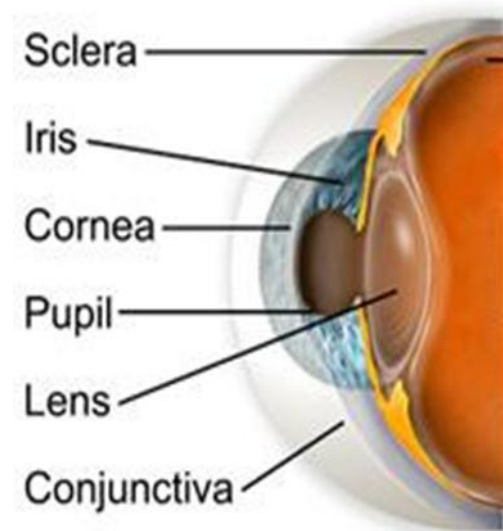




REGLAZING THE EYE

The Surface of the Eye

- The ocular surface is covered by cells from two distinct cell epithelia lineages the conjunctiva and cornea.



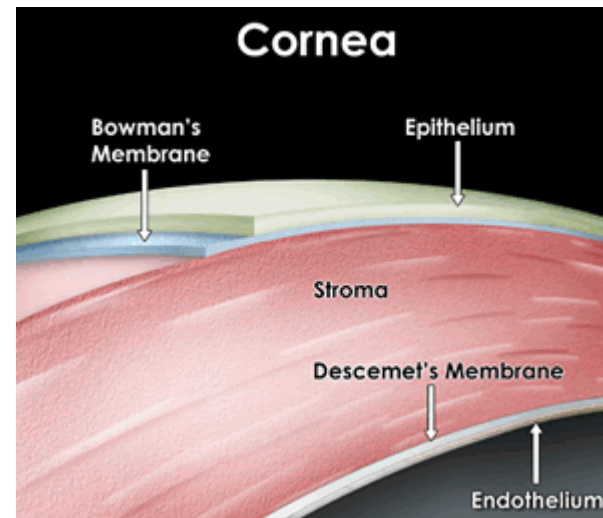
Functions of the Cornea

- 70% of light is refracted through the cornea.
- Protection of the Inner parts of the Eye

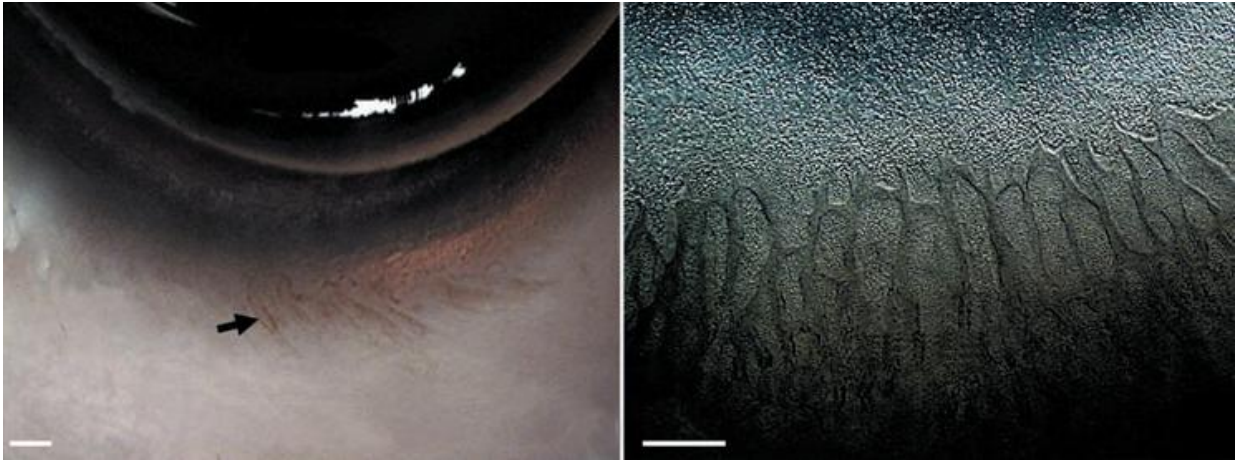


Structure of the cornea

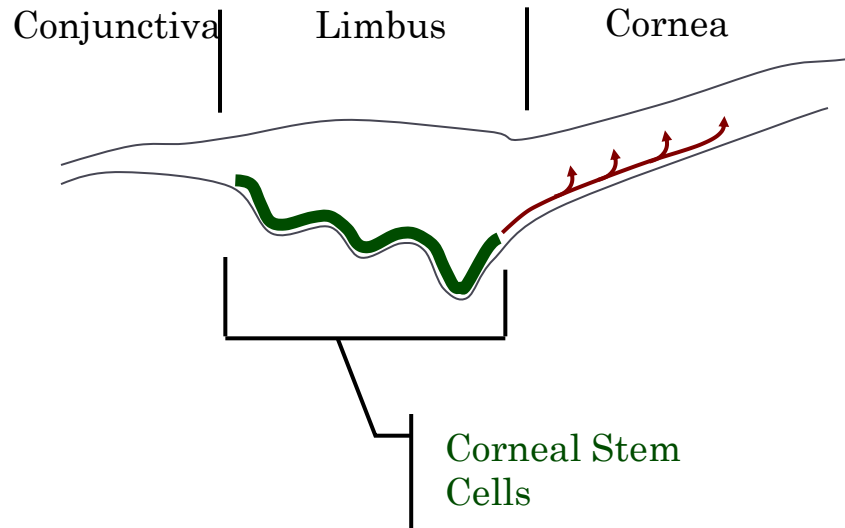
- Corneal epithelium
- Bowman's layer
- Corneal stroma
- Descemet's membrane
- Corneal endothelium



