Strategies to Induce Tolerance or Overcome Immune Suppression

Immune Privilege Niche - Subretinal Space

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Immune Privilege

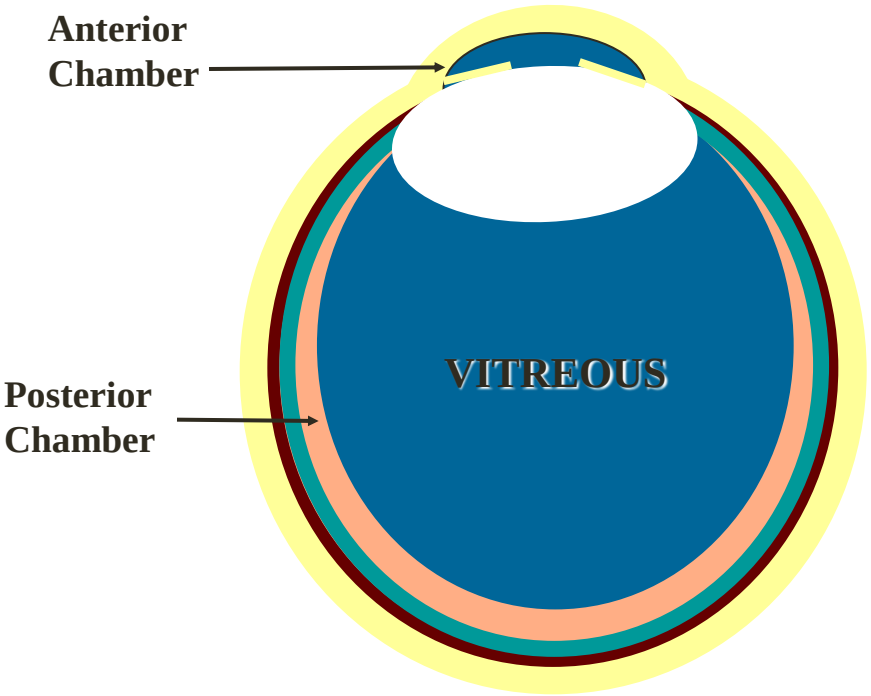
- Immune privilege refers to the fact that foreign tissue grafts placed in the immune privileged site are tolerated and survive for prolonged, often indefinite, intervals, whereas placement of such grafts at conventional body sites leads to acute, irreversible immune rejection.
- Sites include the eye, placenta and testes.
- Immune privilege has evolved from a concept of passive physical barriers to more of an active process.
- Concept of immune privileged site and immune privileged tissue.

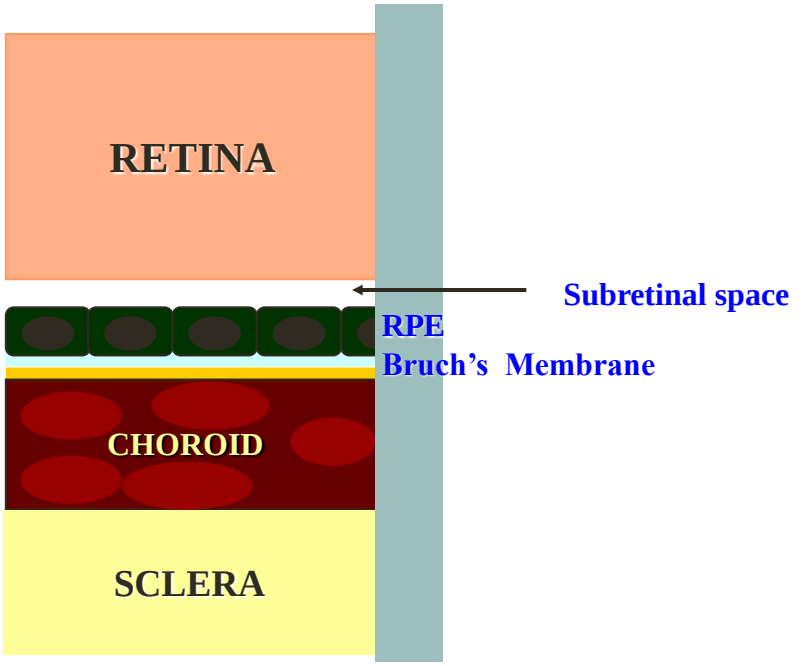
Ocular Immune Privilege

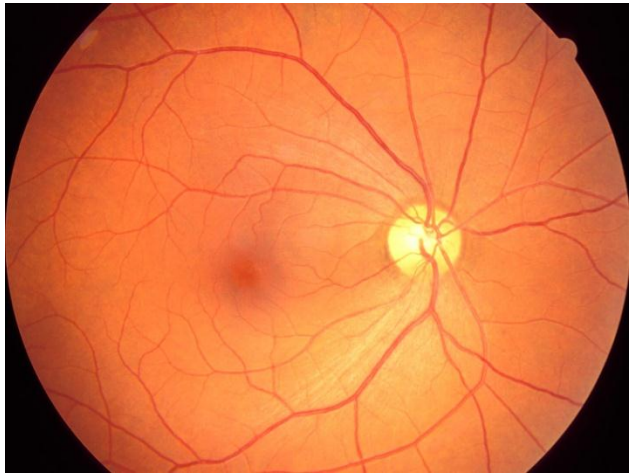
Ocular immune privilege was demonstrated by Sir Peter Medawar in 1948 – skin allografts survive when implanted into the anterior chamber of the eye.

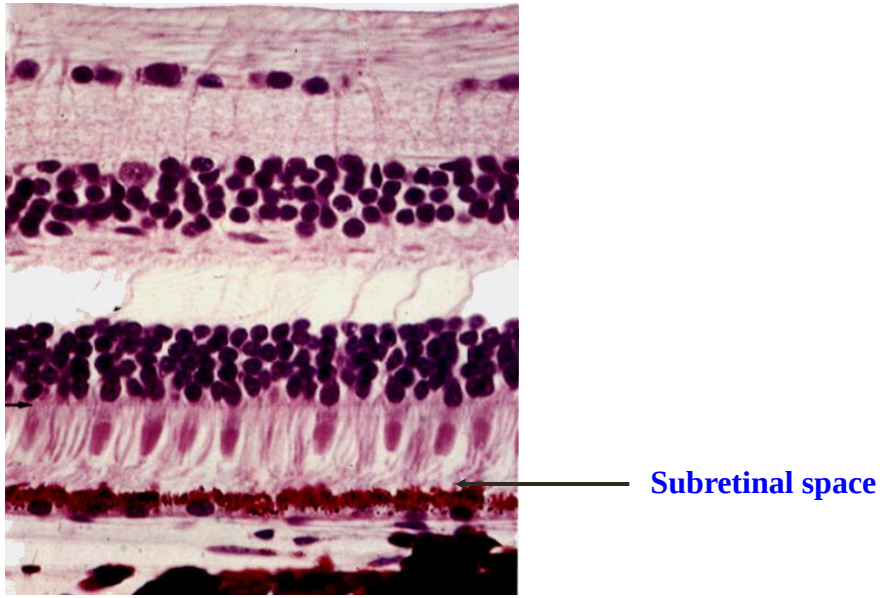
In 1993 Streilein showed that the subretinal space was also an immune privileged site for allografts.

