Scientific Basis of Vision

Stem Cells in the Eye

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What are stem cells

Stem cells are the cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.

Characteristics of stem cells

- They are capable of dividing and self-renewing for long periods;
- They are unspecialized;
- They can give rise to specialized cell types.

Classifications of stem cells

- Embryonic stem cells (ES/hES)
- Somatic / Adult stem cells
- Induced pluripotent stem cells (iPS)

http://stemcells.nih.gov/info/basics/

What are Embryonic Stem Cells

Primitive (undifferentiated) cells derived from a 5-day preimplantation embryo that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

What are adult stem cells

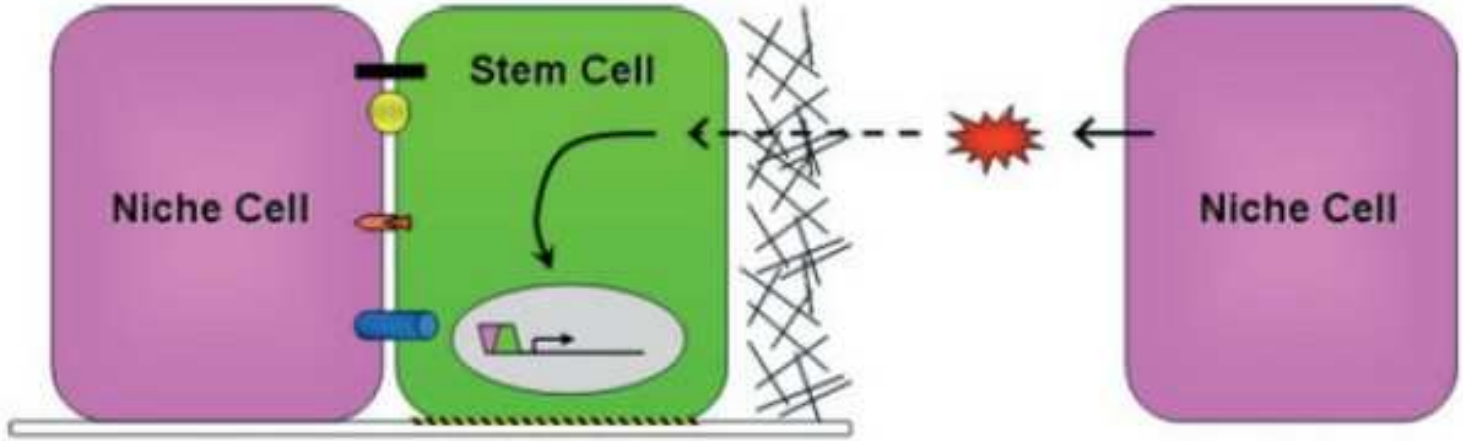
A relatively rare undifferentiated cell found in any organs and differentiated tissues with a limited capacity for both self renewal and differentiation. Such cells vary in their differentiation capacity, but is is usually limited to cell types in the organ of origin.

What are induced pluripotent stem cells (iPSCs)

Somatic cells reprogrammed to enter an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the "stemness" of embryonic stem cells (ESCs). These cells are pluripotent and are capable of generating cells characteristic of all three germ layers.

http://stemcells.nih.gov/info/basics/

Stem Cell Niche



Physical Contact

- *Tight Junction* — N, I
- *Adherens Junction* — D, N
- *Notch Signaling* — C, N, H, I
- *Gap Junction* — D
- *Basement Membrane* — N, E, I
- *Extracellular Matrix* — D, N,

Diffusible Factors

Pathway
Wnt: C, E, H, I
BMP: D, N, E, I
JAK/STAT: D
Growth Factors: N
Hedgehog: I
PGE2: I
O_2: H

- *Transcription Factor Activation*
- *Signal Transduction*

Walker et al. The stem cell niche. J Path. 2009, 217:169

What are Potential Applications of Human Stem Cells

- Regenerative medicine: Generation of cells and tissues for cell-based therapies;
- Test new drugs;
- Explore the complex events during human development and during wound healing, hence to prevent cancer and aging, et al.

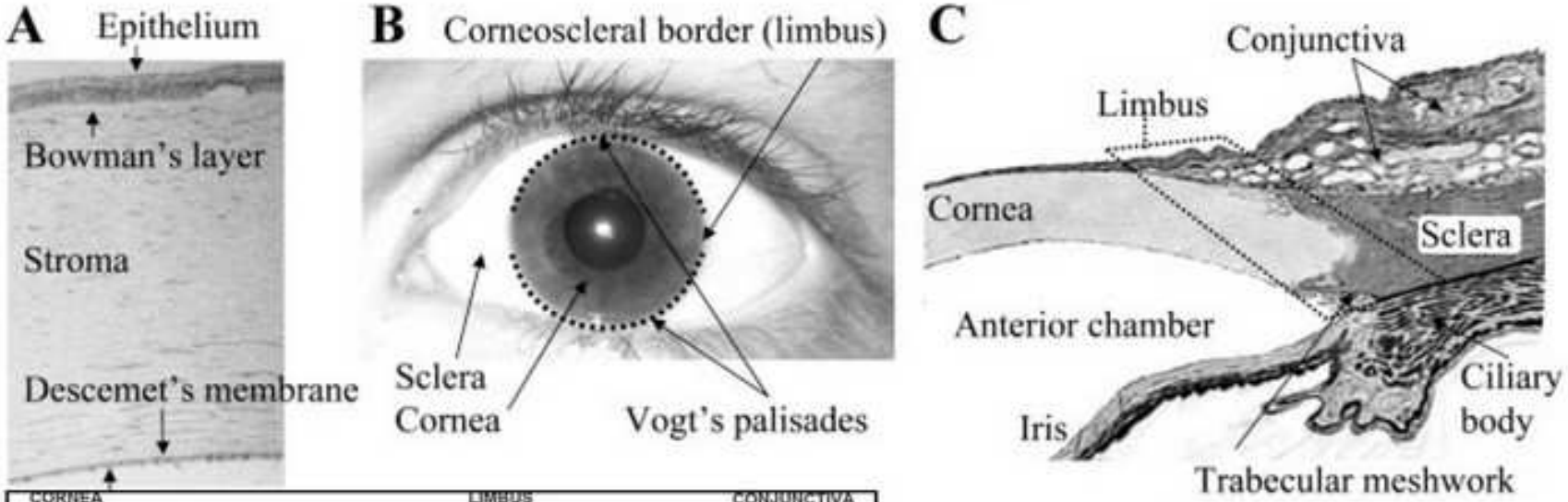
What are Stem Cell Applications in the Eye