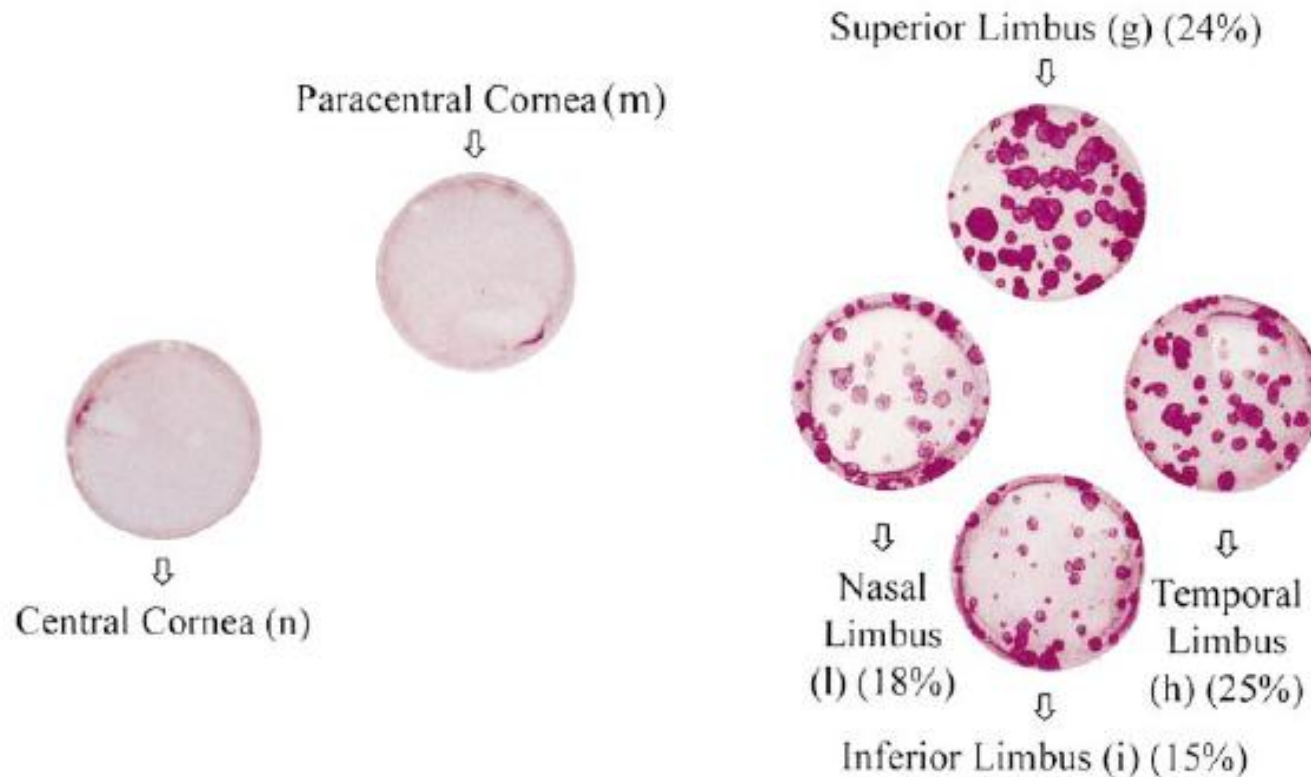
The Limbus



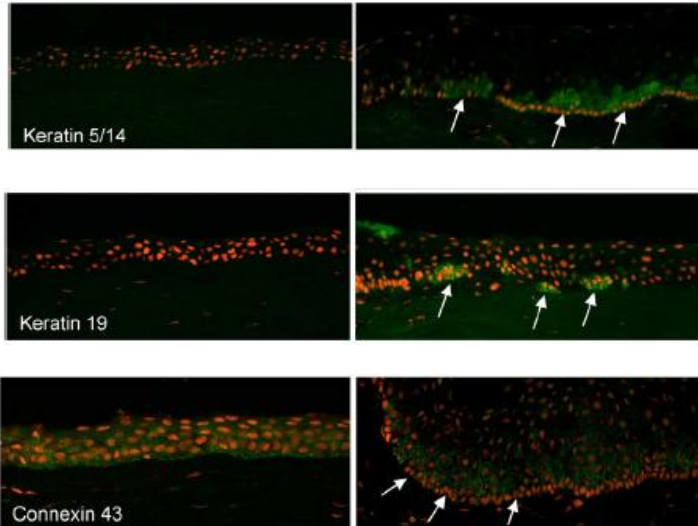
Cornea Stem Cells Located at The Limbus



Evidence That The Limbus Is The Location Of Stem Cells

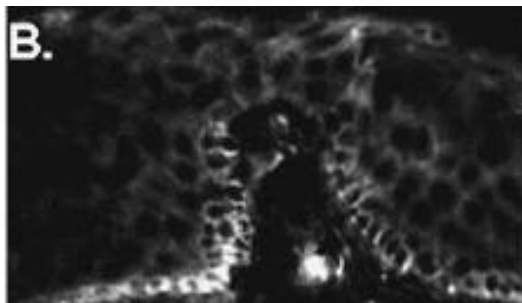
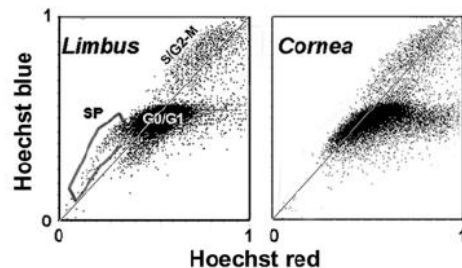


Evidence That The Limbus Is The Location Of Stem Cells



- α -enolase positive
- EGF receptor positive
- CK 5 & 14 positive
- CK 3 & 12 negative
- CK 19 positive
- Vimentin positive
- Integrins β 1, α 6 and α 9 positive
- Transcription factor p63 positive
- Connexin 43 negative
- ABC-G2 positive

Evidence That The Limbus Is The Location Of Stem Cells



- Limbal stem cells have been localised to the Palisades of Vogt in the corneal limbus.
- There are an estimated six such limbal epithelial crypts per human limbus.

Limbal Stem Cell Deficiencies & Treatments

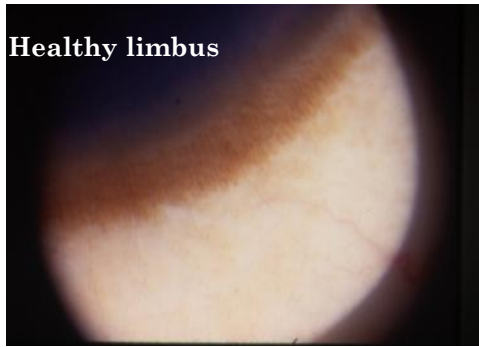
Limbal Stem Cell Deficiency



Chemical / Thermal injury



Stevens Johnsons syndrome (SJS)



Slit image of normal eye



Ocular cicatricial (OCP)



Severe microbial infection

Limbal Stem Cell Transplantation

- Autologous or Donor tissue.
 - Direct limbal tissue transplantation.
 - Cultured limbal stem cell epithelium.

In Vitro Cultivation Of Limbal Stem Cells For Therapy

Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium

Graziella Pellegrini, Carlo E Traverso, Adriano Tito Franzi, Mario Zingirian, Ranieri Cancedda, Michele De Luca

Pellegrini et al., *The Lancet* 3

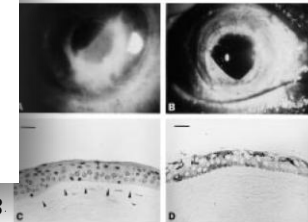
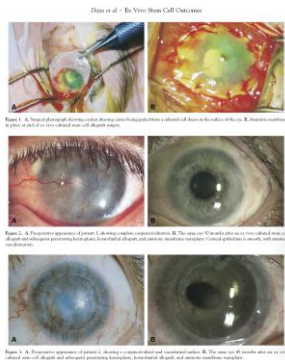


Figure 2: Transplantation and histology of patient 1. A: appearance of injured eye at admission. B: corneal surface about 11 months after grafting (6 months after penetrating keratoplasty). C: regenerated epithelium about 2 years after grafting; haematoxylin and eosin; arrowheads indicate Bowman's layer. D: K3 staining of regenerated epithelium 2 years after grafting. The K3-specific AE5-mAb shows a uniform positive staining. Bars= 20 µm.



