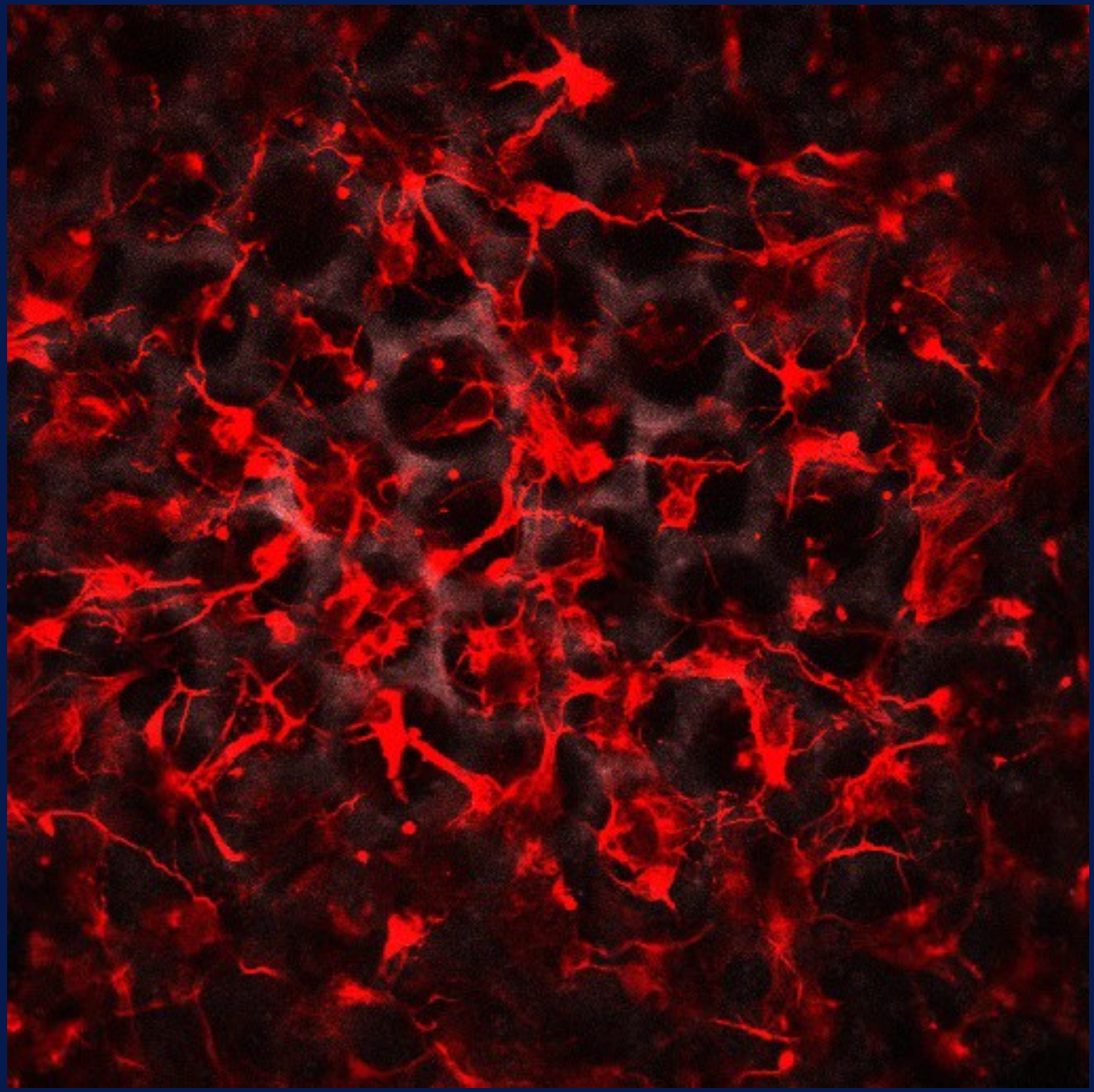
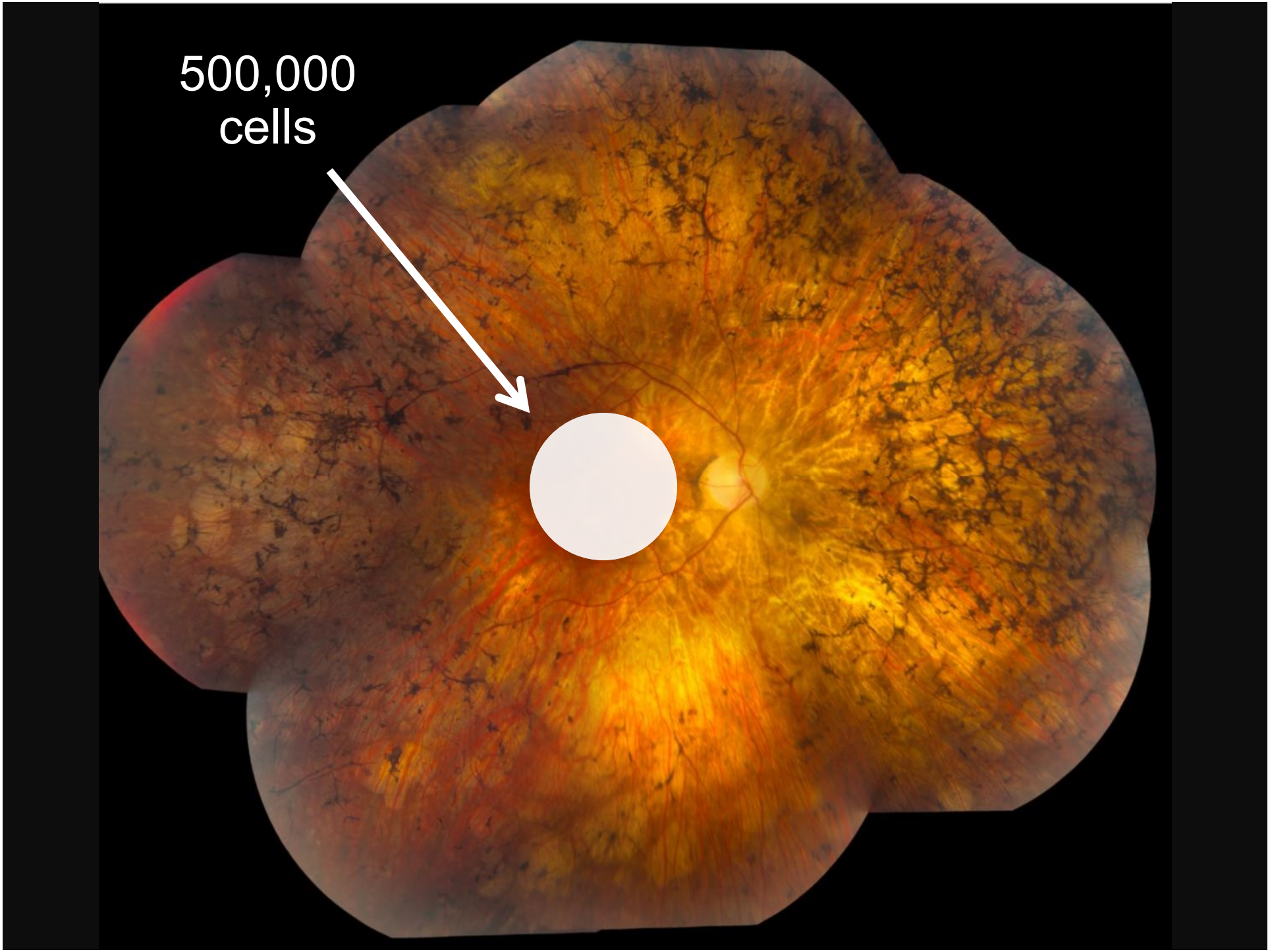
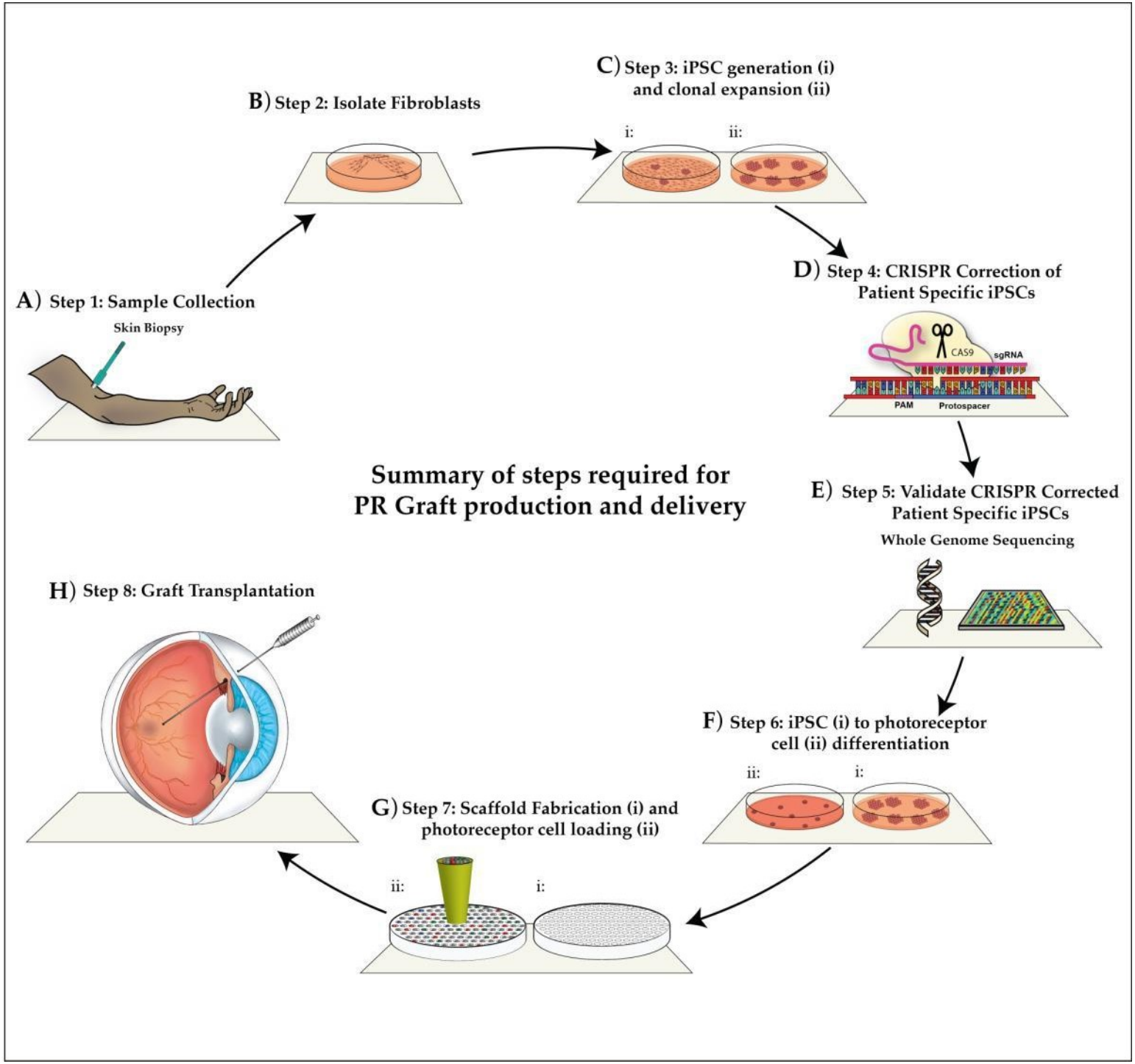
10.0um