- **Corneal limbal stem cells** for limbal stem cell deficiency, corneal bioengineering;
- **Corneal stromal stem cells** for corneal scar, corneal bioengineering;
- **Corneal endothelial stem cells** for Fuchs Dystrophy, corneal endothelial decompensation, corneal bioengineering;
- **Trabecular meshwork stem cells** for glaucoma;
- **Retinal stem cells** for AMD, RP, optic nerve degeneration;
- **hES and iPS** applications in the eye.

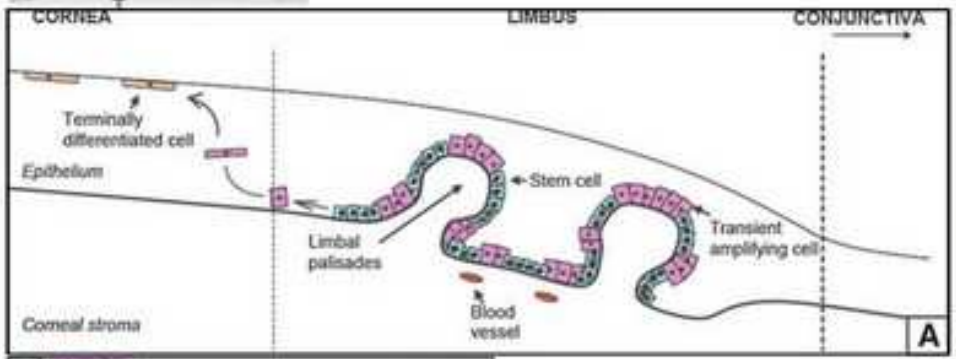
Types of Corneal Transplant

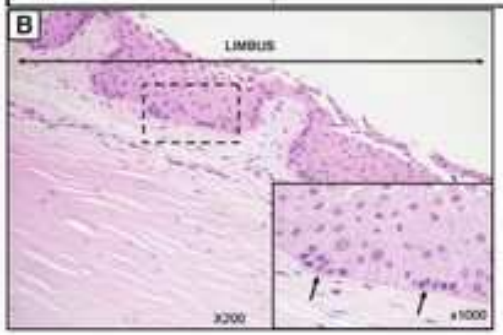
Human donor:	Relative good retention (50-90%) Uncertain supply Not appropriate for all
Fully artificial:	Limited availability Require significant maintenance
Bioengineered:	No components currently suitable for transplant Could solve biocompatibility problems Could solve supply problems

Limbal Stem Cells (LSCs)



Takacs, et al. Cytometry, Part A. 2009, 75A: 54.





Davies, et al. Stem cells and Development. 2010, 19: 1651

Kinoshita, et al. Progress in Retinal and Eye Research. 2001, 20: 639

Limbal Stem Cell Deficiency (LSCD)

Espana, et al. Eye 2004, **18:** 406

Symptoms:

Decreased vision, tearing, Photophobia, blepharospasm, chronic inflammation.

Findings:

Superficial neovascularization, chronic keratitis, scarring, calcification.

Histologically diagnosis:

Impression cytology: goblet cells on the corneal surface.

Treatment:

- Limbal tissue transplantation;
- Ex vivo expanded LSC transplantation;
  1. Amniotic membrane
  2. Fibrin substrate
  3. Temperature-responsive cell culture surface
  4. Mebiol Gel
  5. Poly(lactide-co-glycolide) electrospun scaffold
- Autologous cell transplantation: oral mucosal epithelial cells

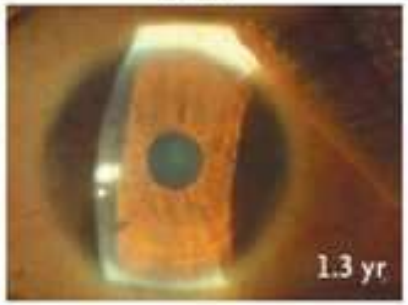
Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration

Rama, et al. N Engl J Med. 2010, 363: 147.