Outcomes and DNA Analysis of Ex Vivo Expanded Stem Cell Allograft for Ocular Surface Reconstruction

Sheraz M. Daya, FACP, FACS,¹ Adam Watson, FRANZCO,¹ Justin R. Sharpe, PhD,² Osama Gileli, FRCS,¹ Andrea Rowe,² Robin Martin, PhD,² S. Elizabeth James, PhD²

Daya et al., *Ophthalmology* 112:470-477. 2005

Cell Tissue Res (2008) 331:135–143
DOI 10.1007/s00441-007-0458-7

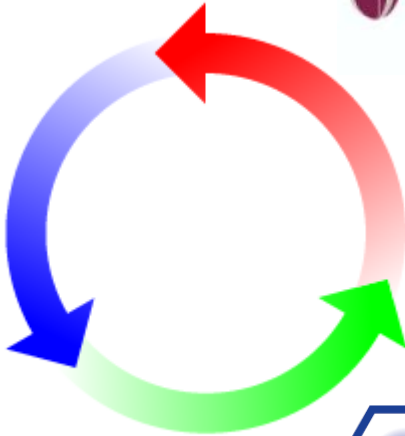
REVIEW

Biological principals and clinical potentials of limbal epithelial stem cells

Maria Notara • Julie T. Daniels

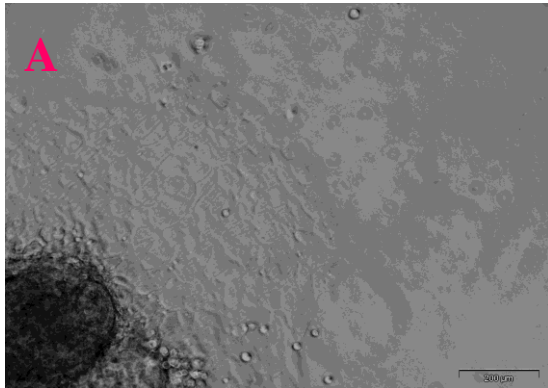
Notara and Daniels, *Cell & Tissue Research* 331(1):135-143, 2007

A Collaborative Effort

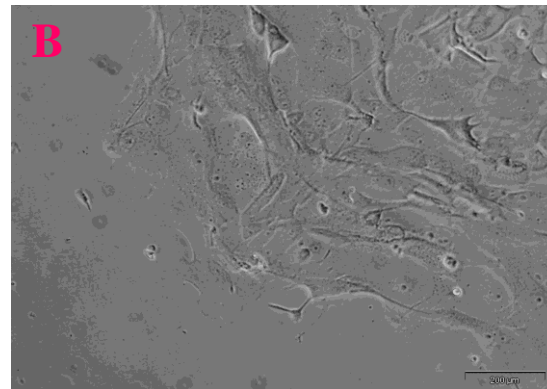


Translational Research Program

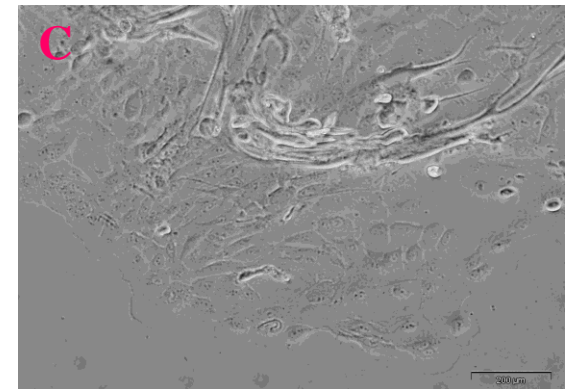
Explant Culture on Tissue Culture Plastic



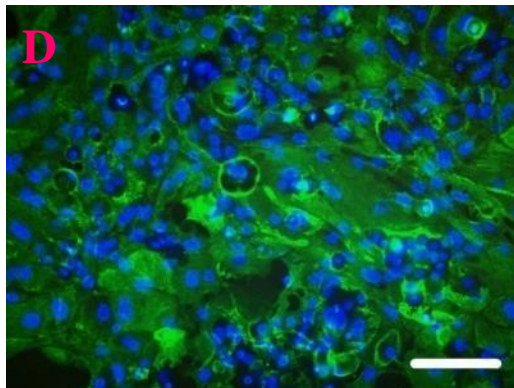
Day 2



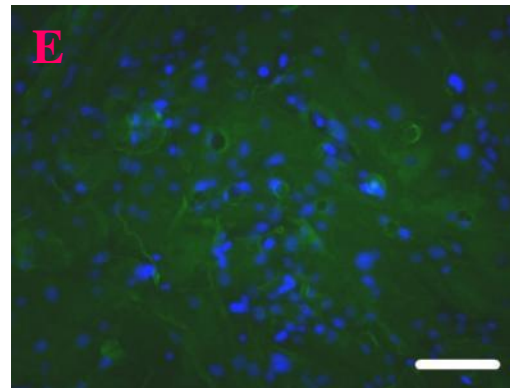
Day 8



Day 16

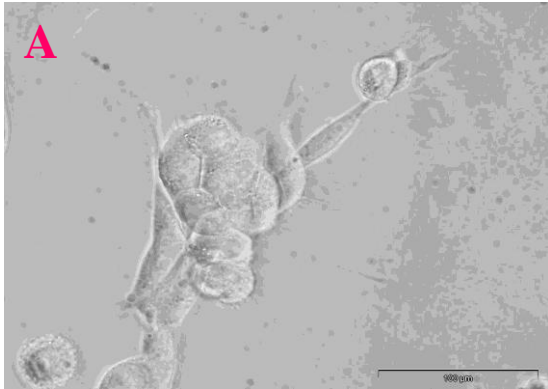


CK3/DAPI

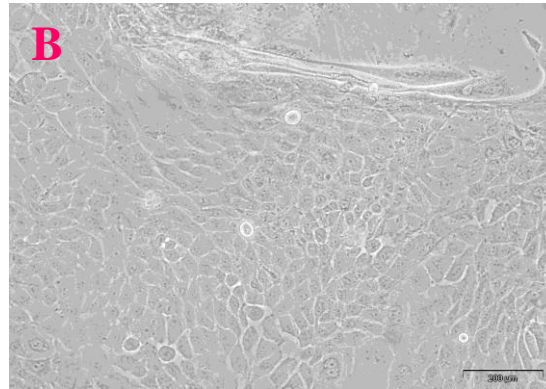


CK19/DAPI

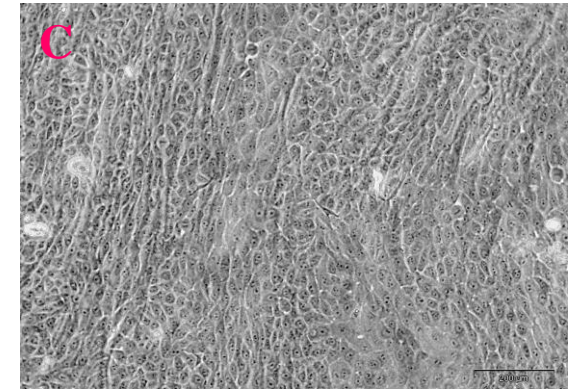
Suspension Culture with 3T3 Feeder Cells.