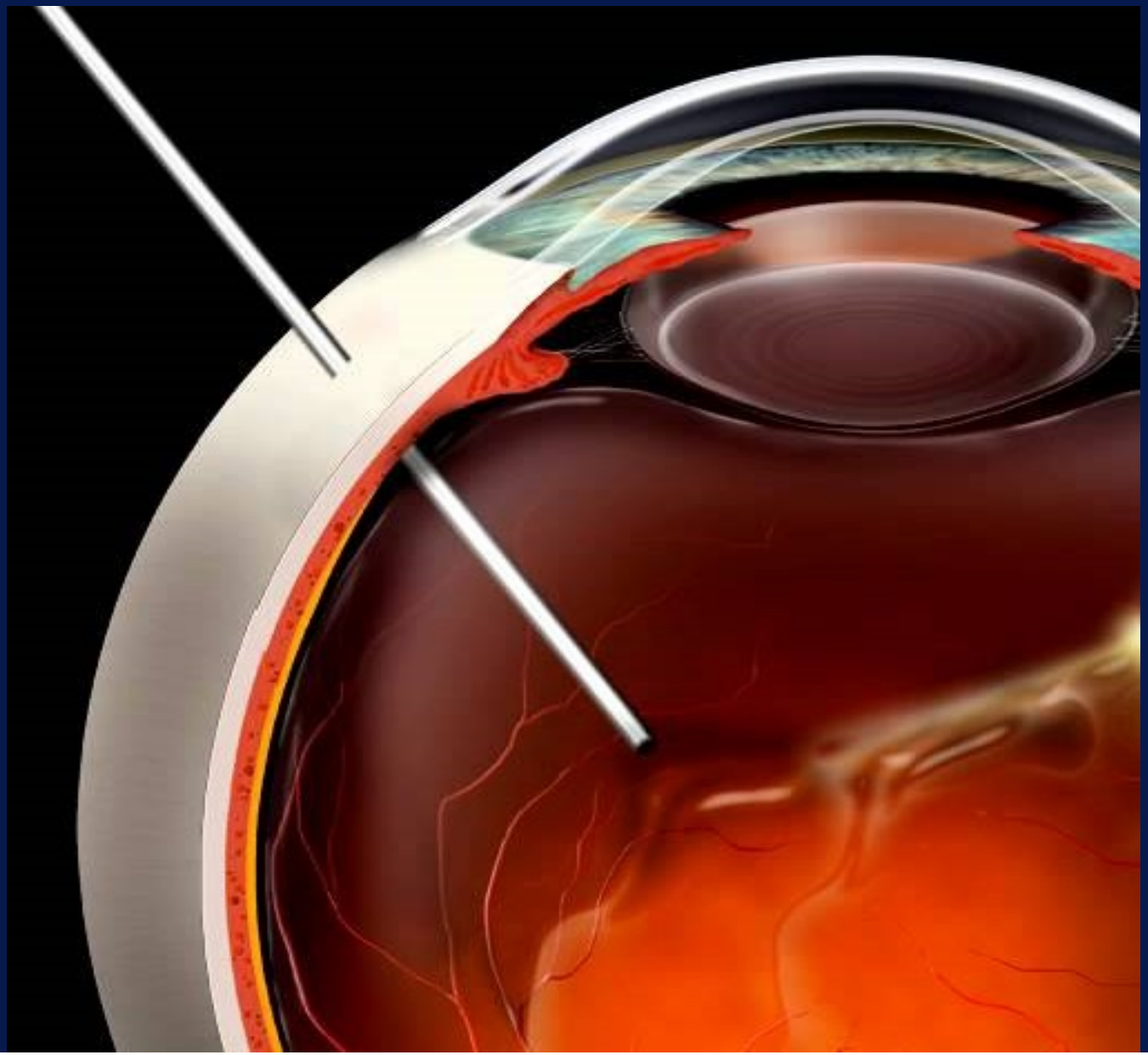

500,000
cells


A) Step 1: Sample Collection
Skin Biopsy
B) Step 2: Isolate Fibroblasts
C) Step 3: iPSC generation (i) and clonal expansion (ii)
i:
ii:
D) Step 4: CRISPR Correction of Patient Specific iPSCs
CAS9
sgRNA
PAM
Protospacer
Summary of steps required for PR Graft production and delivery
E) Step 5: Validate CRISPR Corrected Patient Specific iPSCs
Whole Genome Sequencing
F) Step 6: iPSC (i) to photoreceptor cell (ii) differentiation
ii:
i:
G) Step 7: Scaffold Fabrication (i) and photoreceptor cell loading (ii)
ii:
i:
H) Step 8: Graft Transplantation

How do we delivery gene and stem cell therapy?

