Treating Degenerating Eye Diseases using Stem Cell Derived Eye Tissues

Kapil Bharti, PhD
National Eye Institute
National Institutes of Health

Bharti Lab @Kapilbharti123

Kapil.bharti@nih.gov
Bardet-Biedl syndrome, choroideremia, retinitis pigmentosa, Best disease, macular degeneration (AMD)

- Night blindness is the most common first symptom
- As the disease progresses, there is loss of peripheral vision
- Later there is a loss of central vision as well

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

Sources: https://www.fightingblindness.org/
Homeostatic Unit of the Eye (Photoreceptors/RPE/choroid)
RPE is sandwiched between the photoreceptors and the choroidal blood supply.
Different Diseases Stages Manifest with the Loss of Different Eye Tissues

- Photoreceptor loss
- Capillary loss
- Drusen
Need for Different Tissues at Different Disease Stages

- **Normal**
- **Disruption**
- **Loss**
- **Disarray**

Time

- Prevention: Gene augmentation, Gene editing
- Cell replacement: Cell bypass
- Tissue replacement: Tissue bypass

- RPE Patch
  - PRP only
- RPE/choroid
  - PR/RPE
  - PR/RPE/choroid

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

1. Isolate cells from patient (skin or fibroblasts); grow in a dish
2. Treat cells with "reprogramming" factors
3. Wait a few weeks
4. Pluripotent stem cells
5. Change culture conditions to stimulate cells to differentiate into a variety of cell types

- Blood cells
- Cardiac muscle cells
- Gut cells
Making Patient-Specific Replacement Cell Therapies

Potential treatment

Patients

somatic tissue

+ Reprogramming factors

May need gene editing for monogenic diseases

Cell Therapy

Derived iPS cells

Differentiated PRP cells

Differentiated RPE cells

Differentiated 3D RPE/choroid
Timeline for Development of an FDA Approved Stem Cell Therapy

- **5-8 years:** Proof-of-concept data in animals
- **4-5 years:** IND-enabling studies
  - Pre-clinical toxicology and efficacy in animal models
  - Can be single center and publically funded
- **3-5 years:** FDA approval
  - Need to be multi-center and often require an industry partner
  - Commercial Approval

**Basic research** ➔ **Translational research** ➔ **Preclinical research** ➔ **Phase I** ➔ **Phase II** ➔ **Phase III**
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., Marianieli 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.

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)

- IPS cells → Neuro ectoderm → RPE progenitors → Committed RPE → Immature RPE → Mature RPE

- Low TGF/NODAL, WNT, FGF signaling
- TGF or WNT
- WNT (primary cilium induction)

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

Blood draw from patient - Day 0

CD34+ isolation and cryopreservation

Transfection - Day 8
Passage 1 - Day 30
Mini bank p5 - Day 55
Working bank p10 - Day 80

Passage 11
Differentiation set up up to 3 clones - Day 87

RPE progenitor stage - Day 114

Committed RPE enrichment - Day 129

Scaffold seeding only one clone - Day 129

Clinical product – RPE patch - Day 164

Cryo steps

Patient material

iPSC Mini bank

iPSC Working bank (~ 8 clones)

RPE progenitor bank (10 vials/clone)

Sterility, karyotyping
Orip loss, identity, sequencing

Day 99 differentiation efficiency (go/no go)
Purity assay (go/no go)

Sterility, purity, Identify, function
Functional and Polarized iPSC-RPE Patch on a Scaffold

- RPE monolayer
- Biodegradable 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

STEM121 (human cells)

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

PLGA scaffold is completely degraded in 10 weeks
GLP Pre-clinical Toxicity, Biodistribution, and Tumorigenicity Study

- Human dose
- 2 mm diameter
- 0.5 mm diameter
- Rat equivalent of human dose
- Human dose 70,000 cells

Acute/Sub-acute safety
- 2 wk
- 13 wk

Long-term safety
- 26 wk
- 9 month

Toxicity and Biodistribution Study

Tumorigenicity Study

- 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 iPSC 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
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**Time**
- **Prevention**
- **Gene augmentation**
- **Cell replacement**
- **Tissue replacement**

**Tissue types**
- RPE Patch: PRP only
- RPE/choroid: PRP/RPE/choroid

**Techniques**
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Courtesy: David Gamm (UW, Madison)
Differentiation Pure and Mature iPSC-Endothelial Cells

3 Days
- iPSC cells → Mesoderm

6 Days
- Vascular Specification
- Immature Endothelium

5 Days
- Mature Endothelium

B(P)EL, Activin A, BMP4, VEGF, CHIR → B(P)EL, +VEGF, SB431542 → FGF, IGF-1, EGF, VEGF

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

Min Jae Song, Russ Quinn, Eric Nguyen
Bioprinting to Develop a 3D RPE/ “Choroid” Tissue

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

Min Jae Song, Russ Quinn, Eric Nguyen
Bioprinting to Develop a 3D RPE/ “Choroid” Tissue

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

Min Jae Song, Russ Quinn, Eric Nguyen
3D Bioprinted “Choroid”

Capillaries

Min Jae Song, Russ Quinn, Eric Nguyen
3D Choroid/RPE Tissue

RPE (green) & capillaries (red)

Min Jae Song, Russ Quinn, Eric Nguyen
TIME FOR A 3D BACK of the EYE (Retina/RPE/Choroid Tissue)

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

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