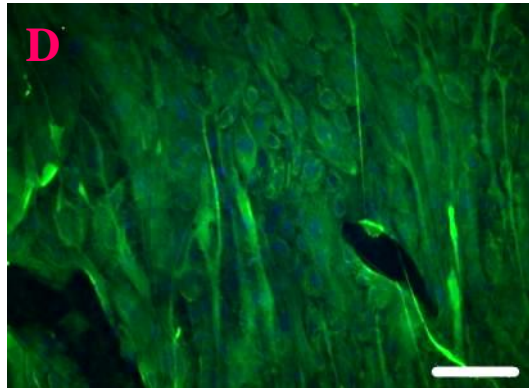
Day 2



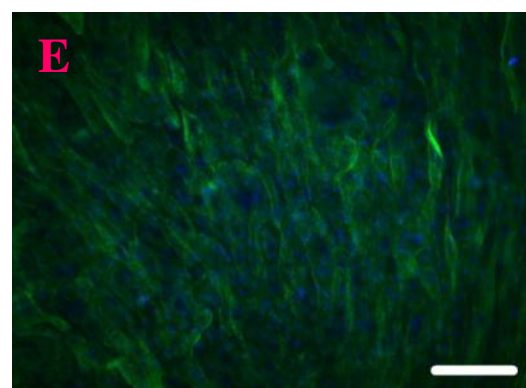
Day 8



Day 16

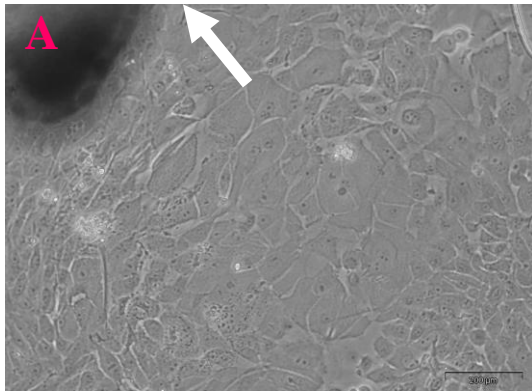


CK3/DAPI

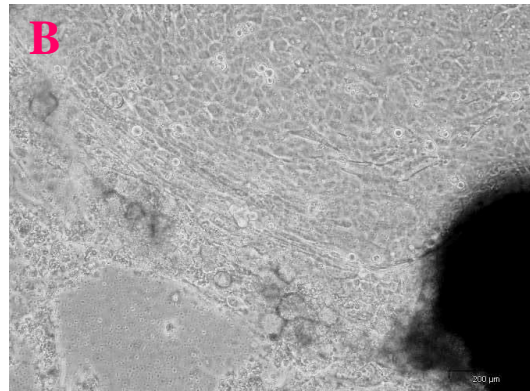


CK19/DAPI

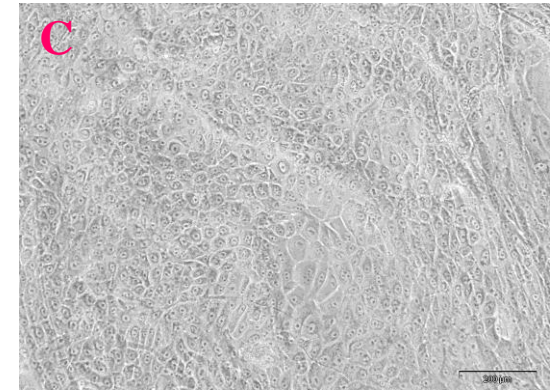
Explant on TC Plastic with 3T3 Feeder Cells



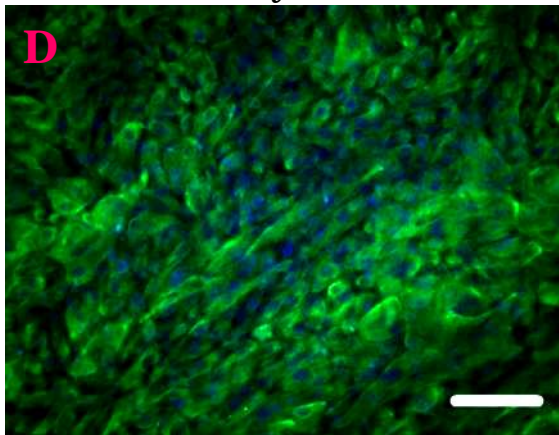
Day 2



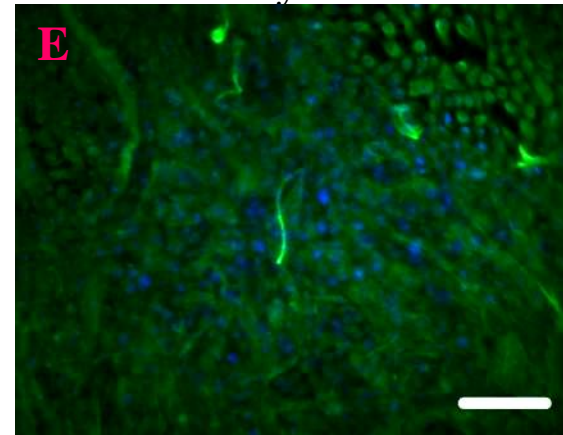
Day 8



Day 16



CK3/DAPI



CK19/DAPI

The Road to Clinic

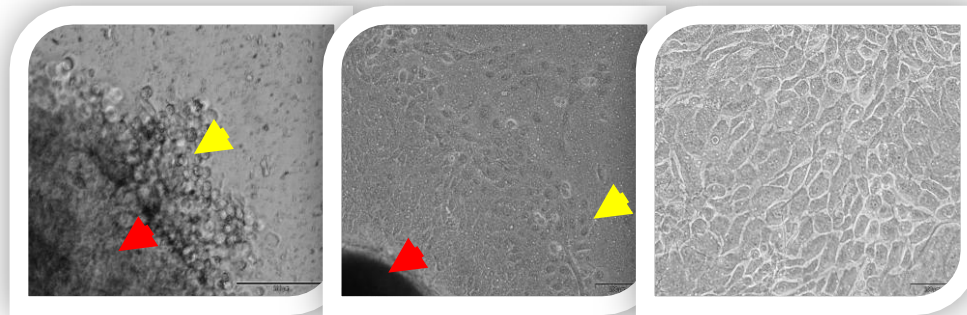
- Regulation EC No 1394/2007 ATMP and amending Directive 2001/83/EC & Regulation (EC) No726/2004.
 - Article 28 (2.7)
- Perform cultures in class B facility.
- GMP materials.