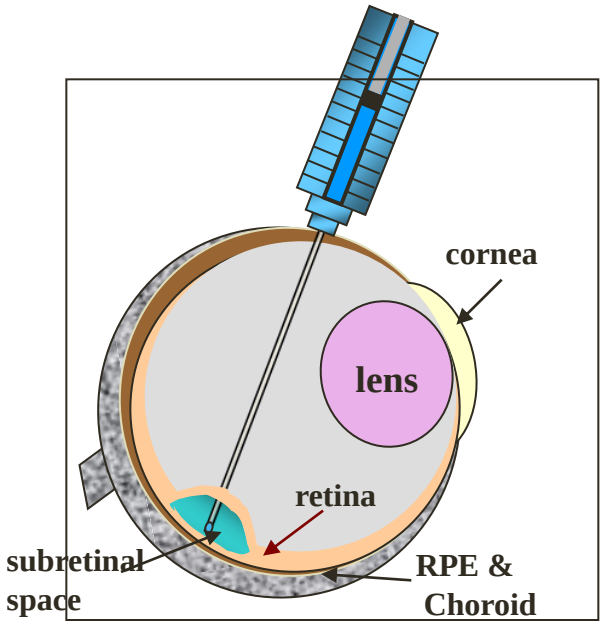
Subsequently the RPE has been shown to be a major source of immune privilege in the subretinal space and also an immune privileged tissue.

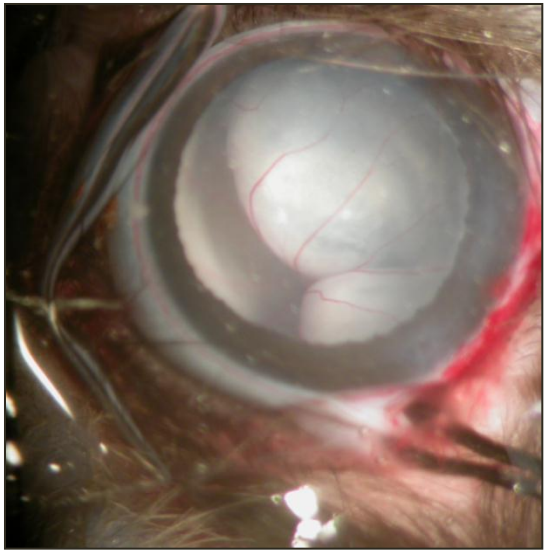

Anterior
Chamber
VITREOUS
Posterior
Chamber


RETINA
Subretinal space
RPE
Bruch's Membrane
CHOROID
SCLERA




Subretinal space





Bleb Area

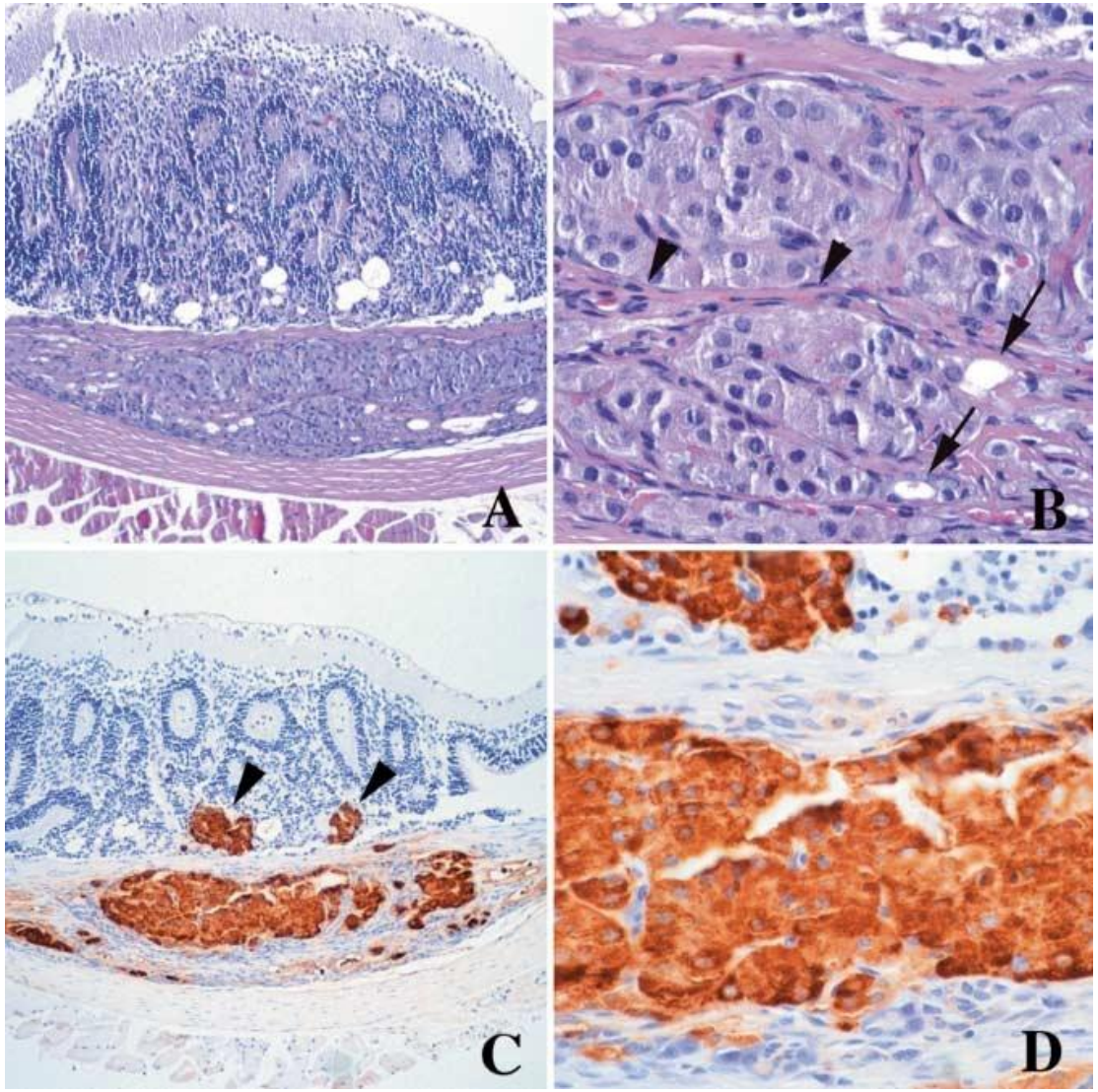
The subretinal space is a potential space in the living eye but the space may be utilized for a graft by injecting fluid between the photoreceptors and RPE to form a subretinal bleb.