Cultures only (on fibrin)

Before

After



Cultures plus keratoplasty

Before

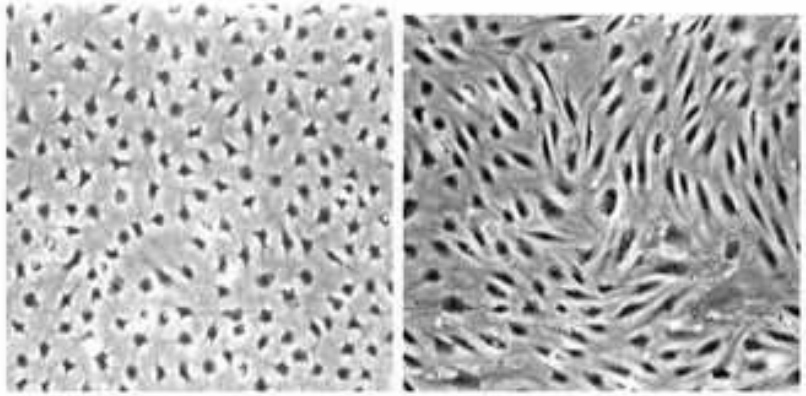
After

Table 1. Clinical Outcomes of Limbal Stem-Cell Grafts in 112 Patients.*

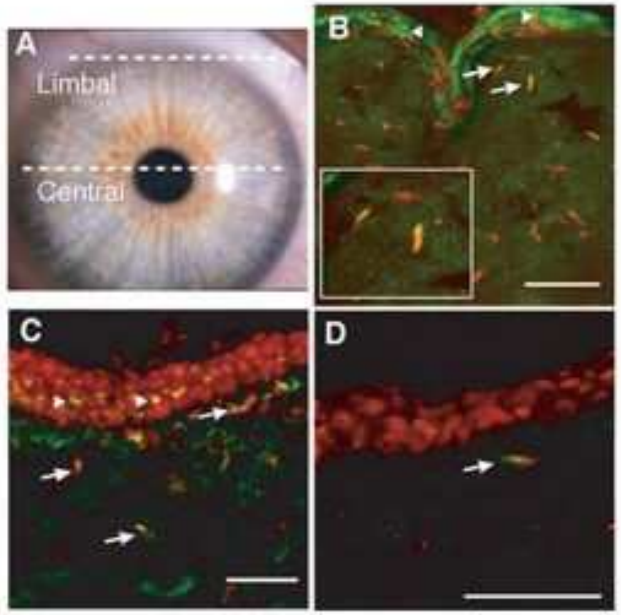
Variable	Number of Eyes	Success	Partial Success	Failure
			number of eyes (percent)	
Outcome of first graft	107	73 (68.2)	18 (16.8)	16 (15.0)
Outcome of subsequent graft	12	9 (75.0)	2 (16.7)	1 (8.3)
Final outcome	107	82 (76.6)	14 (13.1)	11 (10.3)

* Six of the 112 patients were excluded because they did not complete the study. One patient had bilateral limbal stem-cell deficiency and received two grafts.