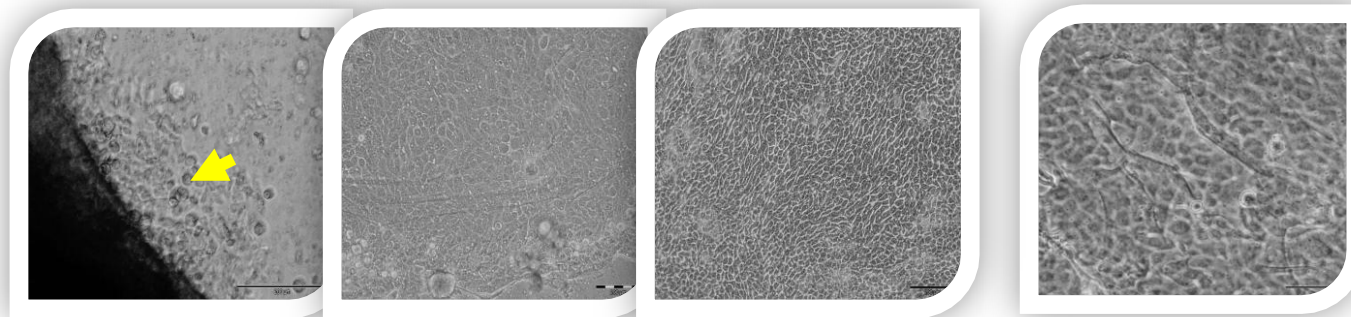
- Explant cultures
- DMEM (with Glutamax 1) + Hams F12 (3:1)
 - 10% FCS
 - 5µg/ml Insulin
 - 0.4µg/ml hydrocortisone
 - 1x10⁻¹⁰M cholera toxin
 - 10ng/ml EGF

- Incubated at 37 °C in 5%CO₂

Cell Morphology



No 3T3



Co-3T3

Day 2

Day 8

Day 16

Day 16

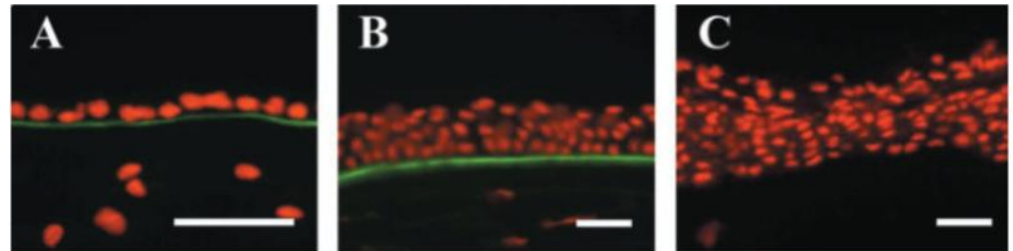
A Suitable Carrier

- Amniotic membrane is the innermost layer of the fetal membrane
- Biological dressing for cornea
 - ▣ infections, sterile melts, and to reconstruct the ocular surface for various procedures.

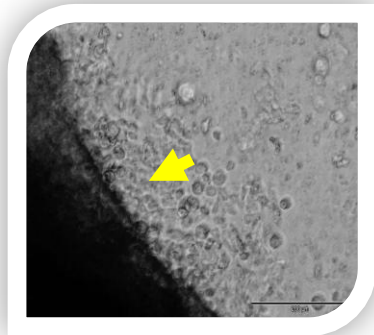


A Suitable Carrier

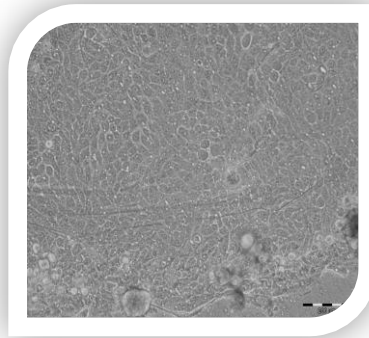
- Anti-adhesive effects
- Bacteriostatic properties
- Wound protection
- Pain reduction
- Re-epithelialisation
- No Immunogenicity.
- Matches Bowman's membrane
 - Collagen I
 - Collagen IV



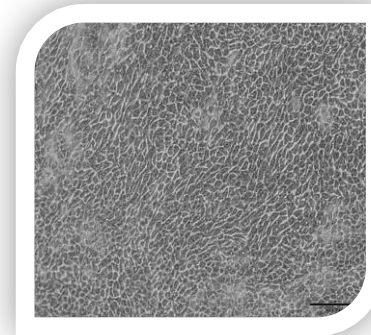
Cell Morphology



Day 2

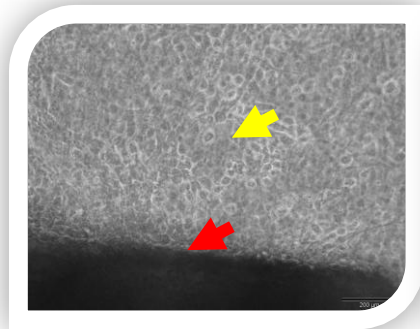


Day 8

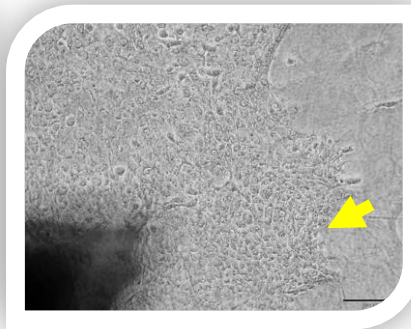


Day 16

Co-3T3



Day 5



Day 14



Day 20

HAM

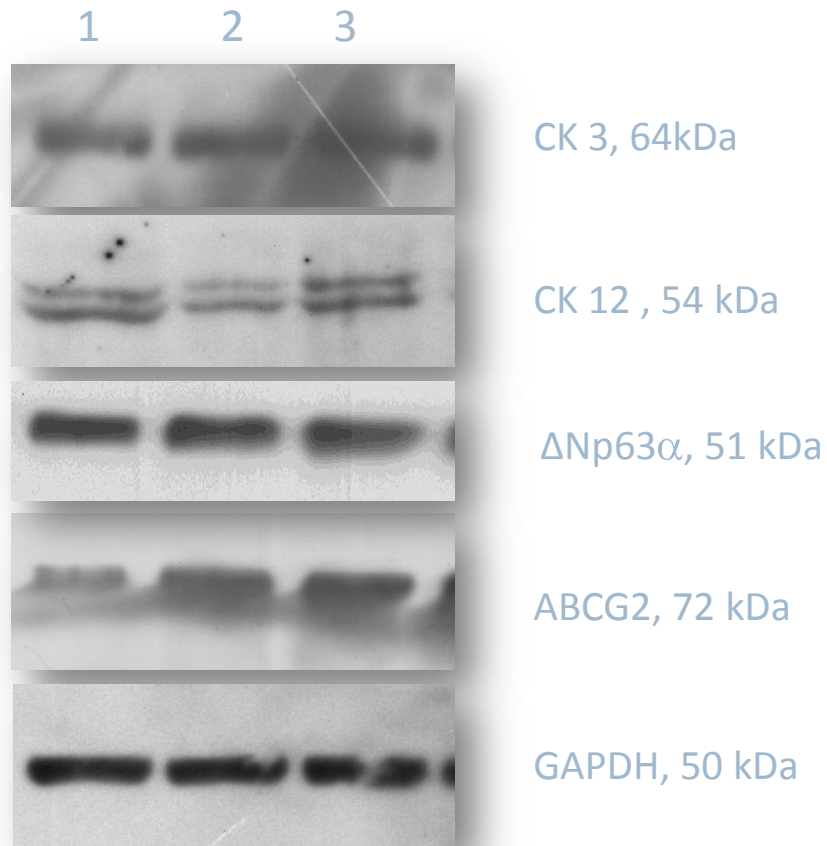
Marker Expression.