- Modern retinal surgery techniques

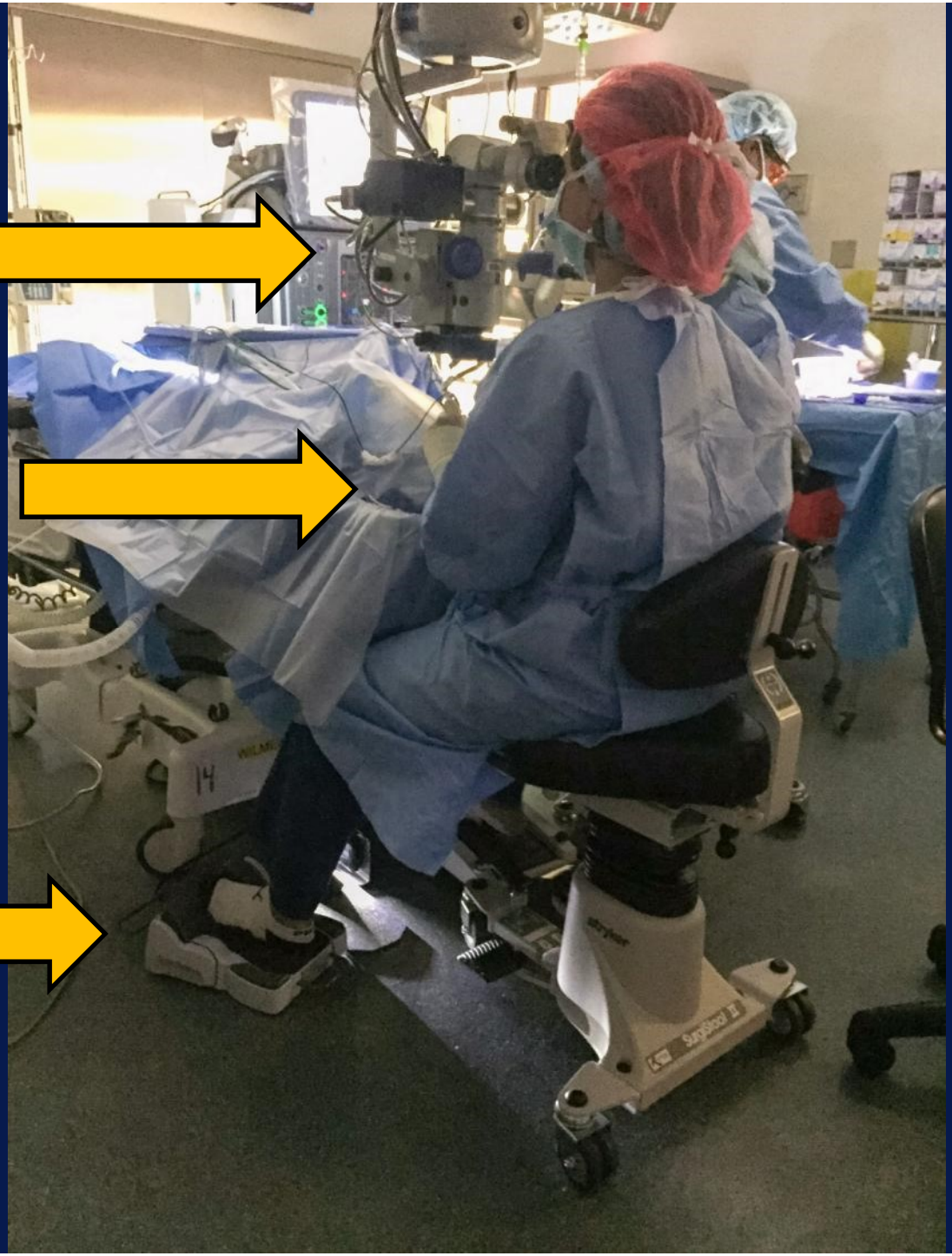
Vitrectomy surgery



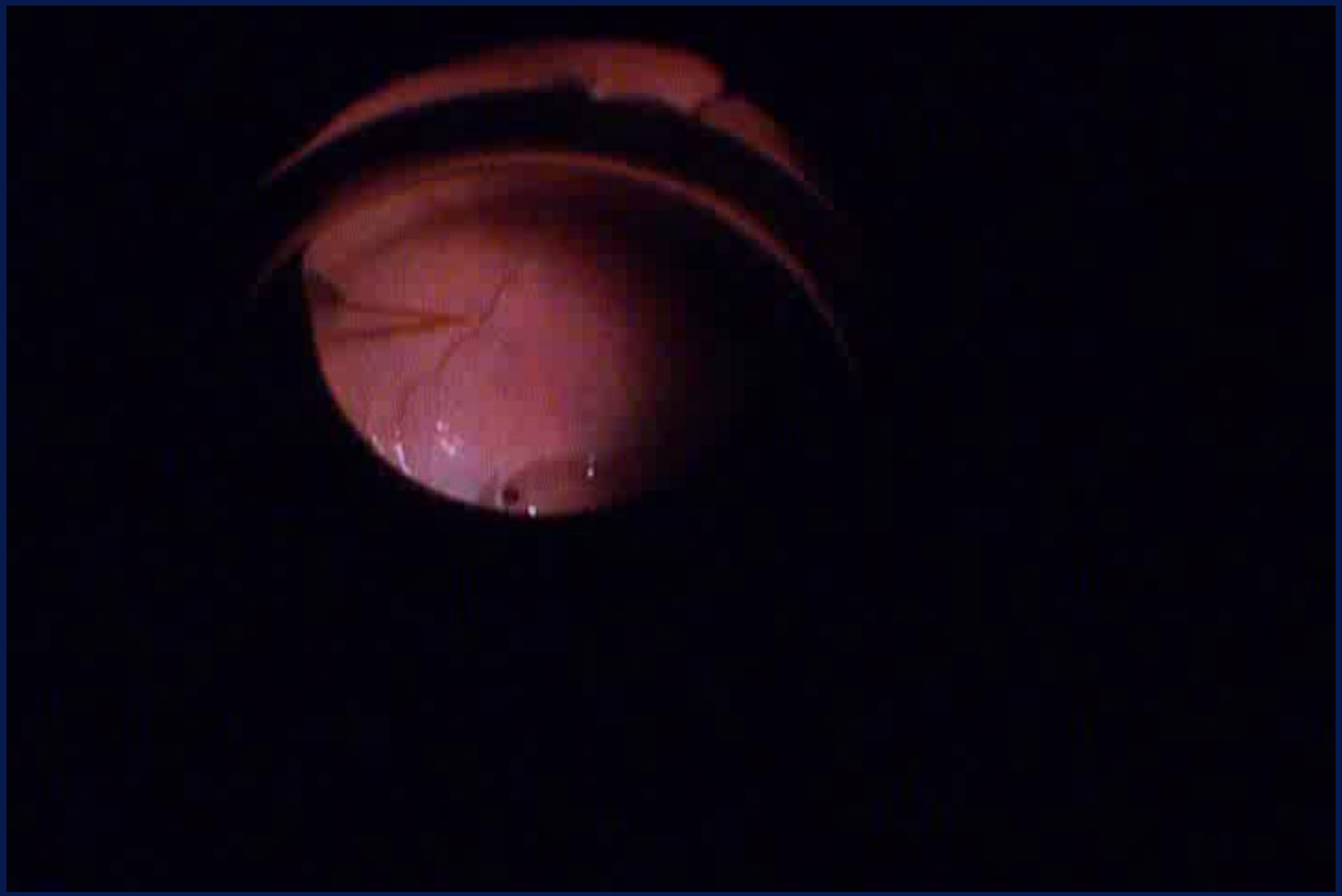
Microscope

Instruments

Foot pedals



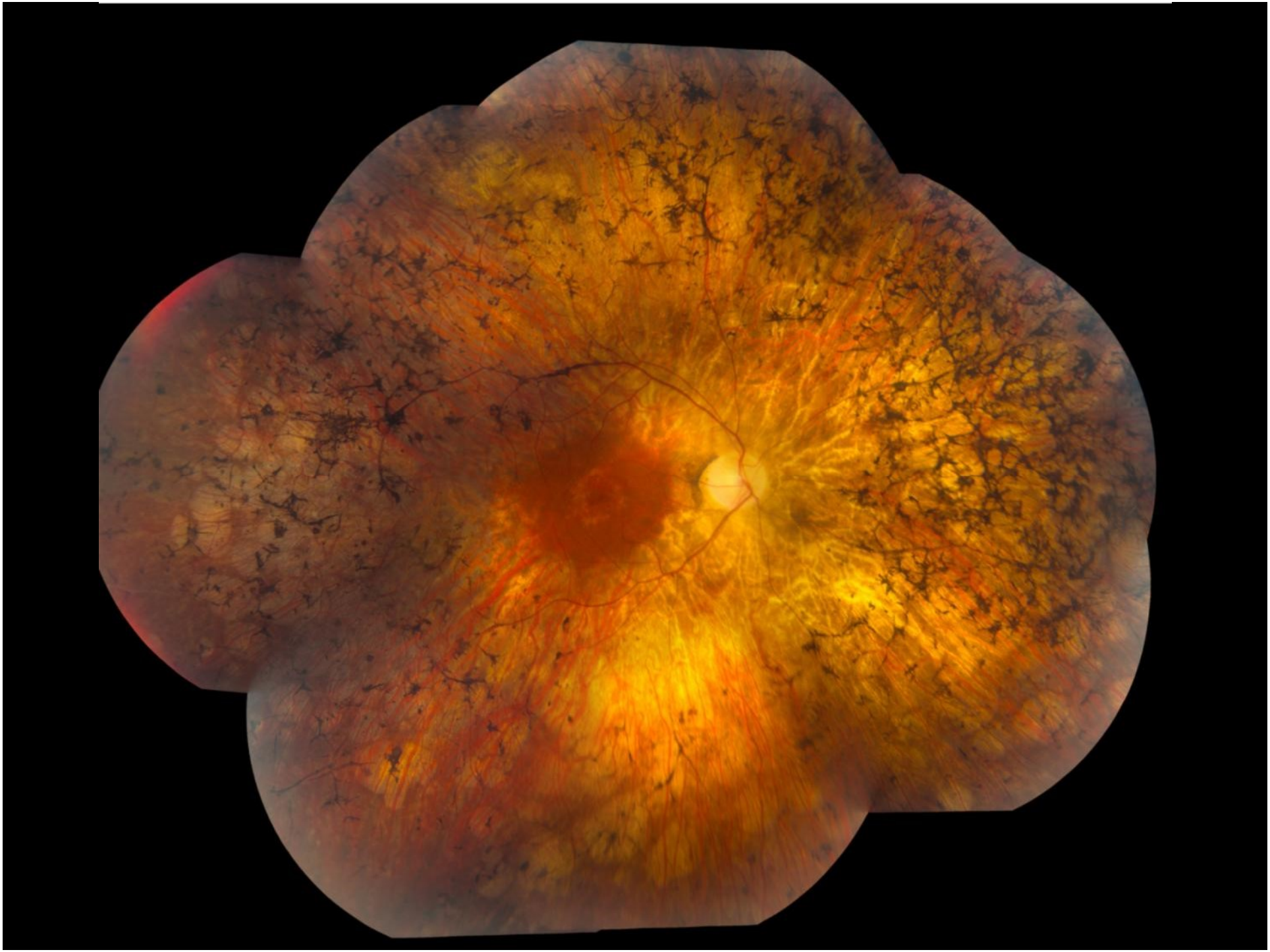
Surgery for gene therapy

