Miltos Balidis MD,PhD,FEBOphth, President of SECRS

INTRODUCING NEW CONCEPTS IN CORNEAL SURGERY

Cairo 2019

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- *Αλκμαίων*
- *Εμπεδοκλής*
- *Ηρόφιλος*
- *Ερασίστρατος*

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Vision

- Obstacles are those frightful things you see when you take your eyes off the goal."

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» *Hannah More*

Stromal Opacity

Replacing with synthetic optical elements / keratoprosthesis

Regeneration of stromal transparency with stem cell remodeling

MSC,ESC,iPSC

Replacement with Biomimetic or bioengineered material

Acellular or cellularized scaffold, Acornea

Replacing stroma sparing the endothelium

DALK , FALK

Stromal multipotent stem cells in the limbus

Stromal stem cells next to basal epithelial cells

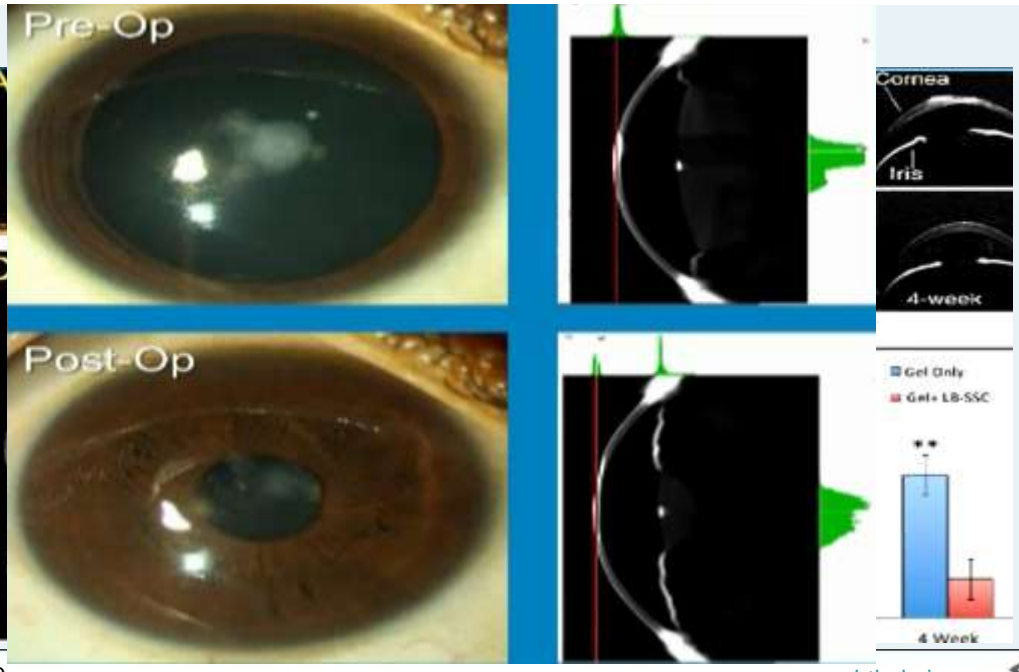
Limbal biopsies contain Stromal Stem Cells

Stromal stem cells differentiate into keratocytes and remodel disorganized stroma

Remodeling of stromal scars by keratocytes, restores transparency

Stromal stem cells

1. Regenerate corneal stroma
2. Remodel activate corneal wounds
3. Differentiate into keratocytes
4. Prevent and reverse scarring