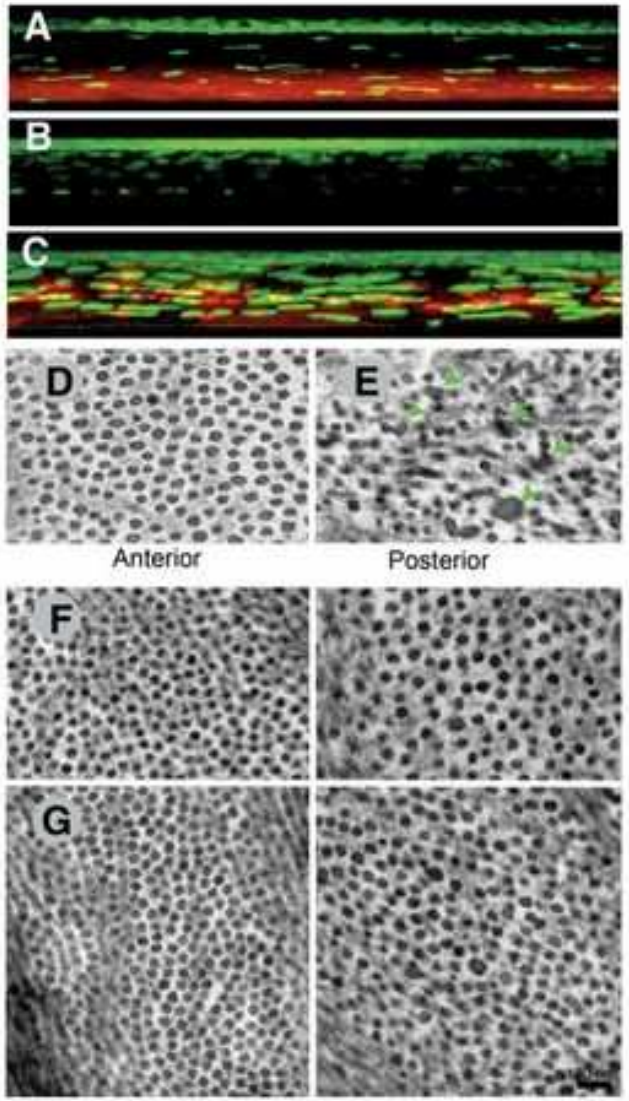
Corneal Stromal Stem Cells



Funderburgh, et al. FASEB J. 2005, 1371

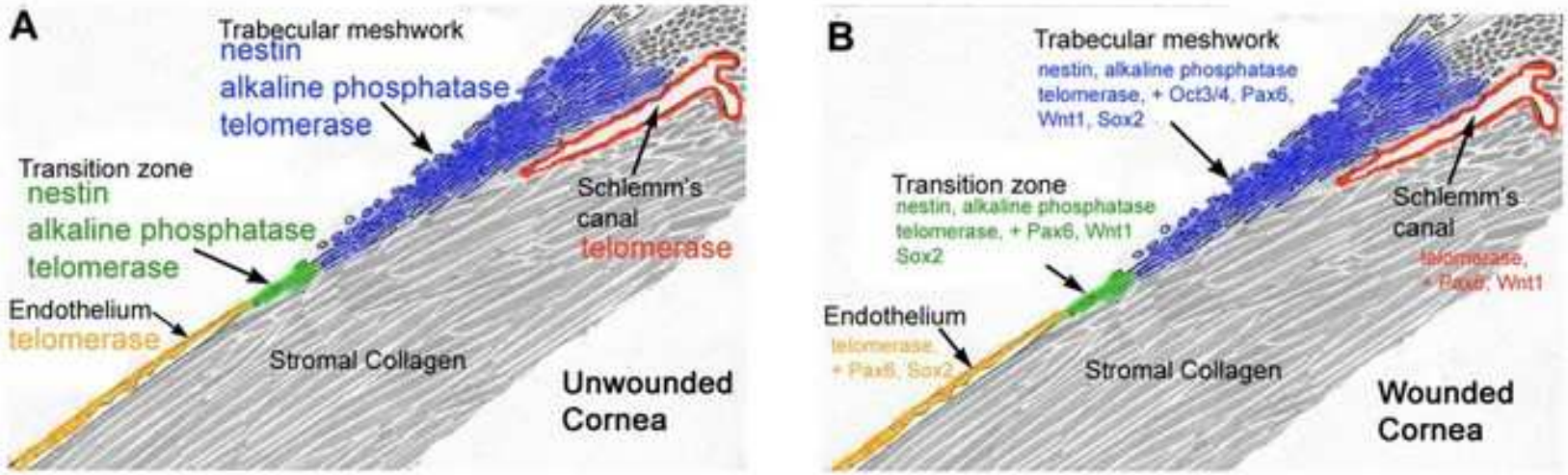


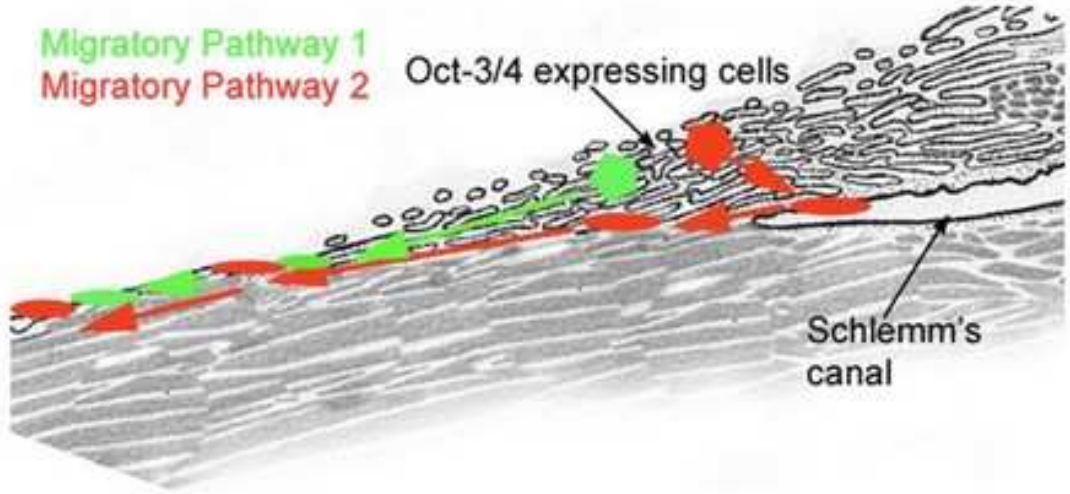
Du, et al. Stem Cells. 2005,23: 1266.



Du, et al. Stem Cells. 2009,27: 1635.