Immune Privilege in Subretinal Space

15/19 pancreatic islet cell allografts survive in subretinal space of outbred SD rats – d60

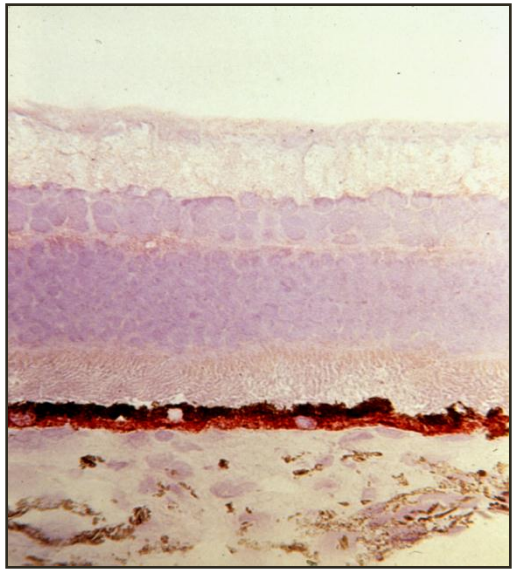
No immune suppression.

Immunoperoxidase stain for insulin.

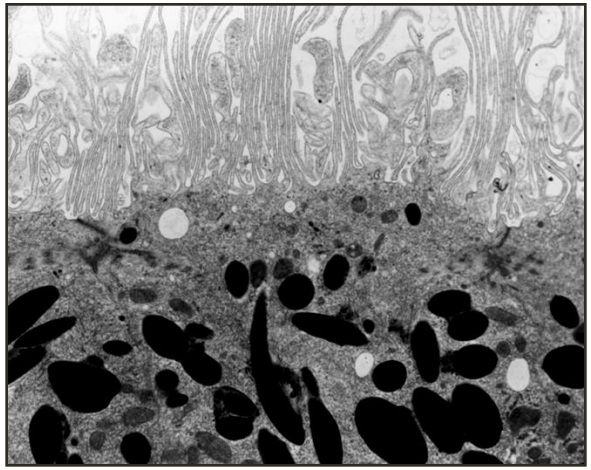


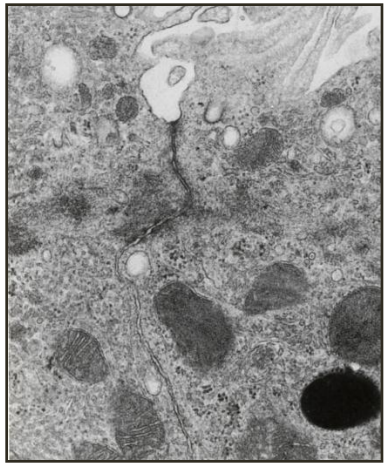
Survival of Allografted Pancreatic Islets in Subretinal Space in Rats
Makoto Inoue et. al. *Ophthalmic Research*, 2003;35:48-53

Physical Factors Promoting Immune Privilege in the Subretinal Space



Lack of lymphatics in retina/RPE (although present in choroid)



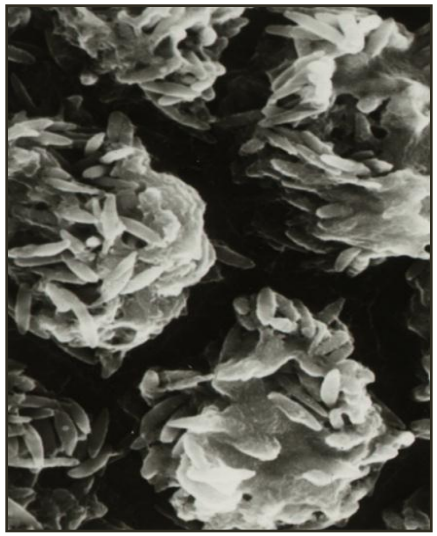


Outer blood retina barrier mediated by tight junctions between RPE

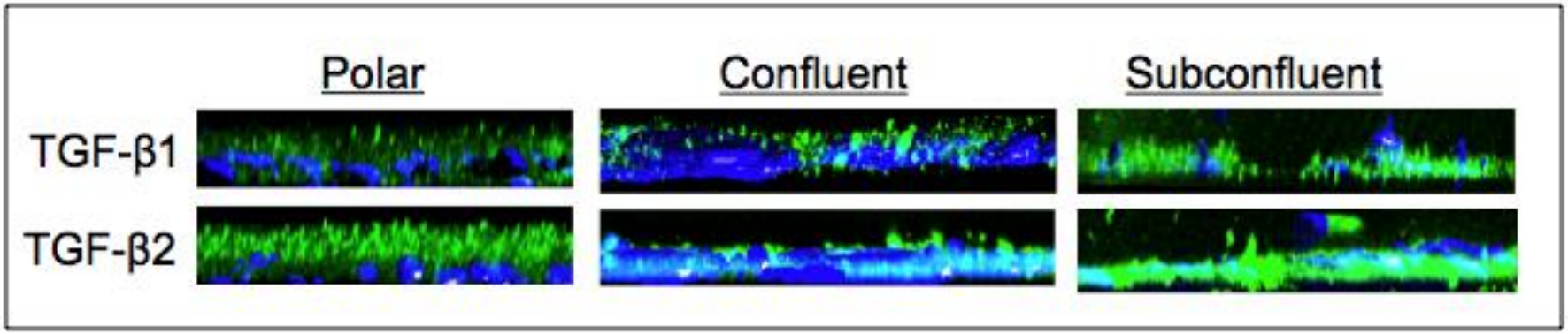
RPE express anti-inflammatory factors that promote immune privilege

Soluble Factors:
TGF-beta
Pigment Epithelium Derived Factor
AlphaB Crystallin
Soluble TNF-R