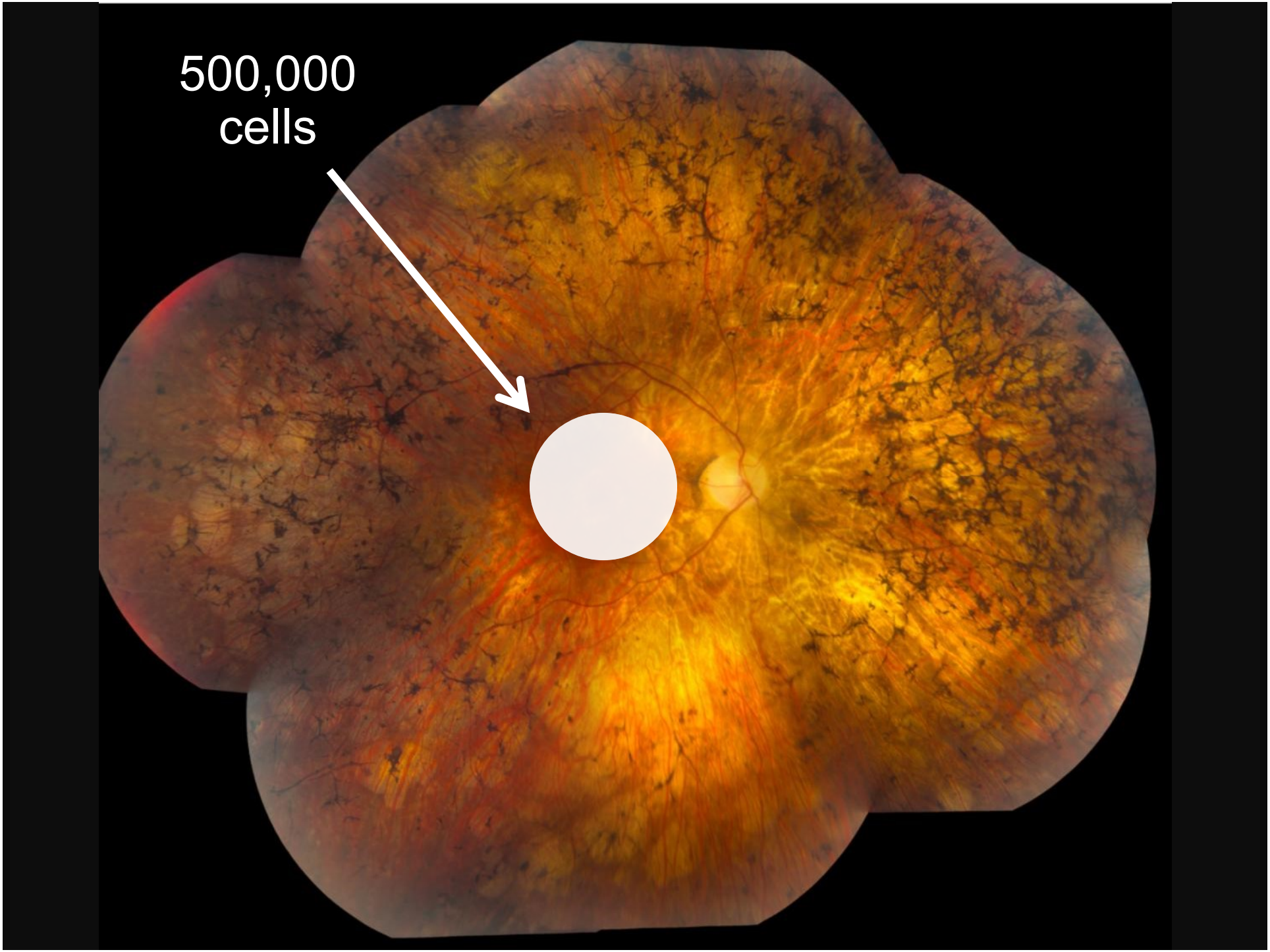


Video courtesy Steve Russell, MD

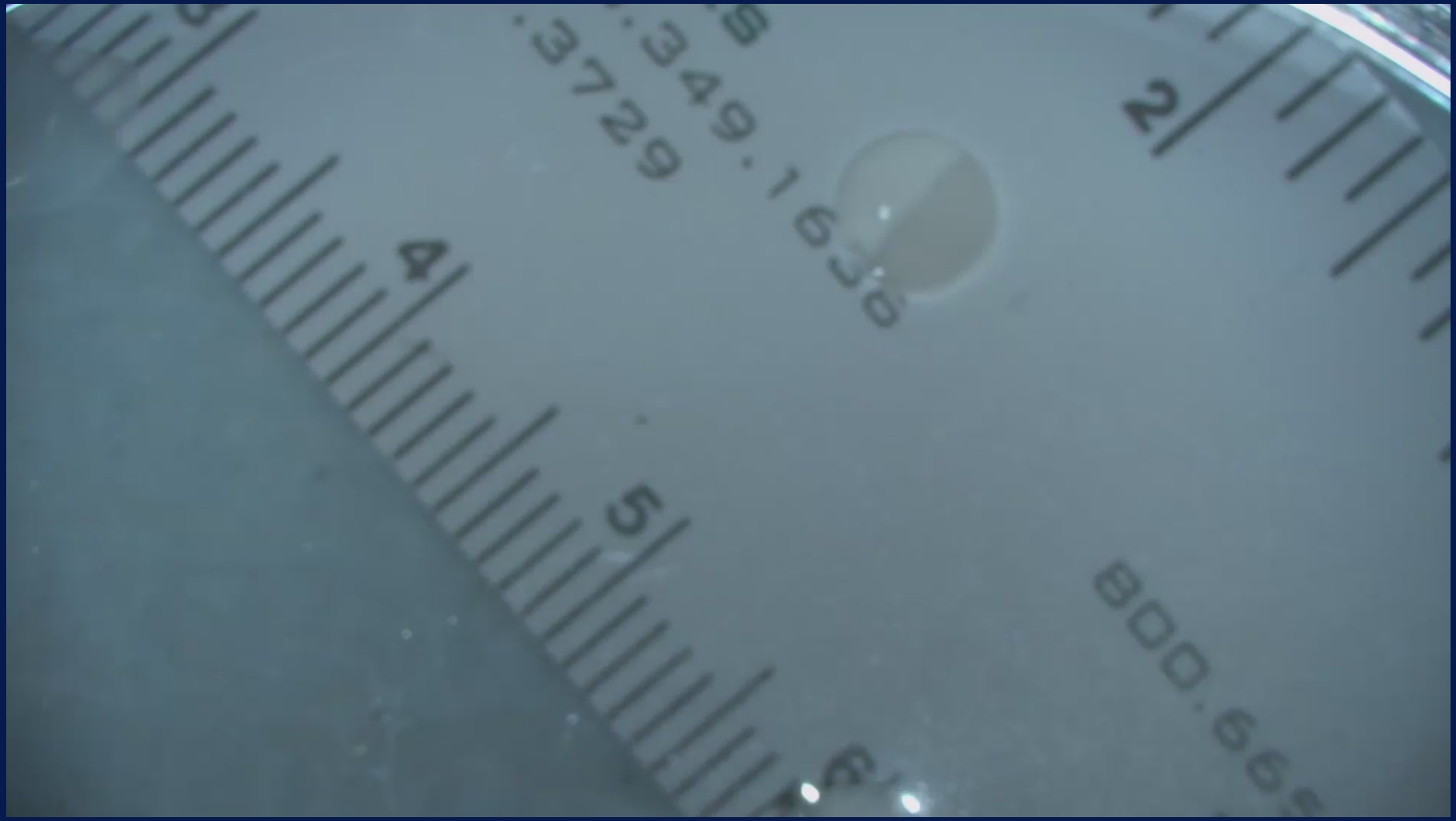
Surgery for stem cell therapy

- How do we deliver the polymer and stem cells under the center of the retina?