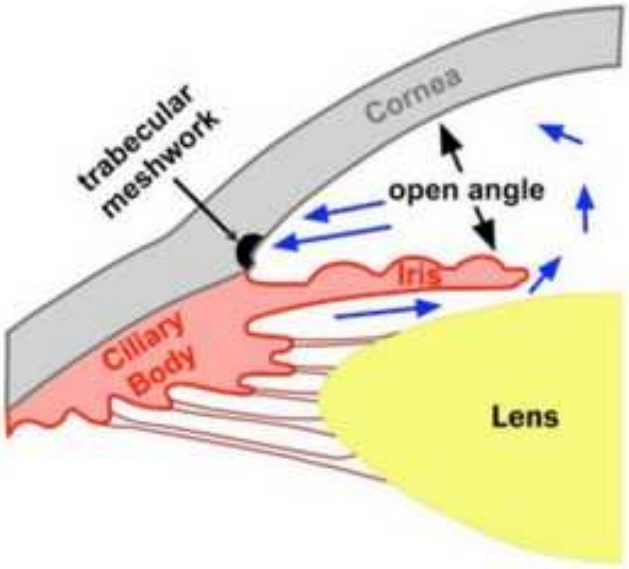
Corneal Endothelial Stem Cells



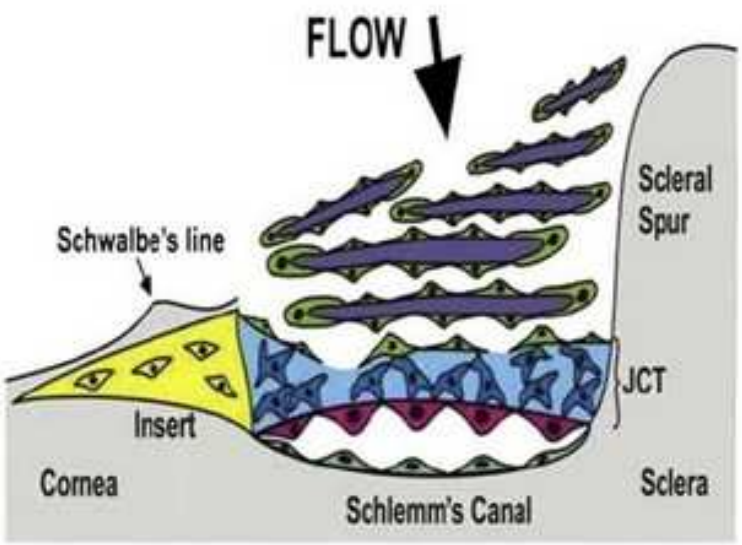


McGowan et, al. Stem cell markers in the human posterior limbus and corneal endothelium of unwounded and wounded corneas. Mol Vis. 2007, 13: 1984.

Trabecular Meshwork Stem Cells (TMSCs) for Glaucoma



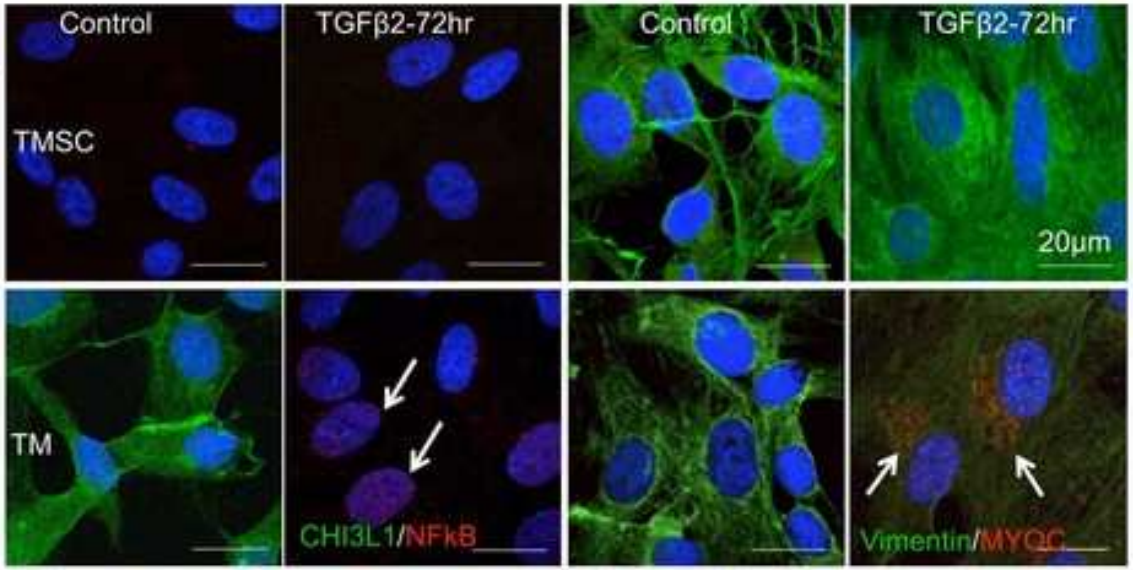
http://www.medrounds.org/glaucoma-guide /2006/06/section-4-d-angle-closure-glaucoma.html



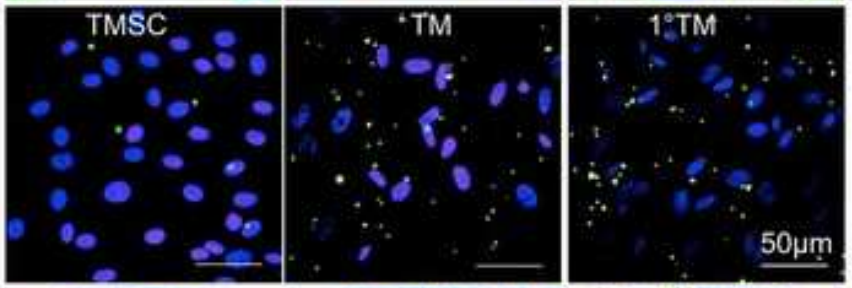
Kelley et al. Stem cells in the trabecular meshwork: present and future promises. Exp Eye Res. 2009, 1-5.

Trabecular Meshwork Stem Cells (TMSCs)