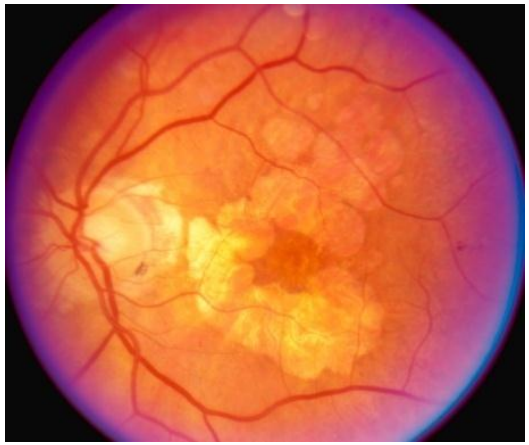
Cell Surface:
TGF-beta
CD95 (FAS) ligand
CD59
CD46



Apical microvilli of RPE projecting into subretinal space



Age-Related Macular Degeneration



Atrophic AMD

Geographic Atrophy

Atrophic AMD is characterized by dysfunction and death of RPE in the macula. Confluent areas of RPE loss are termed geographic atrophy.

There is no current effective therapy for atrophic AMD.

HESC represent of source of RPE for cellular therapy of AMD

A clinical trial for hESC-RPE cell suspensions for advanced dry AMD is ongoing (NCT01344993; Advanced Cell Technology)

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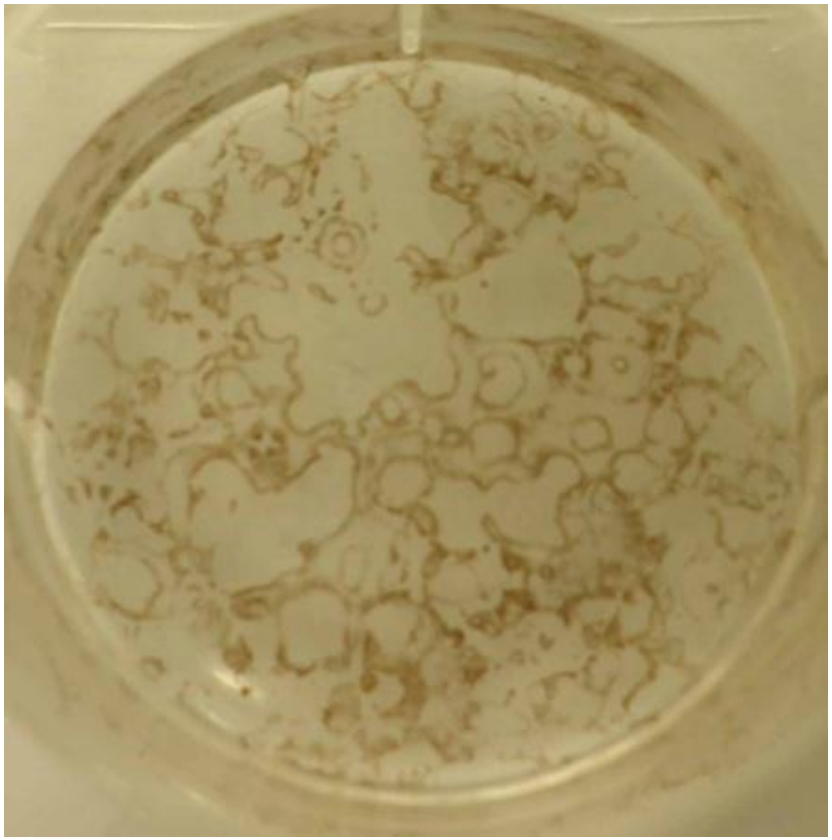
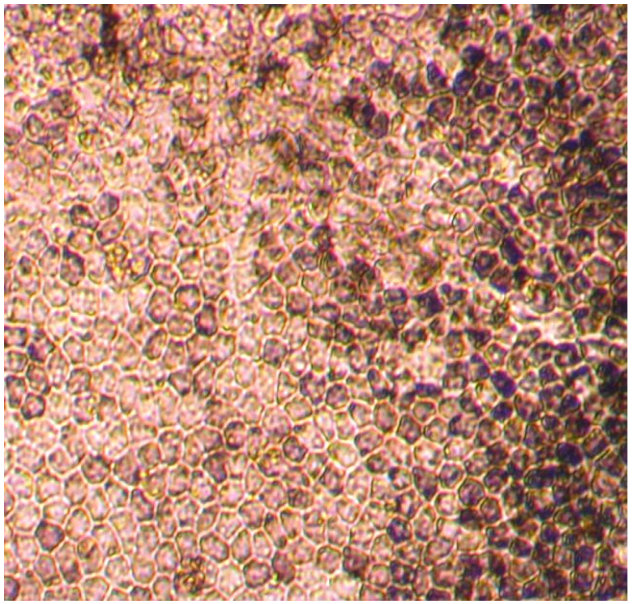
hESC spontaneously differentiate into pigmented RPE in culture