Marker	Specification
Δ Np63 α	Nuclear
ABCG2	ATP-binding cassette transporter
p63	Nuclear *
Cytokeratin 14	Limbal basal cells
Cytokeratin19	Limbal corneal epithelial cells*
Vimentin	Structural protein
α -2 Integrin	Membrane
β -1 Intergrin	Membrane

Marker	Specification
Cytokeratin 3	Major cytokeratin in corneal epithelium [♣]
Cytokeratin 12	Major cytokeratin in corneal epithelium [♣]
Connexin43	Cell-cell contact (tight junction protein)
ZO1	Cell-cell contact
Occludin	Cell-cell contact
E-cadherin	Membrane

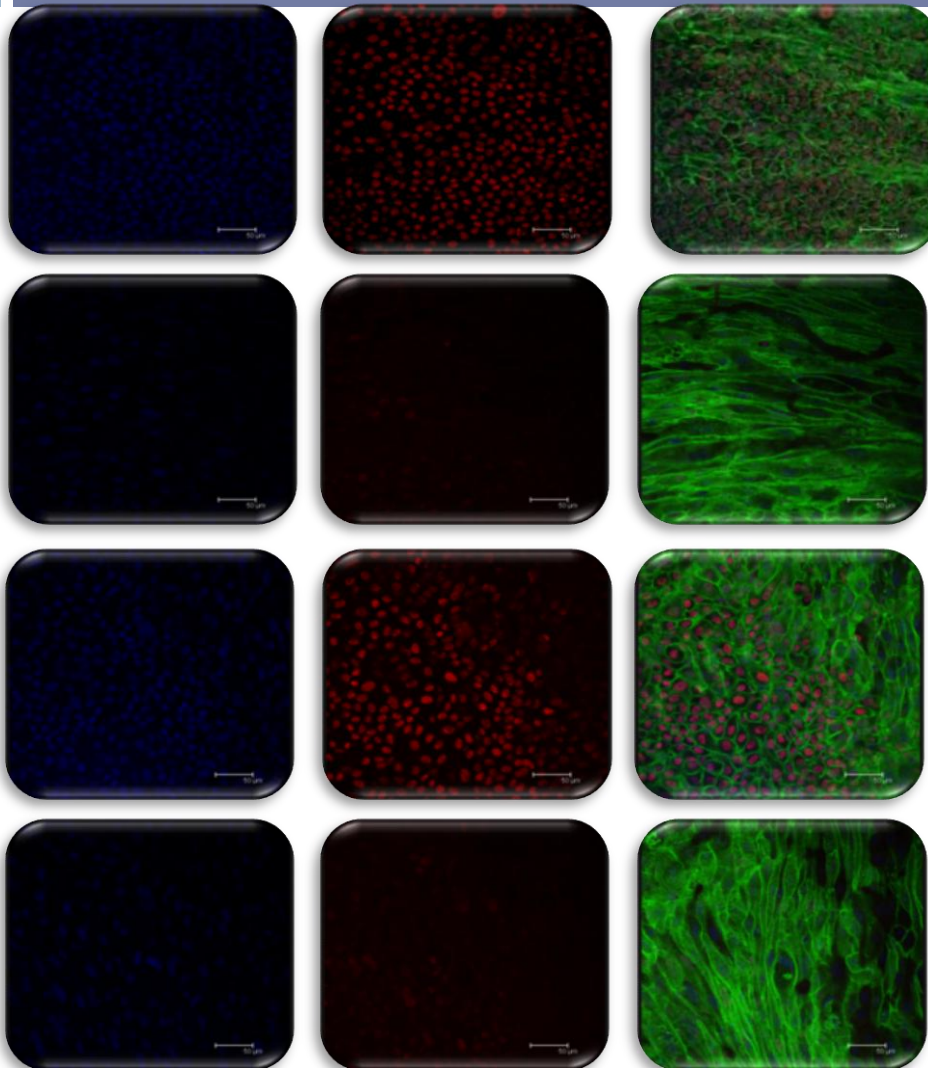
**Differentiation* (2005) 73:61-68, *IOVS* (2006) 47: 4780-86, [♣]*IOVS* (2006) 47:3820-27, *FEBS lett* (2004) 565:6-10.

Marker Expression.