TMSCs are resistant to TGFβ2, a risk factor associated with glaucoma

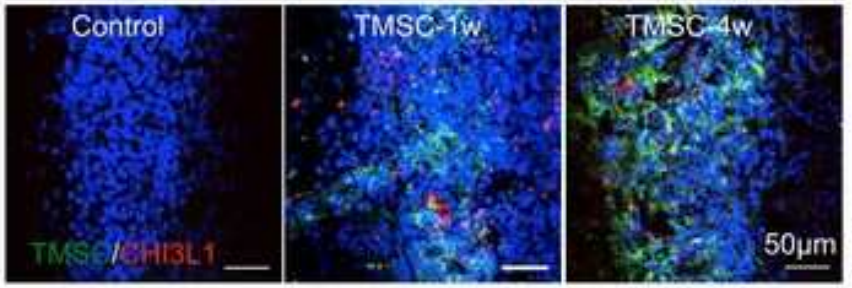


TMSCs can become TM cells with phagocytic function



Phagocytosis

TMSCs can home to the TM region after anterior chamber injection



Injection in AC

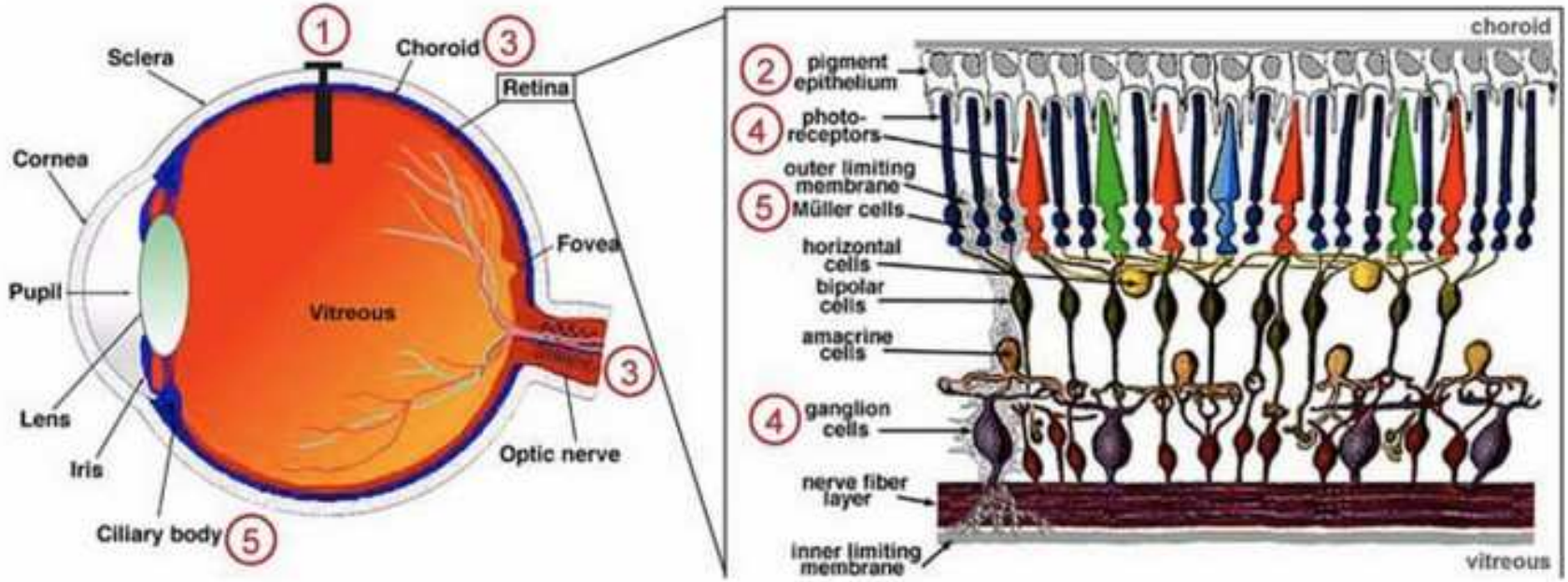
Unpublished data

Stem Cell-based Therapies for Retinal neurodegenerative Diseases

- **Age-related macular degeneration (AMD)**: loss of macular function from the degenerative changes of aging.

  Dry AMD: progressive atrophy of the retinal pigment epithelium (RPE) with subsequent loss of the choriocapillaris and photoreceptors within the macula.