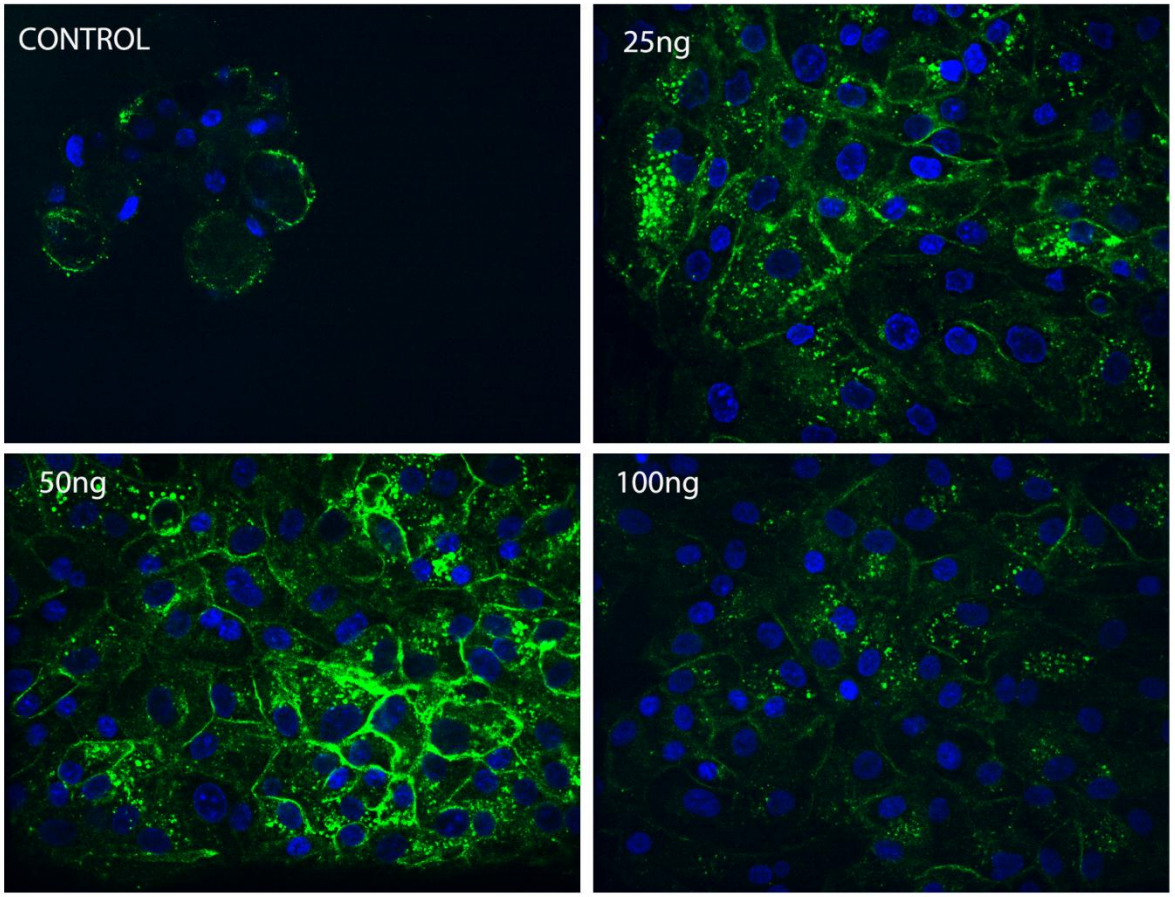




Courtesy of Dennis Clegg, UCSB

Pigmented colonies can be picked after 6-8 weeks in culture and can be expanded to pure cultures of RPE

HLA Class I Expression on HESC-RPE after treatment with Interferon-γ



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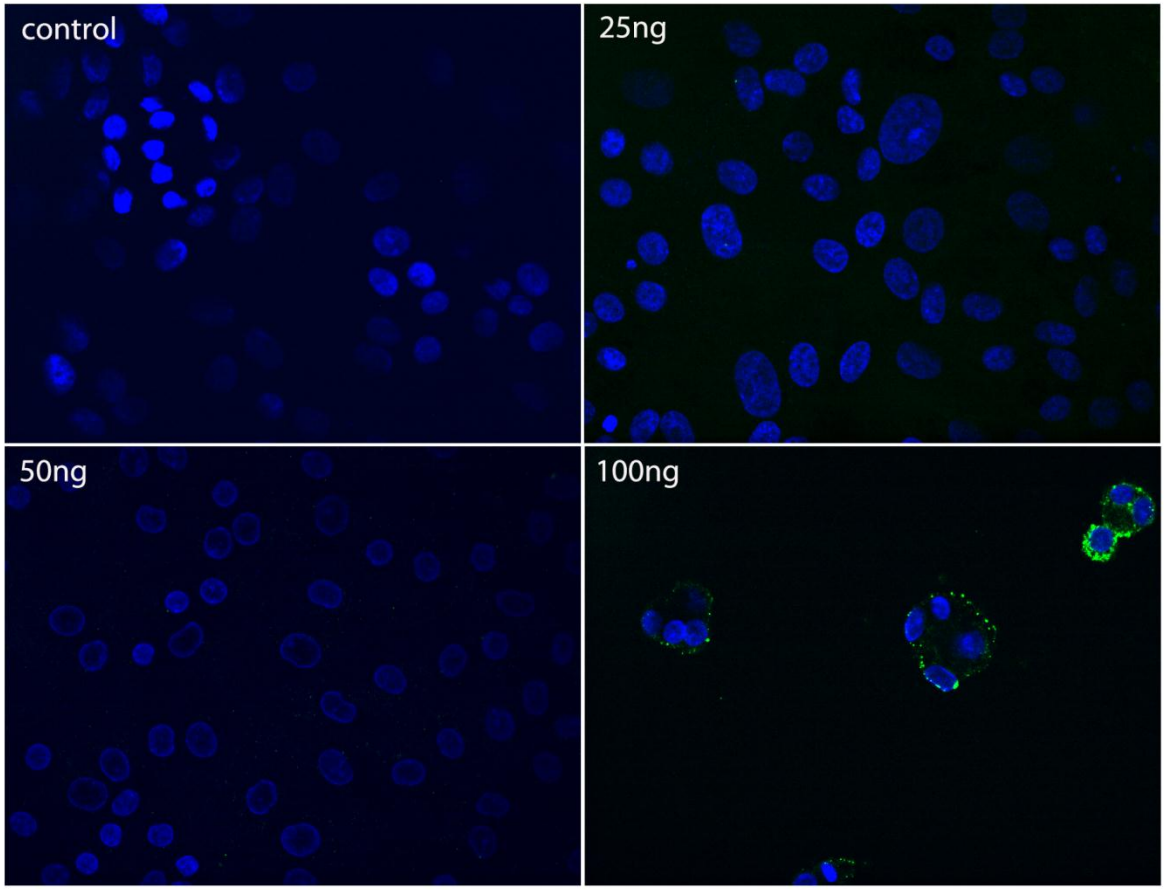
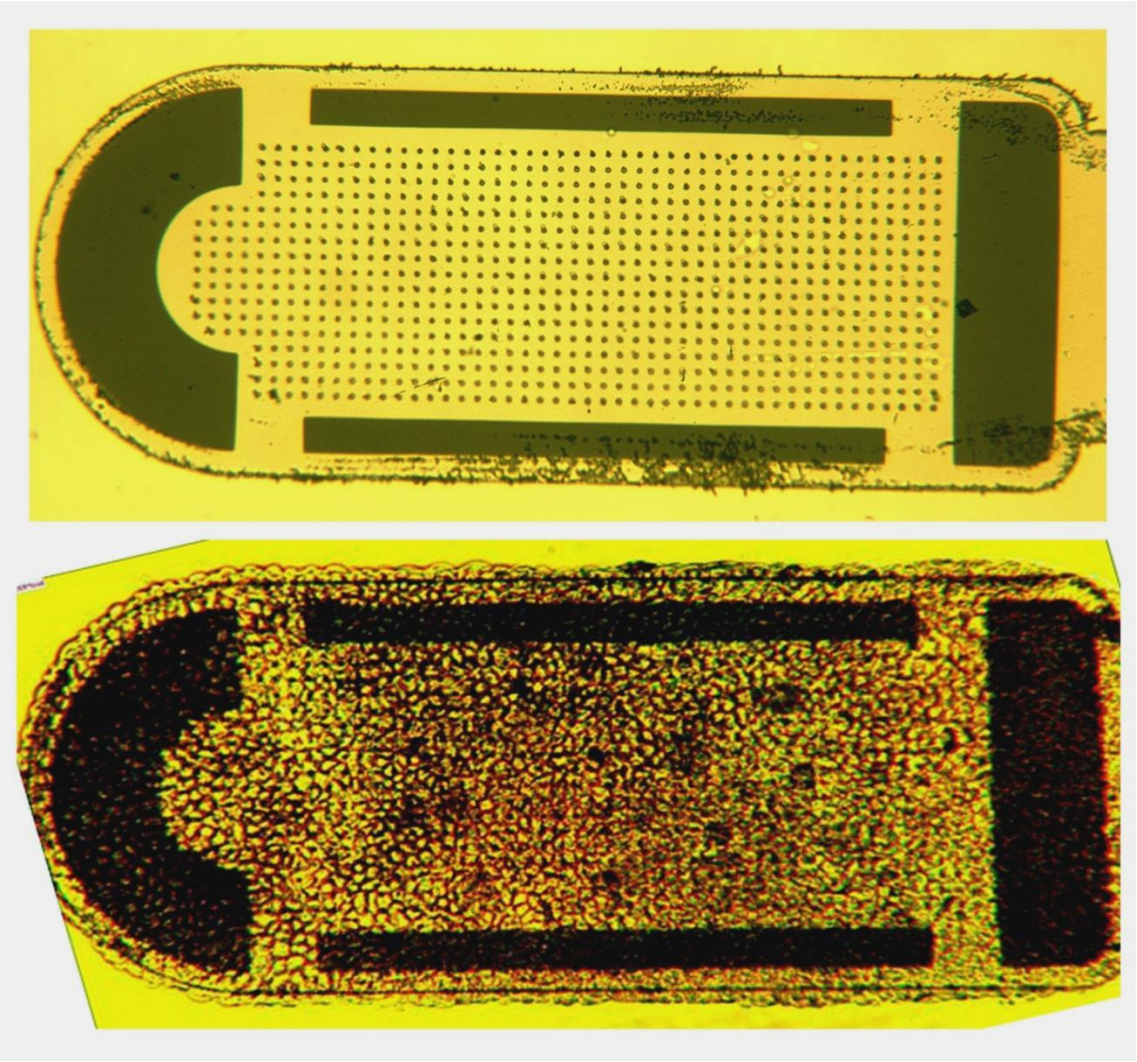
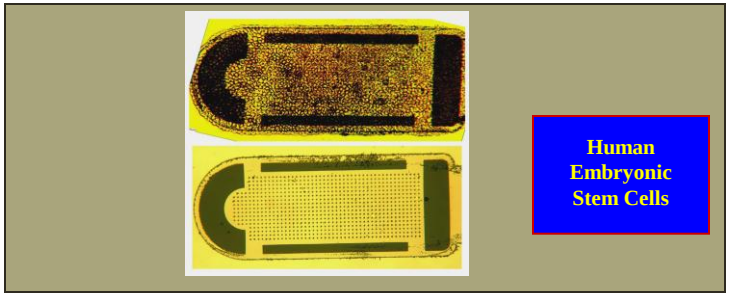