Restoration of Corneal Transparency by Mesenchymal Stem Cells

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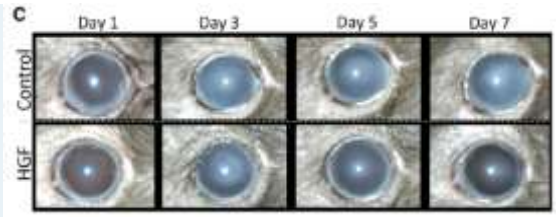
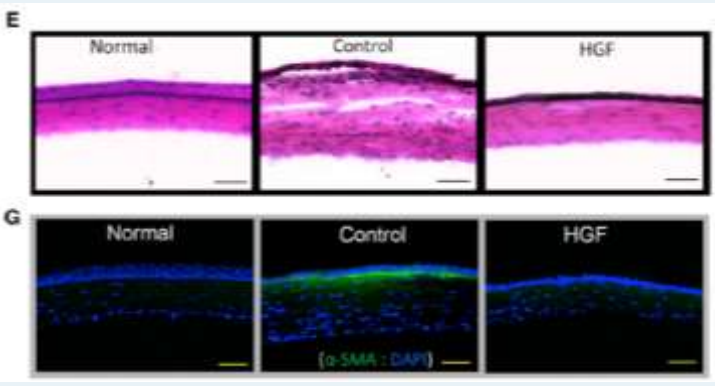
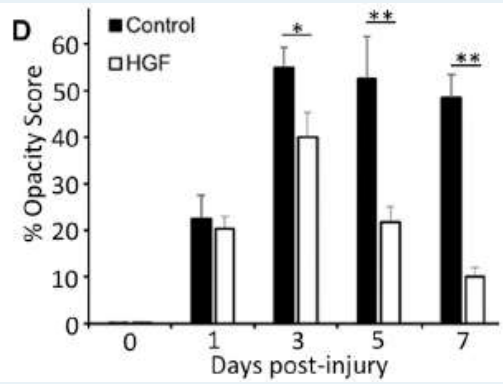
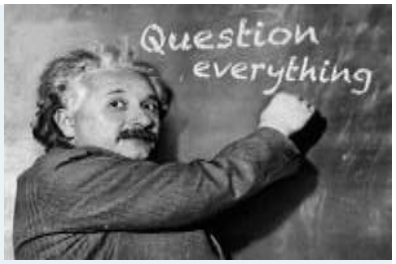
Topical Hepatic Growth Factor alone is Sufficient to restore corneal transparencyin Ocular Injury







Pluripotent stem cell (iPS) transplantation



- Are the cells identical to the original cells?
- How long do they remain effective
- Long term potential side effects?
  - Oxidative stress associated with reprogramming?
- Do we still need retroviruses?

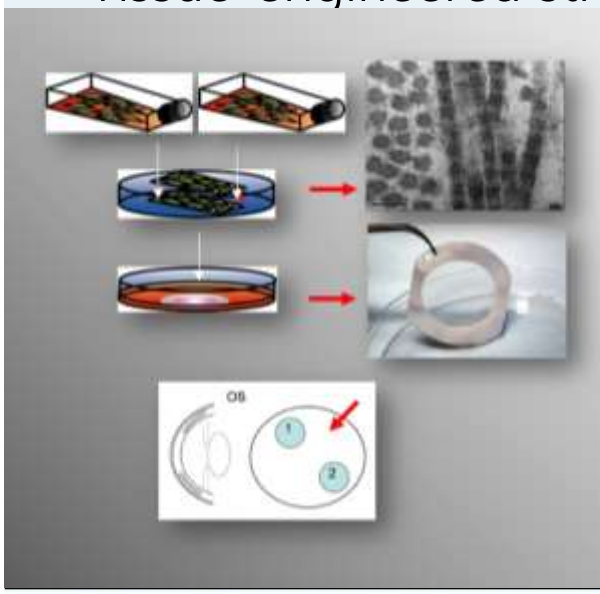
Hotspots of De Novo Point Mutations in Induced Pluripotent Stem Cells Yoshihara, M et al , Cell Reports, vol. 21, no. 2, pp. 308–315, 2017

Stromal Opacity

Stromal substitutes

- Acellular inert material impearmeable to cells
- Acellular biomaterial as scaffold
- Biomaterial enhanced with cells at production
- Stromal substitutes entirely engineered from cells