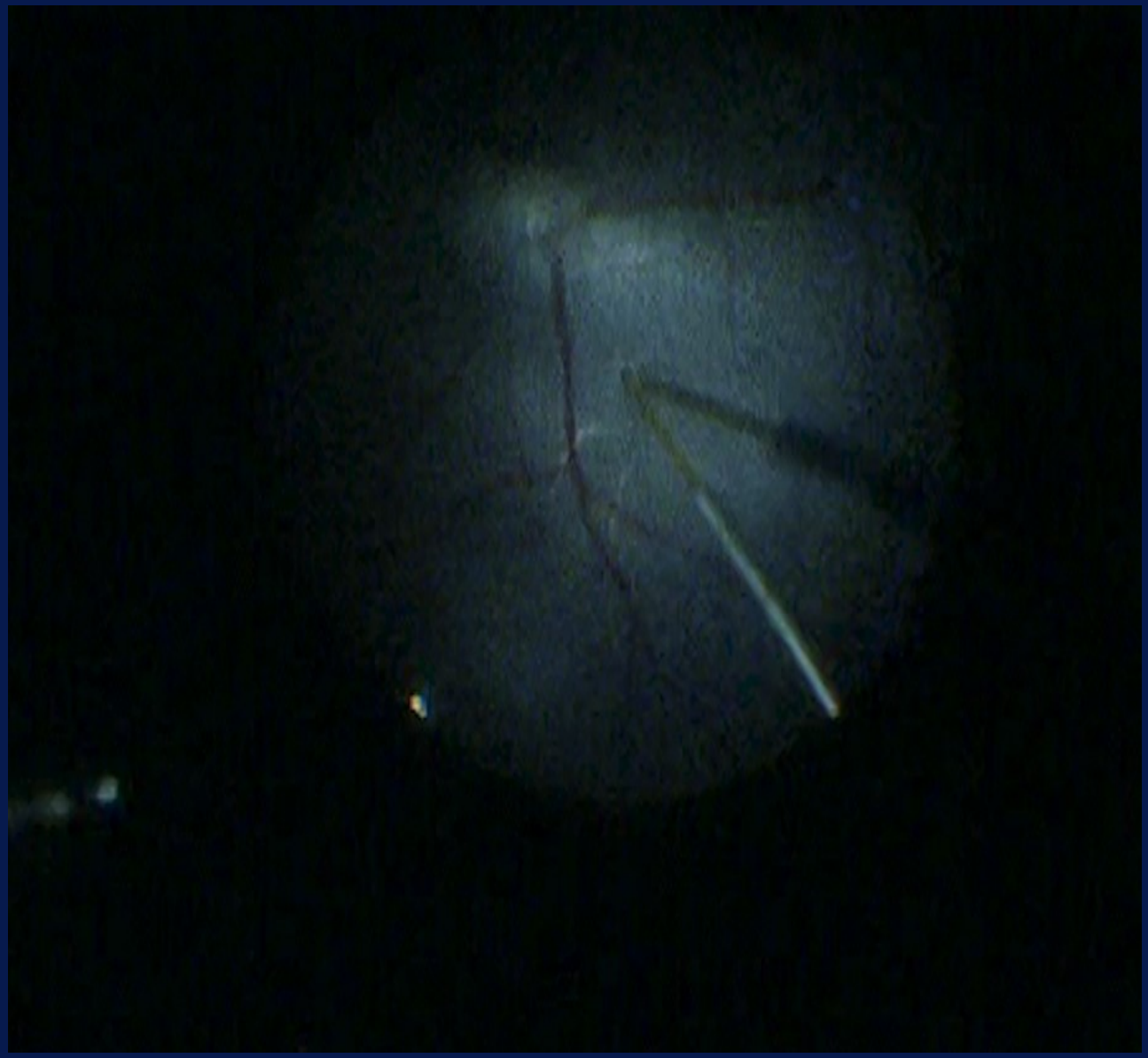



500,000
cells

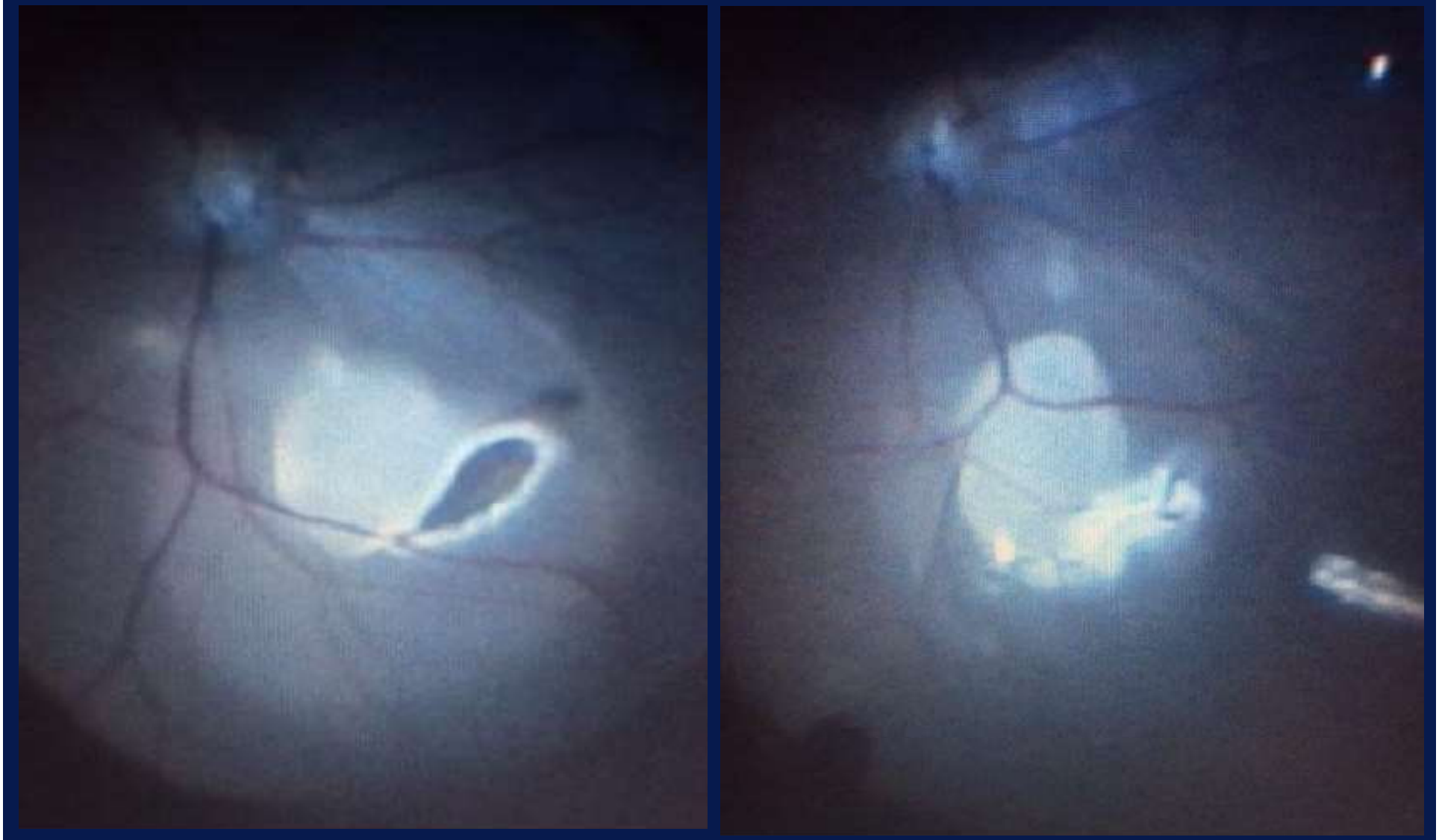
Loading the polymer



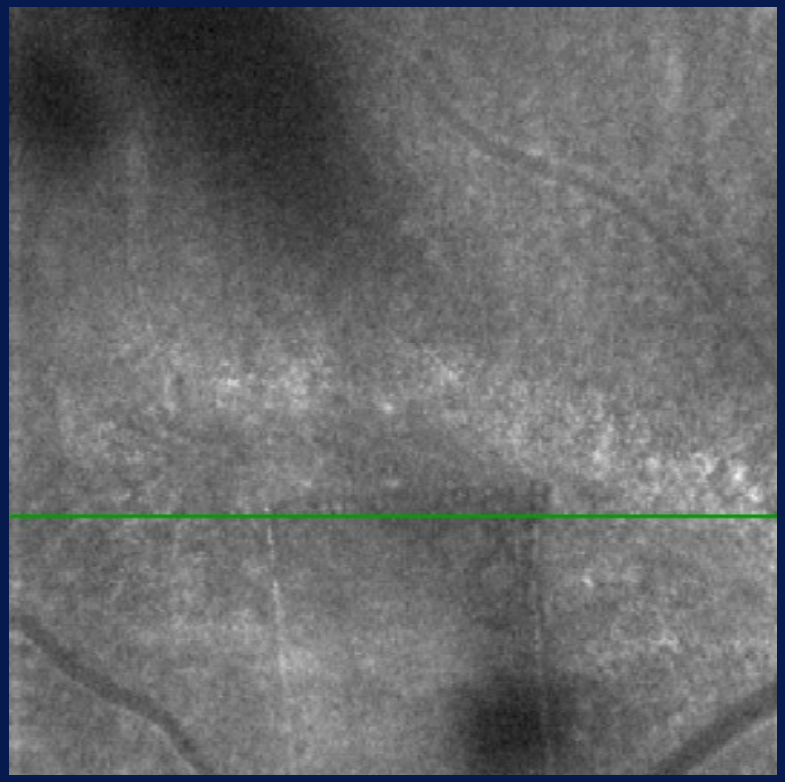
Video courtesy Elliott Sohn, MD

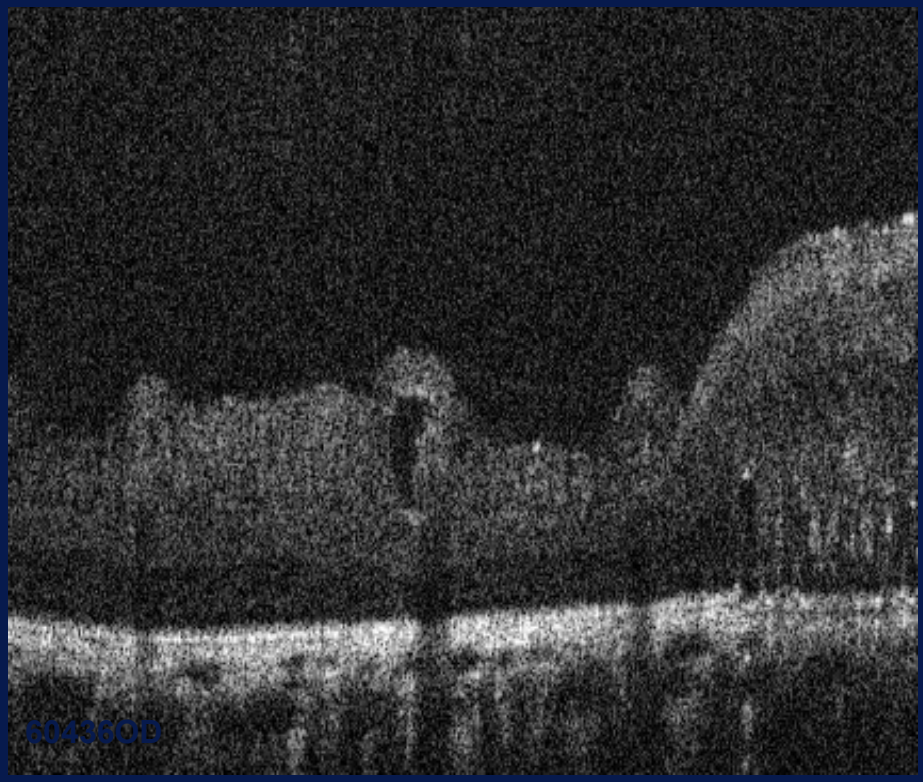


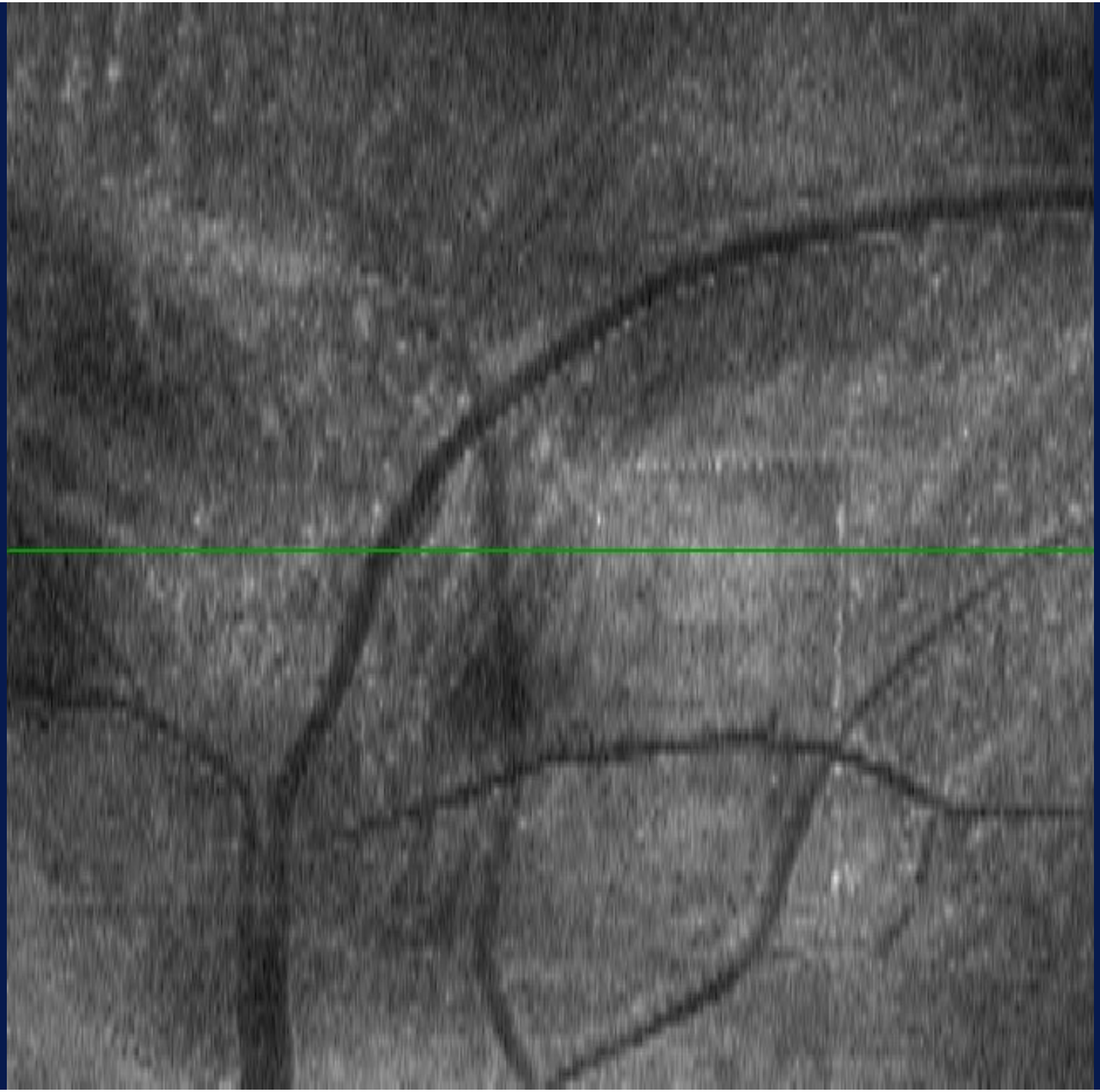