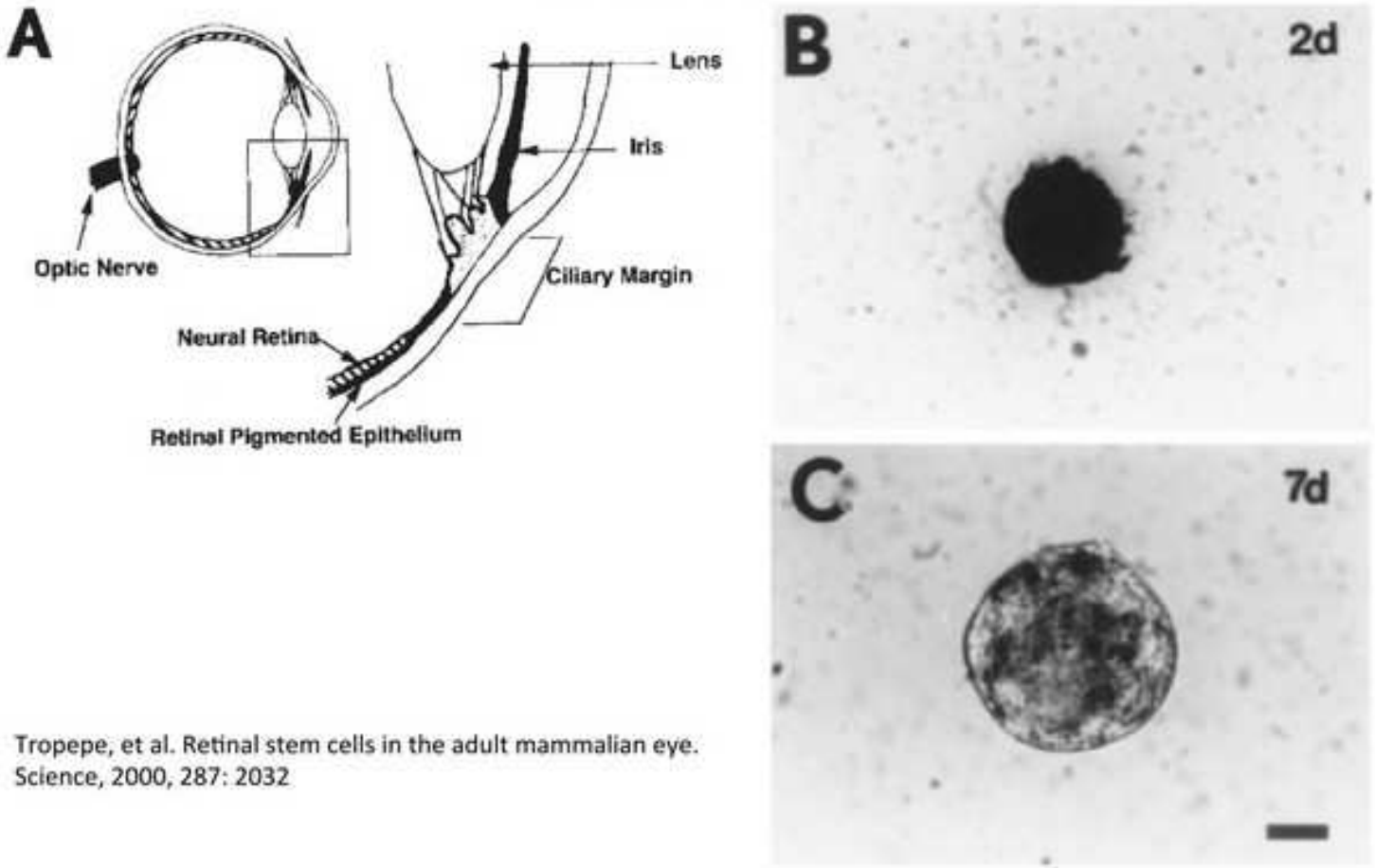
  Wet AMD: bleeding or fluid leakage from abnormal vessels grown from the choriocapillaris beneath the RPE and macula (choroidal neovascularization, CNV)
- **Retinitis pigmentosa (RP)**: a group of genetic disorder, affecting either photoreceptors or RPE, which cause blindness via progressive photoreceptor degeneration.
- **Glaucoma**: optic neuropathy with loss of retinal ganglion cells (RGC) and axons, causing optic nerve degeneration, resulting in visual field loss.
- **Ischemic retinopathy** (diabetic retinopathy, retinopathy of prematurity, vascular occlusions): A group of diseases in which the retinal vasculature is damaged leading to deprivation of oxygen and nutrients, and sometimes pathological neovascularization.



1. Implantable cell-encapsulation device for chronic intraocular delivery of secreted neurotrophins in AMD & RP*
2. Transplantation of ES-derived RPE for recovery of RPE function & photoreceptor protection in AMD*
3. Vascular stem cell repair and therapeutic angiogenesis in ischaemic retinopathies
4. Transplantation of stem/progenitor cells for photoreceptor replacement in outer retinal disease, & RGCs in inner retinal disease
5. Modulation of retinal stem cells, either Müller cell or ciliary body origin, to elicit endogenous retinal repair/regeneration

Bull dt al. Towards stem cell-based therapies for retinal neurodegenerative diseases. Stem Cells. 2011, 29: 1170.