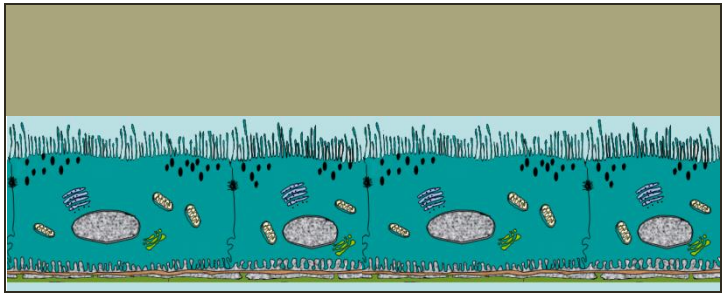
Our Approach: HESC (H9)-Derived RPE are Grown as a polarized monolayer on Non-Biodegradable Parylene Inserts

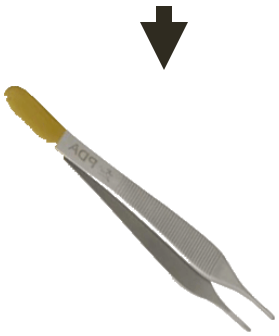
Insert with no cells

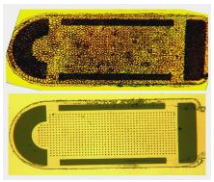
Insert with H9-derived RPE 4 weeks after seeding