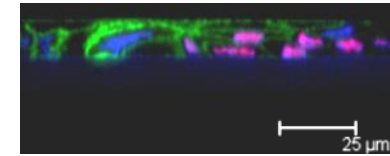


1. *Insert no-3T3*
2. *HAM*
3. *Insert Co-3T3*

Δ NP63 α Expression



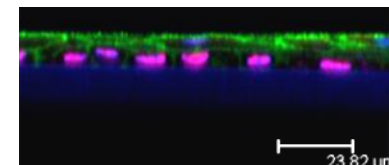
Basal



No 3T3

Apical

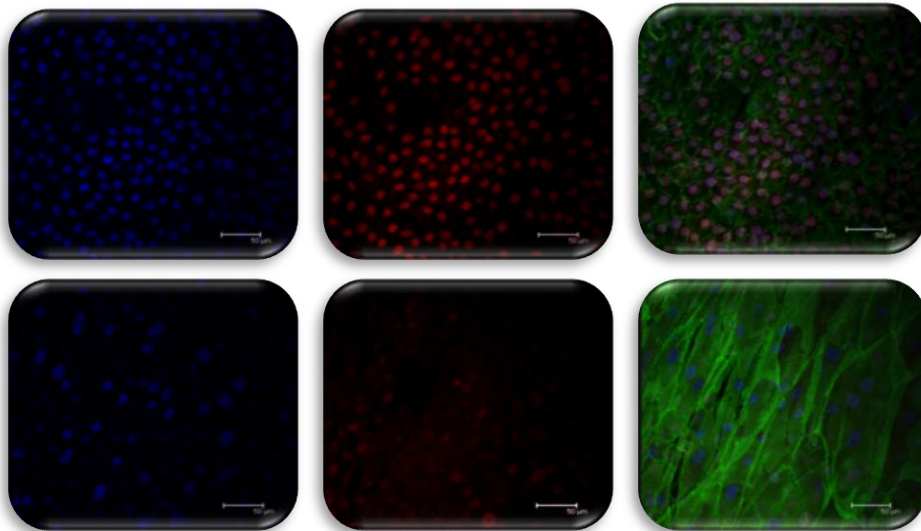
Basal



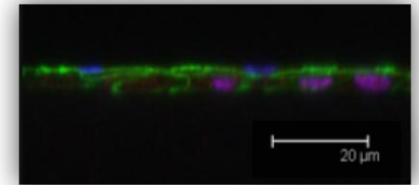
Co 3T3

Apical

Δ NP63 α Expression



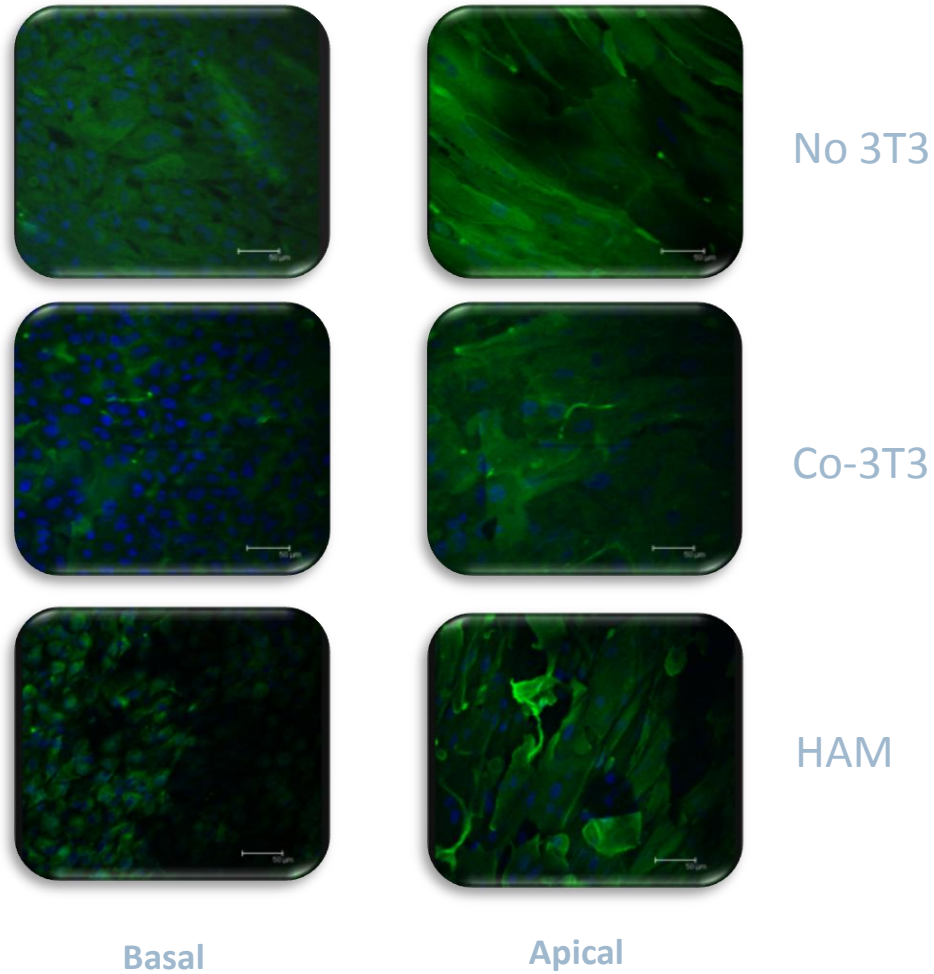
Basal



Apical

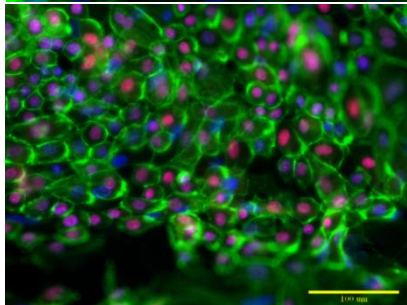
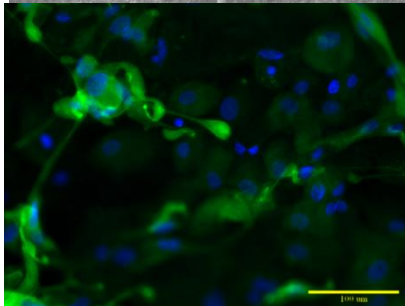
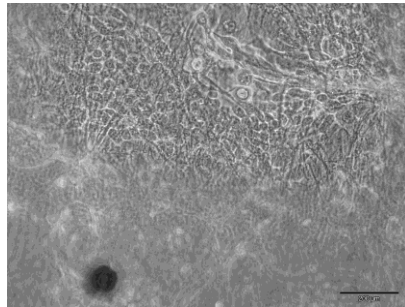
HAM

CK3 Expression



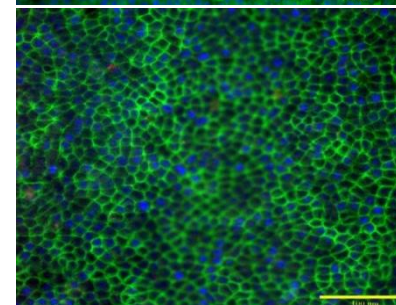
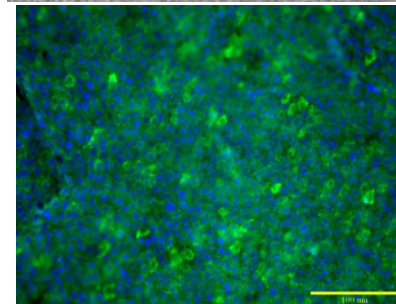
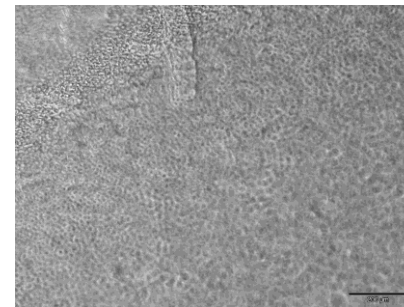
- *HAM - Transplant Services Foundation*
- *Acelagraft - Celgene Cellular Therapeutics*
 - Decellularized and dehydrated human amniotic membrane (DDHAM)
- *Ambio5 - IOP Ophthalmics*
 - All antigens are removed; cells are devitalized; and final grafts are terminally sterilized

Acelagraft



CK3

Δ NP63 α
E-Cadherin



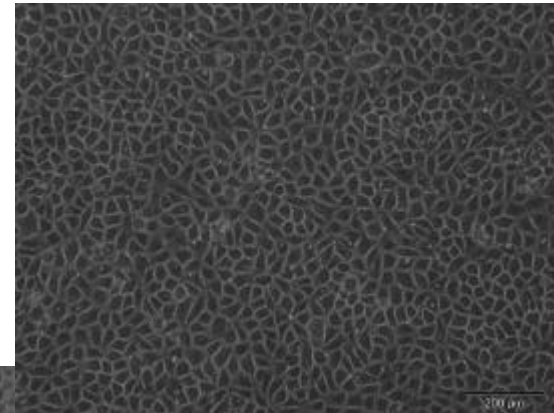
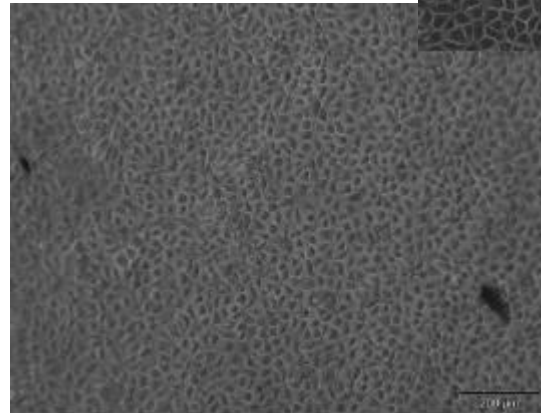
Ambio5