Retinal Stem Cells

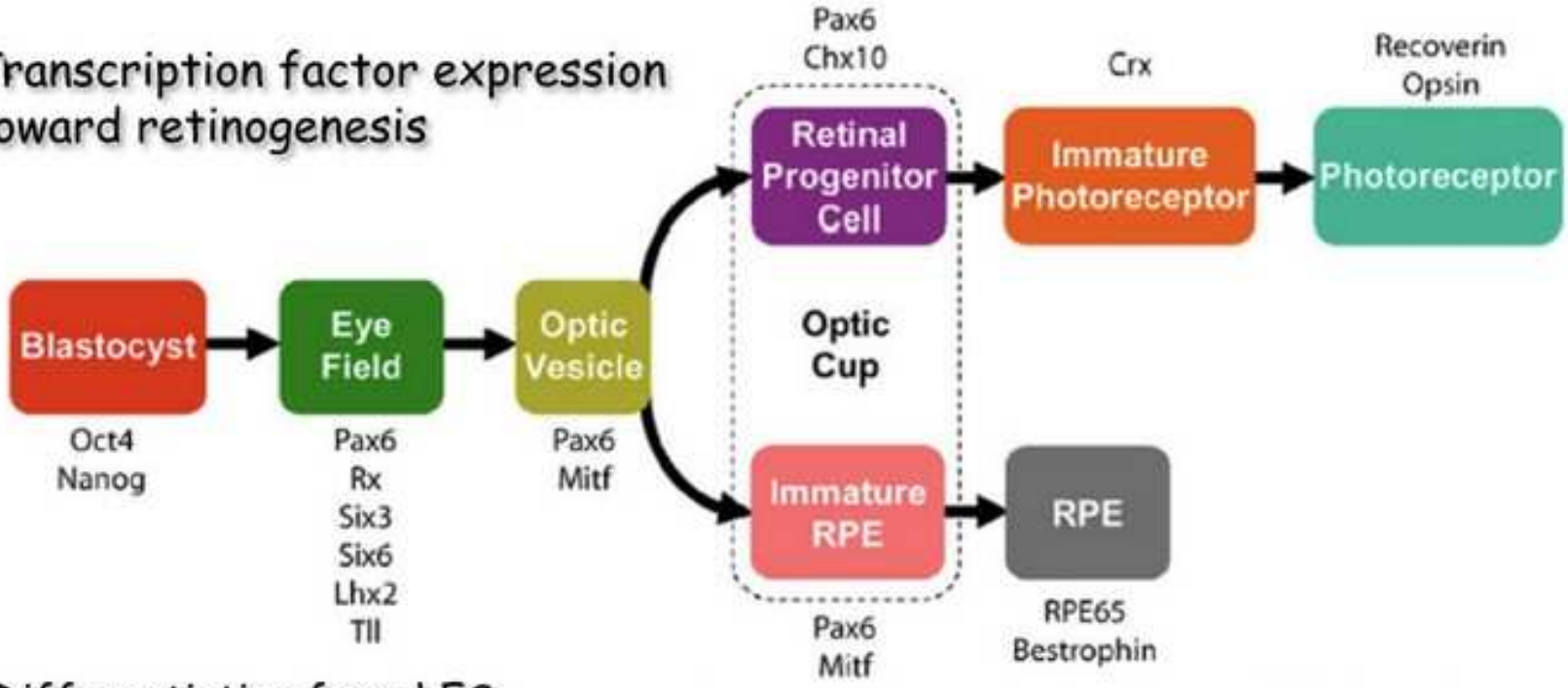


Tropepe, et al. Retinal stem cells in the adult mammalian eye. Science, 2000, 287: 2032

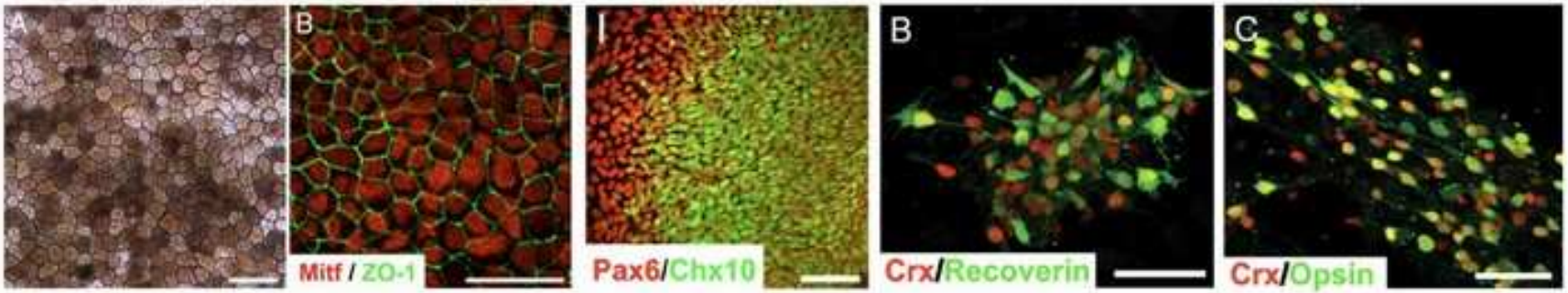
Facile isolation and the characterization of human retinal stem cells.
Coles, et al. PNAS, 2004, 101: 15772.

Cells previously identified as retinal stem cells are pigmented ciliary epithelial cells.
Cicero, et al. PNAS, 2009, 106: 6685.

Transcription factor expression toward retinogenesis



Differentiation from hES

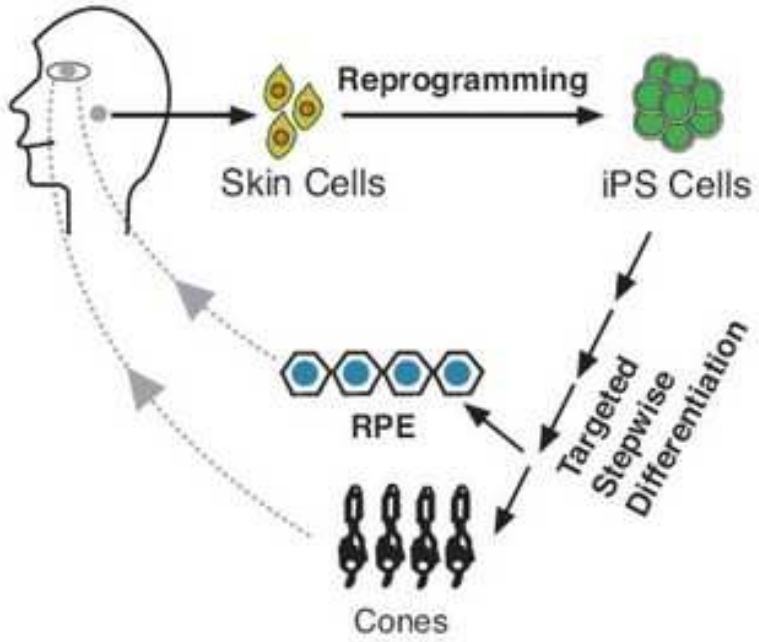


RPE-day 30 | Optic cup-day 30 | Photoreceptors-day 80

Meyer et al. Modeling early retinal development with human embryonic and induced pluripotent stem cells. PNAS. 2009, 106: 16698

Autologous stem cell based-therapy

Oral mucosal stem cells: for LSCD
Mesenchymal stem cells: multipotent
Adipose derived stem cells: multipotent
Induced pluripotent stem cells: pluripotent
Activating endogenous repair mechanisms



Wang SZ. Tales of retinogenesis told by human stem cells. PNAS. 2009, 106: 16543