5 mm polymer under a pig retina

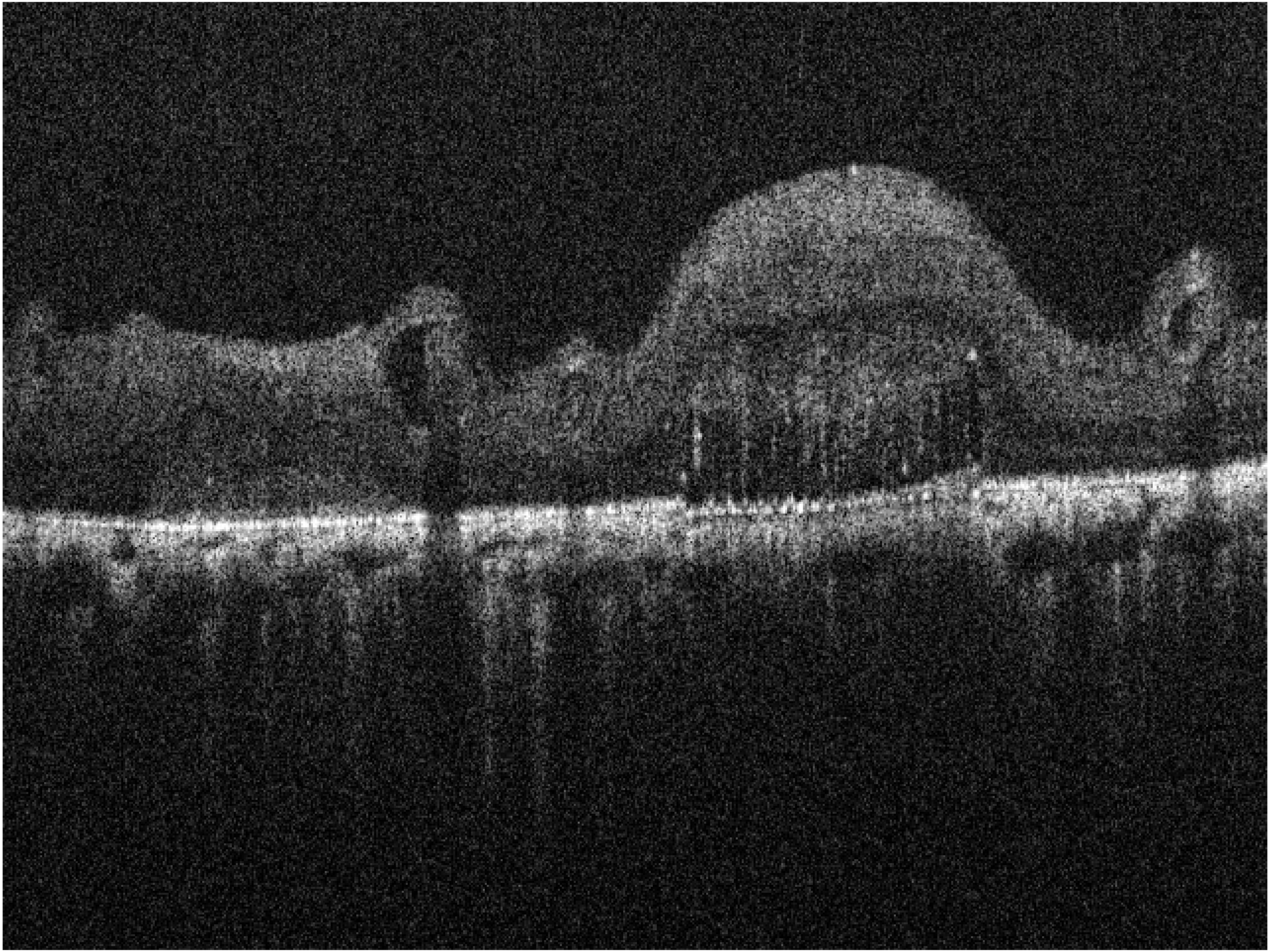


OCT scan of a polymer transplanted in a pig model of retinitis pigmentosa

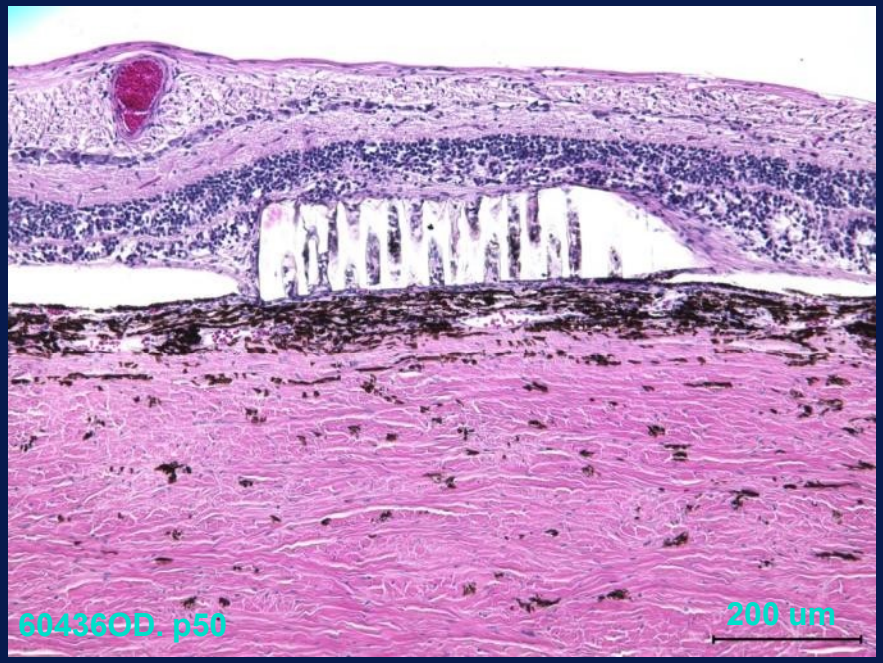


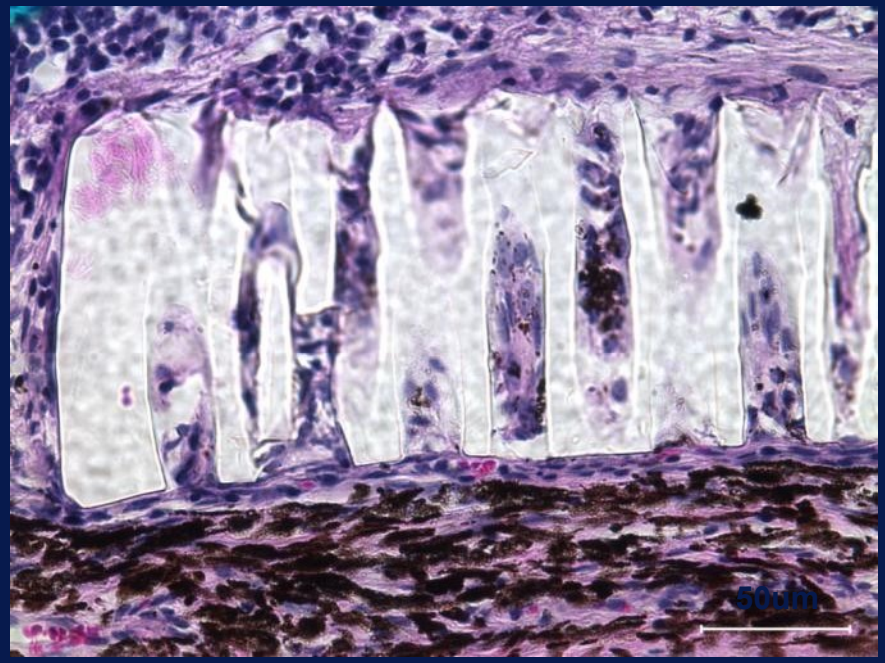


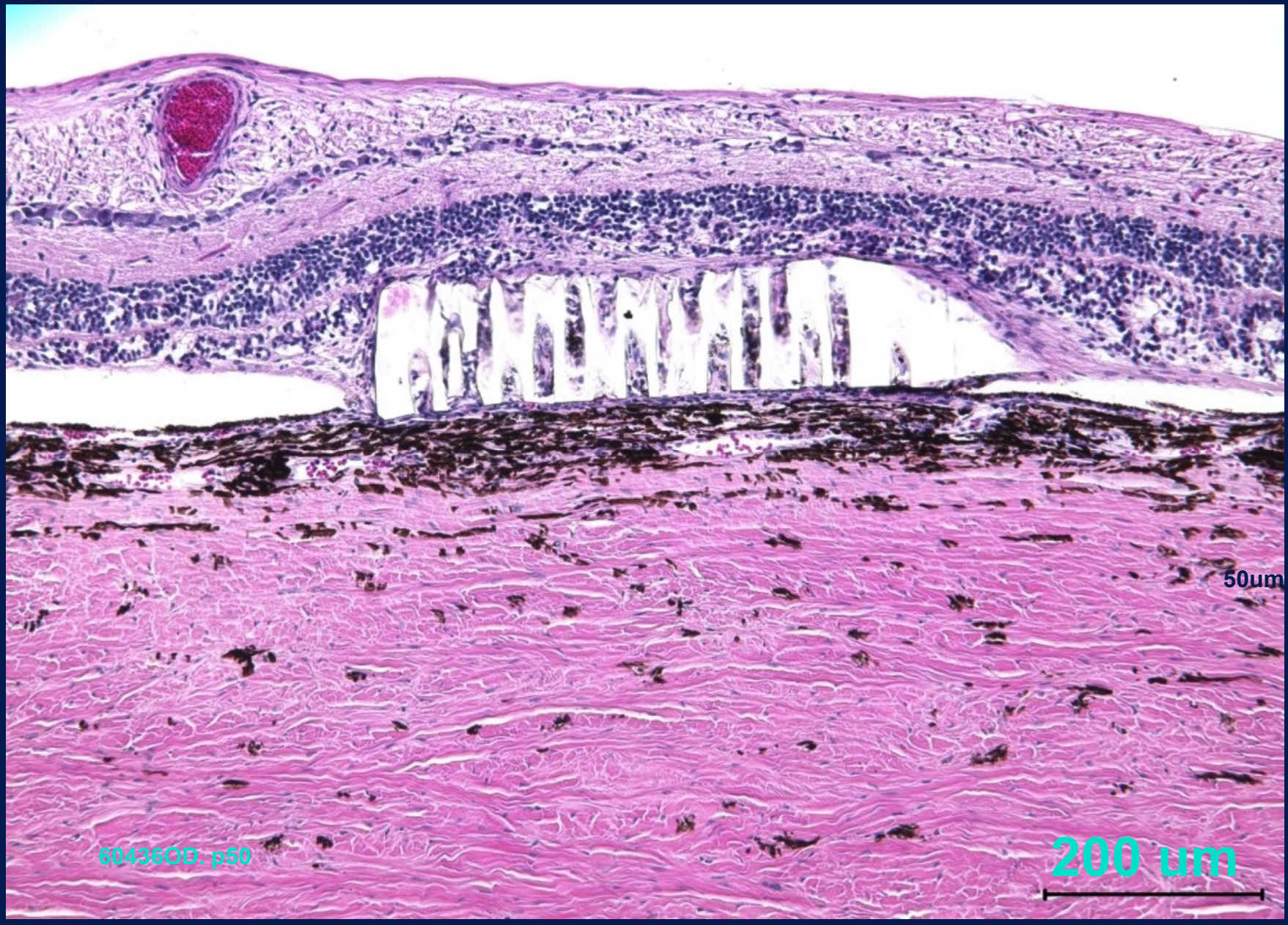