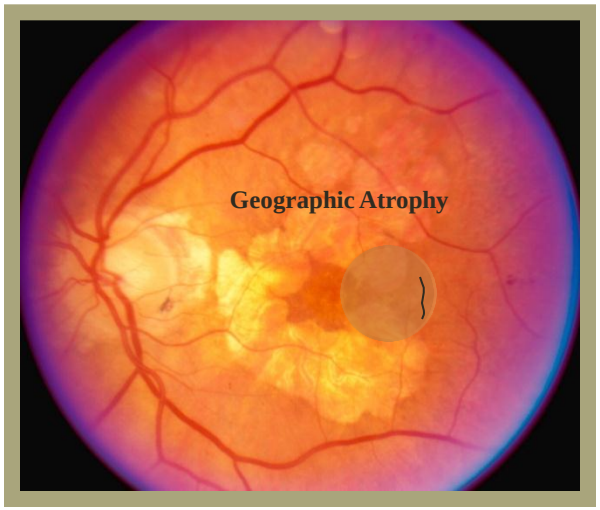


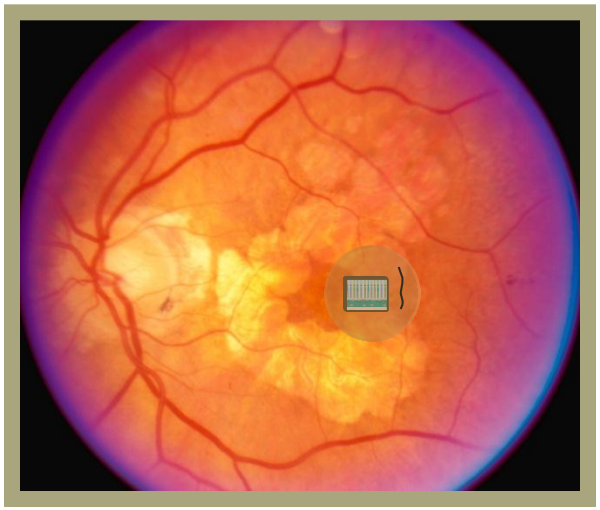

Human
Embryonic
Stem Cells





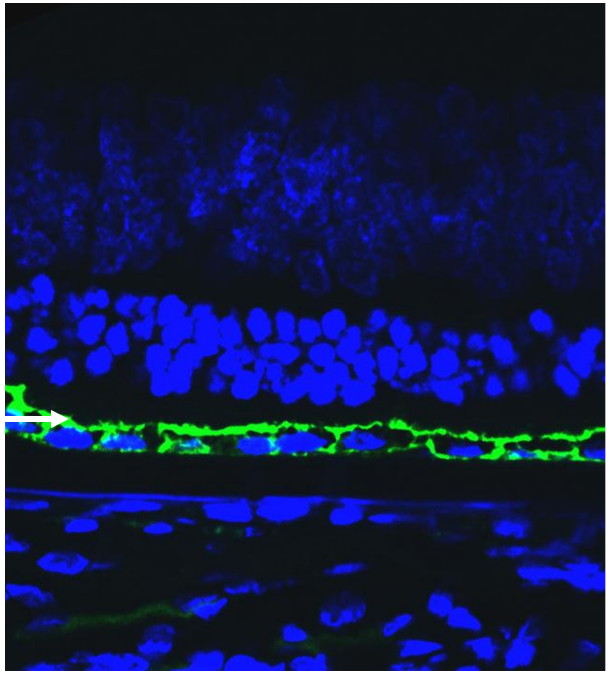


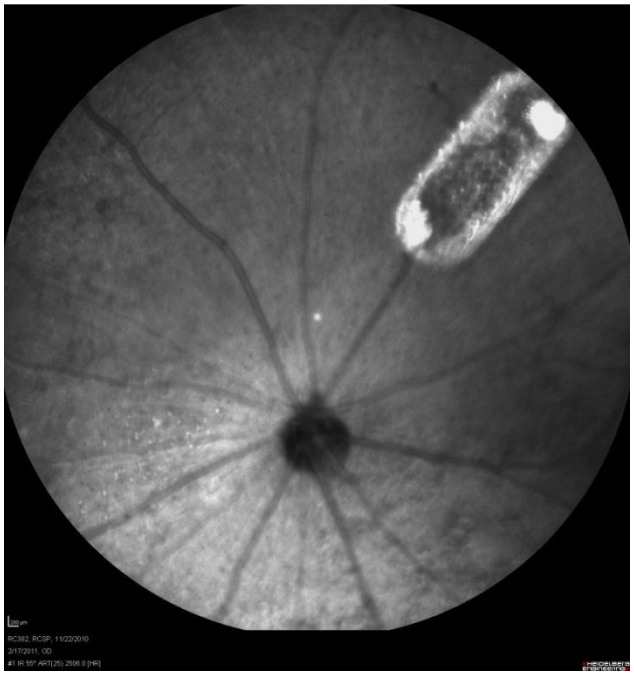

Geographic Atrophy



hESC-RPE implanted into the subretinal space of retinal degeneration (RCS) rats survive as an intact monolayer.

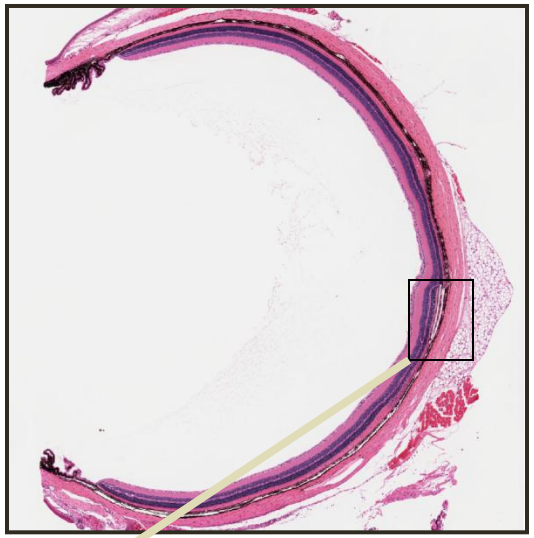
TRA-1-85 human specific Ab labelling

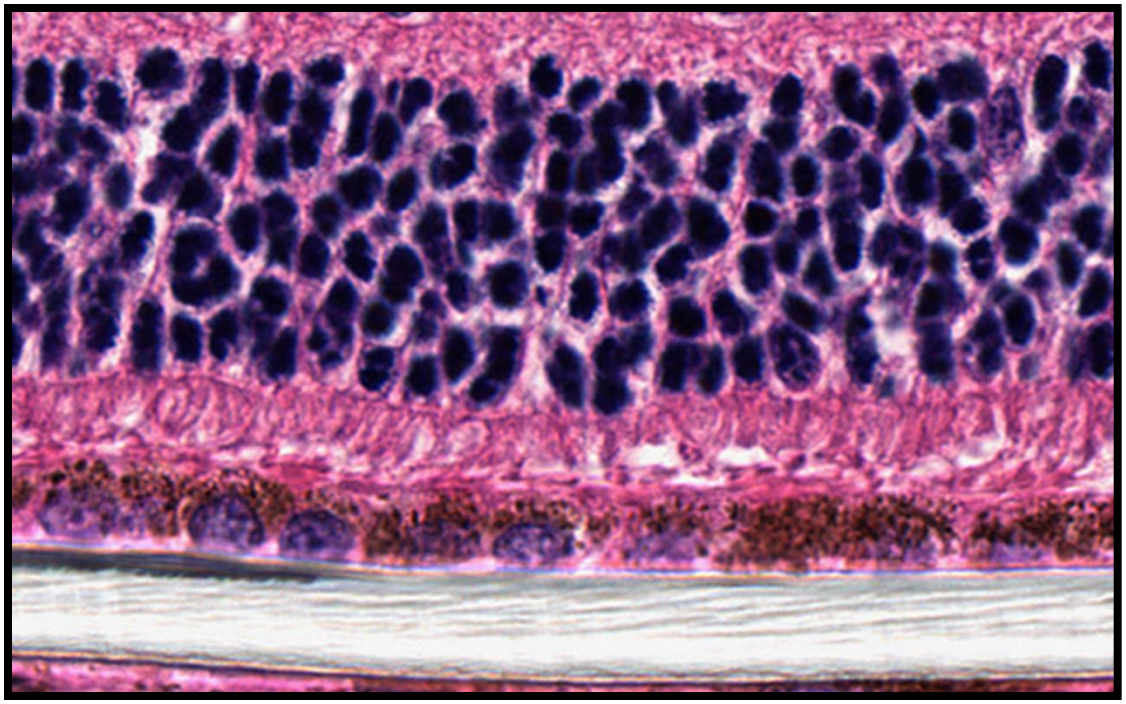




The placement of the implant in the subretinal space without damage to Bruch's membrane and choroid is essential for survival of grafted cells. Grafts with perfect placement may survive without immunosuppression. Current protocols include systemic immunosuppression with cyclosporin and short term therapy with systemic dexamethasone .

hESC-RPE implanted into the subretinal space of RCS rats survive without an inflammatory response, and integrate into the retina where they rescue host photoreceptors.