Future Directions

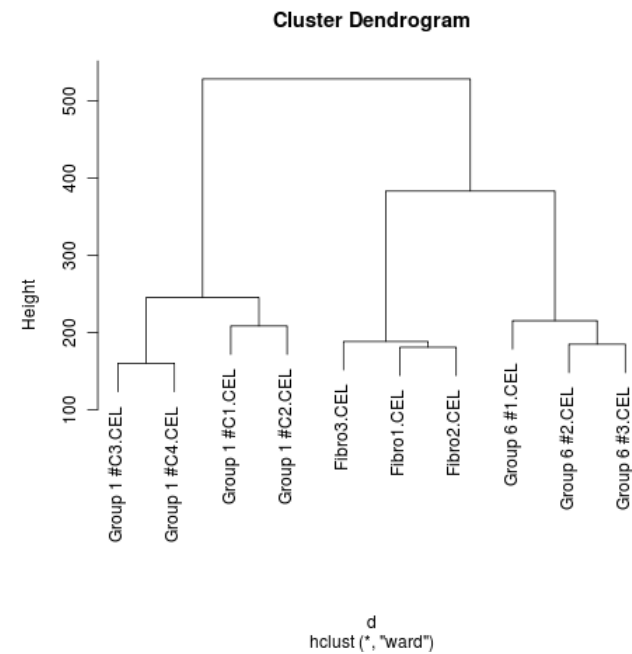
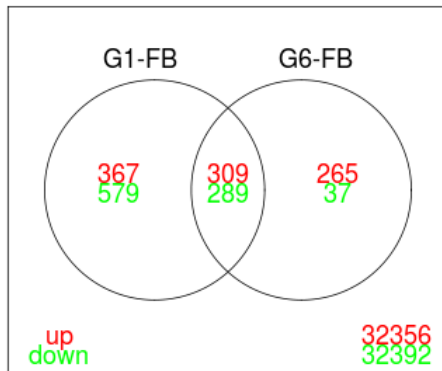
Development of Alternative Carrier



Prof. Fergal O'Brien



Are there differences?

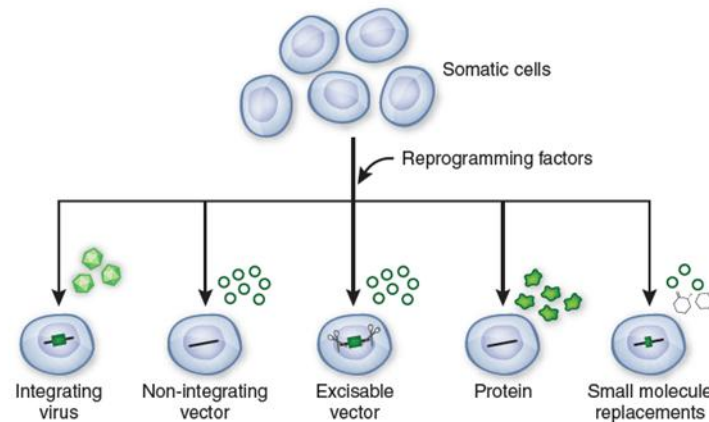


Are there differences?



Name	# of Entities	Expanded # of Entities	Overlap
metabolic process	2574	2574	109
apoptosis	768	768	41
cell adhesion	688	688	34
phosphorylation	526	526	30
response to drug	495	495	28
positive regulation of cell proliferation	429	429	25
negative regulation of cell proliferation	391	391	25
intracellular signal transduction	379	379	21
intracellular signaling pathway	377	377	21
axon guidance	327	327	29
interspecies interaction between organisms	319	319	21
inflammatory response	316	316	17
innate immune response	278	278	18
cell-cell signaling	273	273	16
response to hypoxia	242	242	22
platelet activation	239	239	22
response to organic cyclic substance	227	227	16
positive regulation of apoptosis	222	222	18
nerve growth factor receptor signaling pathway	221	221	14
angiogenesis	196	196	20
actin cytoskeleton organization	188	188	12
negative regulation of endopeptidase activity	165	165	13
organ morphogenesis	162	162	12
embryonic development	157	157	11
chemotaxis	149	149	12
response to estradiol stimulus	142	142	15
central nervous system development	140	140	11
response to ethanol	139	139	11
response to glucocorticoid stimulus	138	138	14
response to organic substance	136	136	10
cell-cell adhesion	128	128	10
cell migration	125	125	11
intracellular protein kinase cascade	116	116	15
negative regulation of peptidase activity	112	112	10
leukocyte migration	110	110	9
integrin-mediated signaling pathway	106	106	9
epidermis development	98	98	9
wound healing	98	98	8

iPS Technology

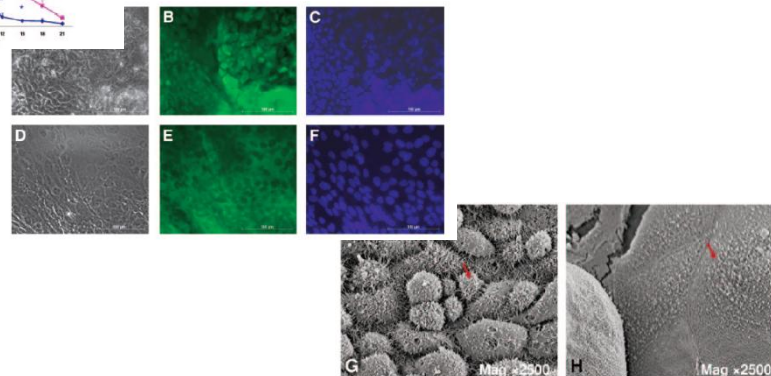
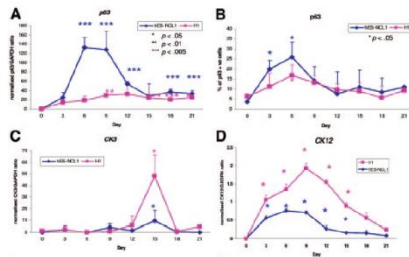
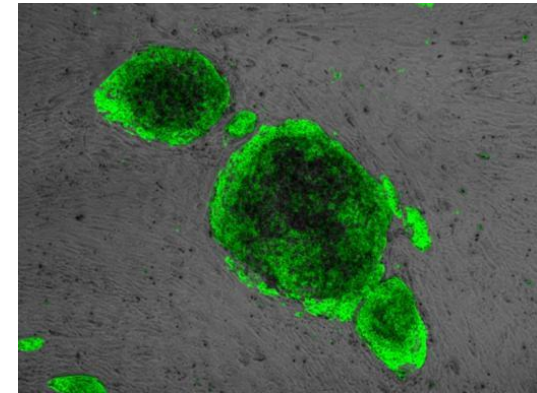


Nature Methods volume 7 (1) 2010

- Initially used viruses that integrated into the genome.
- Non integrating adenoviruses
- Transient plasmid transfection
- Episomal vectors

Potential of iPS based therapies

- Differentiation not as easy it appears.



Thank you



- Dr. Clair Gallager
- Dr. Kishore Reddy
- Prof. Martin Clynes



- Mr William Power
- Prof. Conor Murphy
- Dr Andra Bobart



- Ms Sandra Shaw
- Dr. Elva Eatkins
- Dr. William Murphy



- Research Committee Royal Victoria Eye & Ear Hospital



- HRB Partnership Award 2007 PA/2007/08



- This research is partially funded by 3U Biomedical Research (DCU-NUI Maynooth-RCSI) This research was [partially] funded by 3U Biomedical Research (DCU-NUI Maynooth-RCSI)

From the Creators of **TOY STORY**
YOU WON'T BELIEVE YOUR EYE



Disney · PIXAR
MONSTERS, INC.
In Cinemas Soon