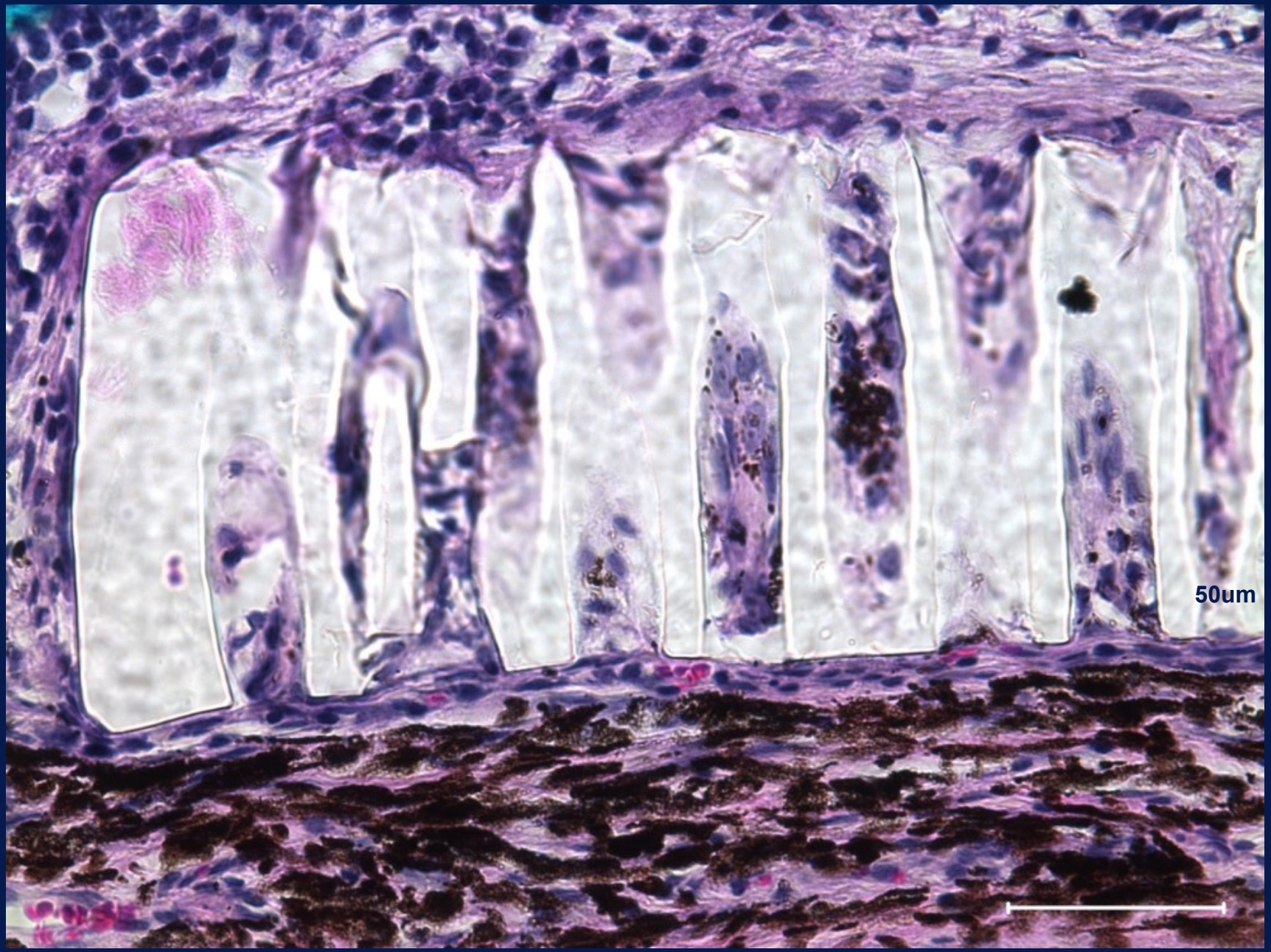


Microscopic Sections of 3D polymer






50um
60436OD. p50
200 um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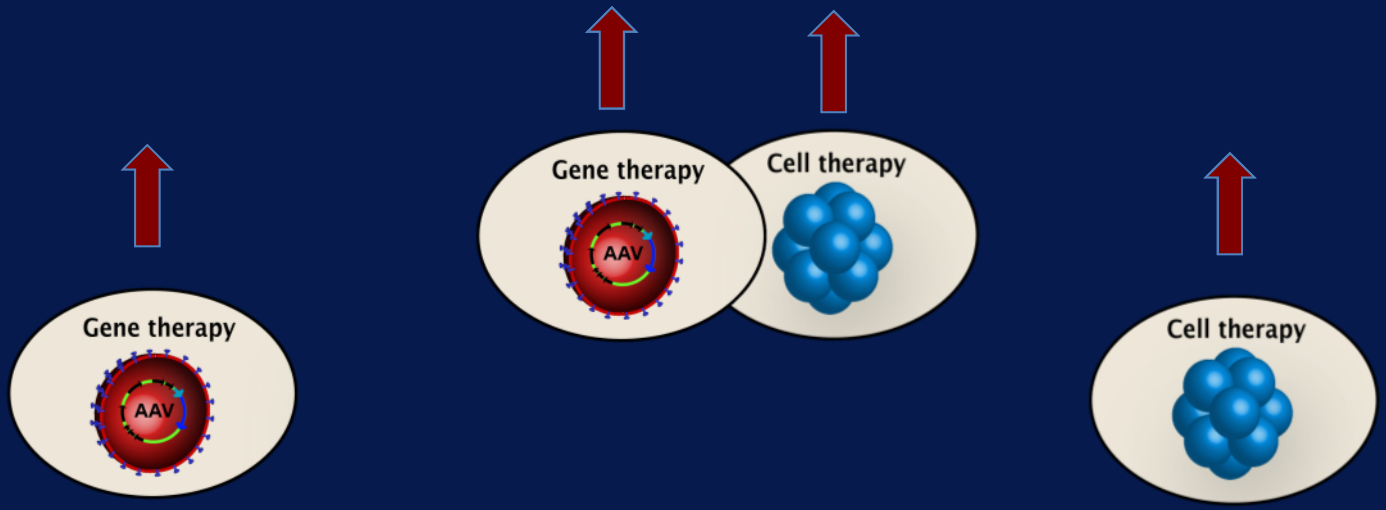
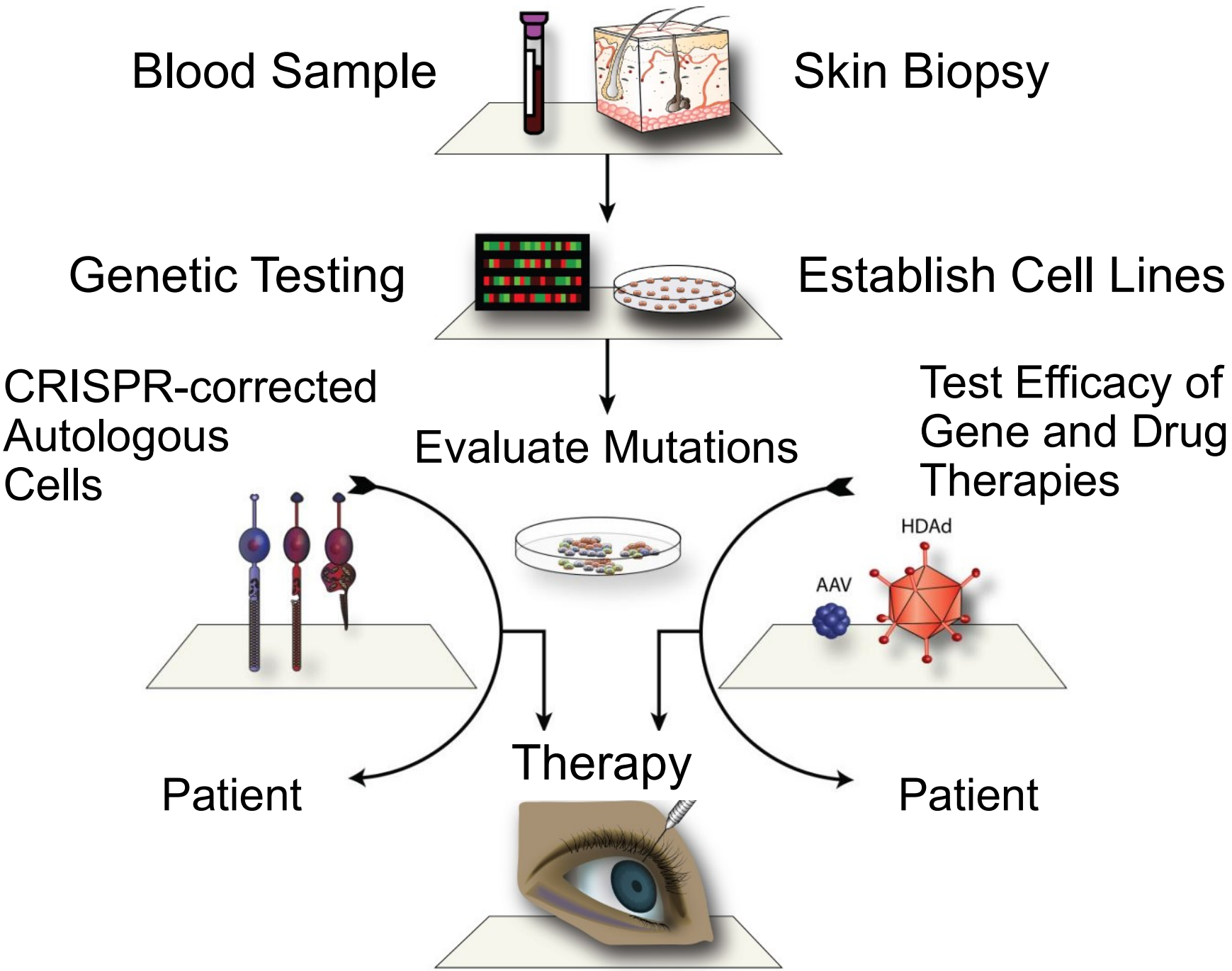
Summary of our treatment strategy

Treatment

Disease Course

Mild Moderate Severe




Blood Sample
Skin Biopsy
Genetic Testing
Establish Cell Lines
CRISPR-corrected Autologous Cells
Evaluate Mutations
Test Efficacy of Gene and Drug Therapies
HDAd
AAV
Patient
Therapy
Patient

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