ONL

IS

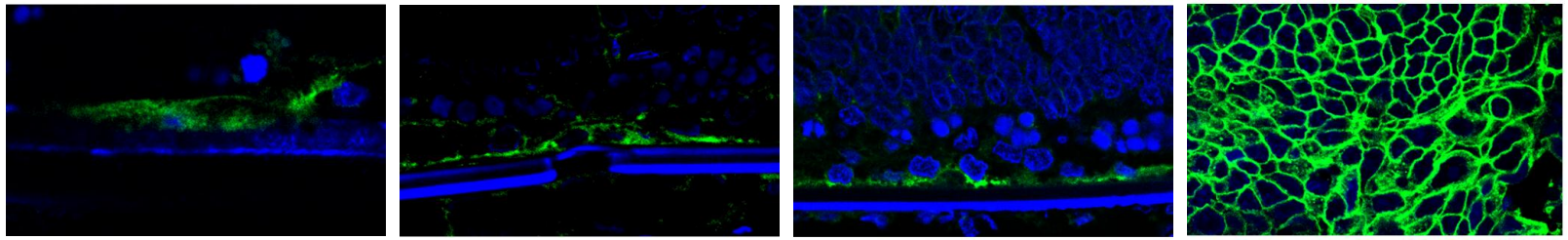
OS

hESC RPE

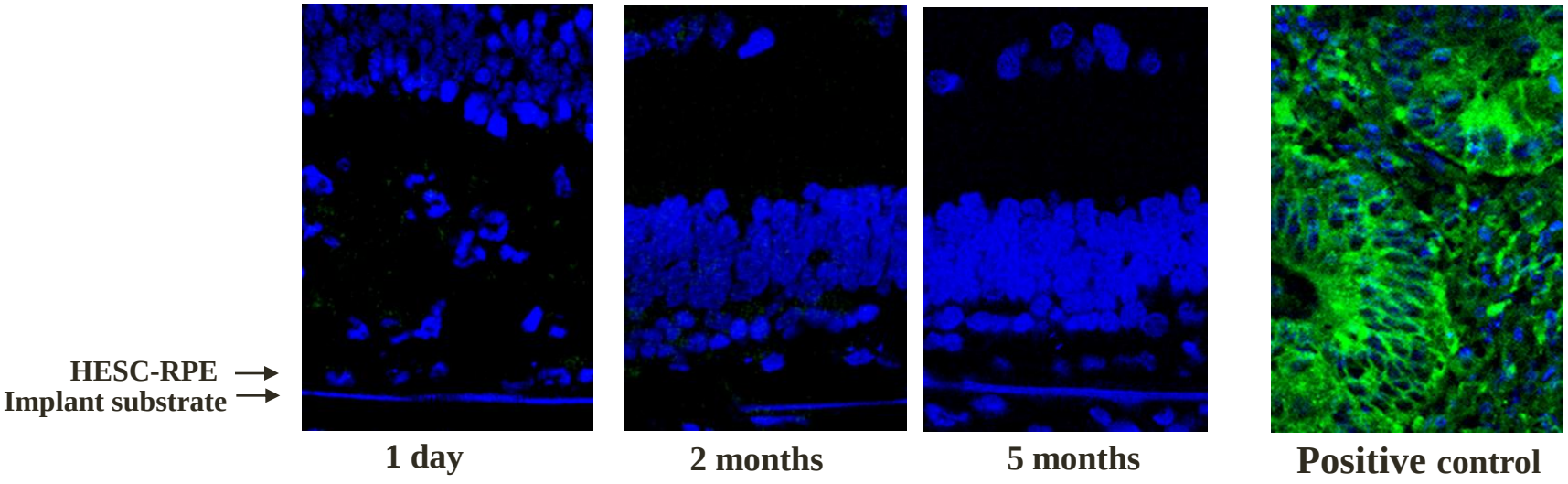
parylene

HLA Expression after Subretinal Implant of HESC-RPE into Rat

Class I



Class II



Current Strategies for Immune Suppression in hESC-RPE cell Therapy

1. **ACT Trial :** NCT01344993

Short term systemic immunosuppression with tacrolimus beginning prior to treatment with mycophenolate mofetil (MMF) added and continued (personal communication with ACT)

2. **London Project to Cure Blindness:**

Systemic prednisolone (oral) beginning 1 week prior to treatment (taper over one month) with local triamcinolone (intravitreal and scleral) for 3-6 months coverage (personal communication with Pete Coffey).

Current Strategies for Immune Suppression in hESC-RPE cell Therapy

3. **Other possibilities**:

   Local immunosuppression with Ozurdex (Allergan Inc.) dexamethasone drug delivery system. This biodegradable intravitreal implant provides sustained delivery to the retina and vitreous and is approved by the FDA for treatment of macular edema associated with retinal vein occlusion and noninfectious posterior uveitis.

Major Considerations:

1. Local vs systemic immunosuppression
2. Timing and length of immunosuppression
3. Methods to detect rejection of the transplanted cells

The California Project to Cure Blindness

- USC Doheny Eye Institute (Mark Humayun, PI; David Hinton Co-PI; Vas Sadda, Biju Thomas, Martin Pera)
- UCSB Macular Degeneration and Stem Cell Centers (Dennis Clegg, Co-PI; Lincoln Johnson)
- UCL London Project to Cure Blindness (Pete Coffey, Partner PI funded by MRC)
- Caltech Biology and Chemistry (Scott Fraser, Bob Grubbs, Yu-Chong Tai)
- City of Hope Center for Biomedicine and Genetics GMP Facility (Larry Couture)
- Geron Corporation (JaneLebkowski)

FUNDING: CIRM Disease Team Grant