Tissue-engineered stromal substitute



LOEX TE-Self-assembled stroma

- ✓ Dispase digestion + removal of endo and epithelium
- ✓ Keratocyte isolation (feline or human)
- ✓ Fibroblast culture medium:
  - DMEM
  - 10% fetal calf serum
  - 25 µg/mL gentamycin; 100 IU/mL pen G
  - Seeded: 50 µg/mL ascorbic acid
  - 28 days (feline) or 35 days (human)
  - Thick sheets stacked (6 layers)
- ✓ Secreted their own ECM
- ✓ Multilayered structure of self-assembled ECM layers
- ✓ Implant entirely TE prior to transplantation
- ✓ *In vivo* transplantation in feline model
  - 8 animals (16 grafts; diam 4 mm)
  - 1 eye/animal
  - Follow up: 4 months

2014
2013
2014

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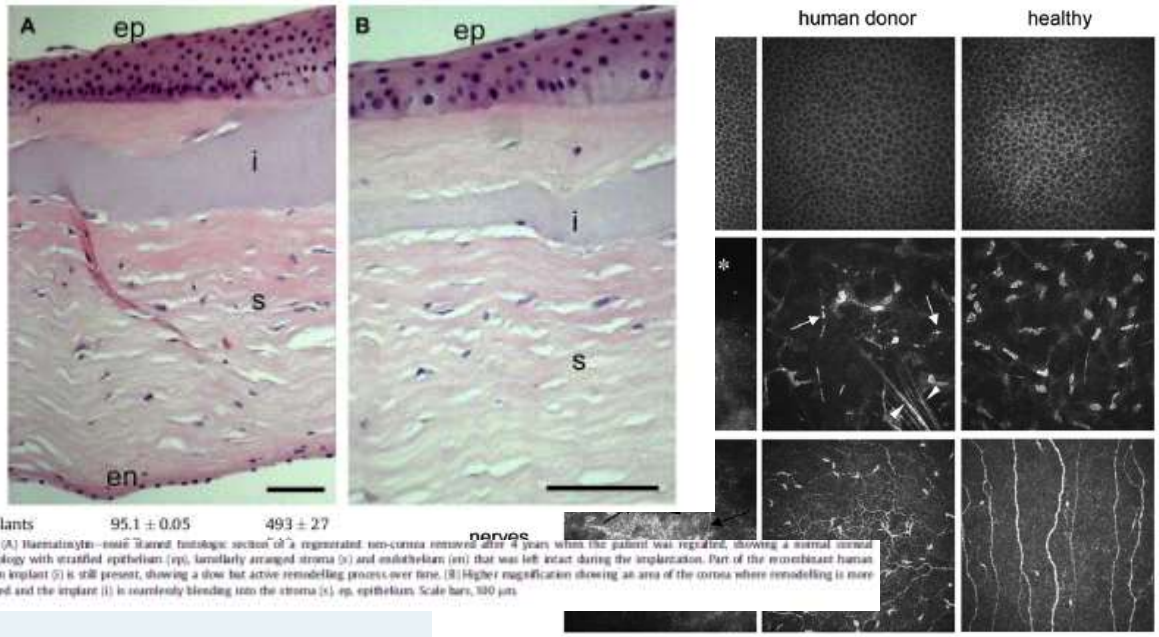

Implants	95.1 ± 0.05	493 ± 27

nerves

Fig. 5. (A) Haematoxylin–eosin stained histologic section of a regenerated non-cornea removed after 4 years when the patient was regrafted, showing a normal corneal morphology with stratified epithelium (ep), lamellarly arranged stroma (s) and endothelium (en) that was left intact during the implantation. Part of the recombinant human collagen implant (i) is still present, showing a slow but active remodelling process over time. (B) Higher magnification showing an area of the cornea where remodelling is more advanced and the implant (i) is seamlessly blending into the stroma (s). ep, epithelium. Scale bars, 100 µm.

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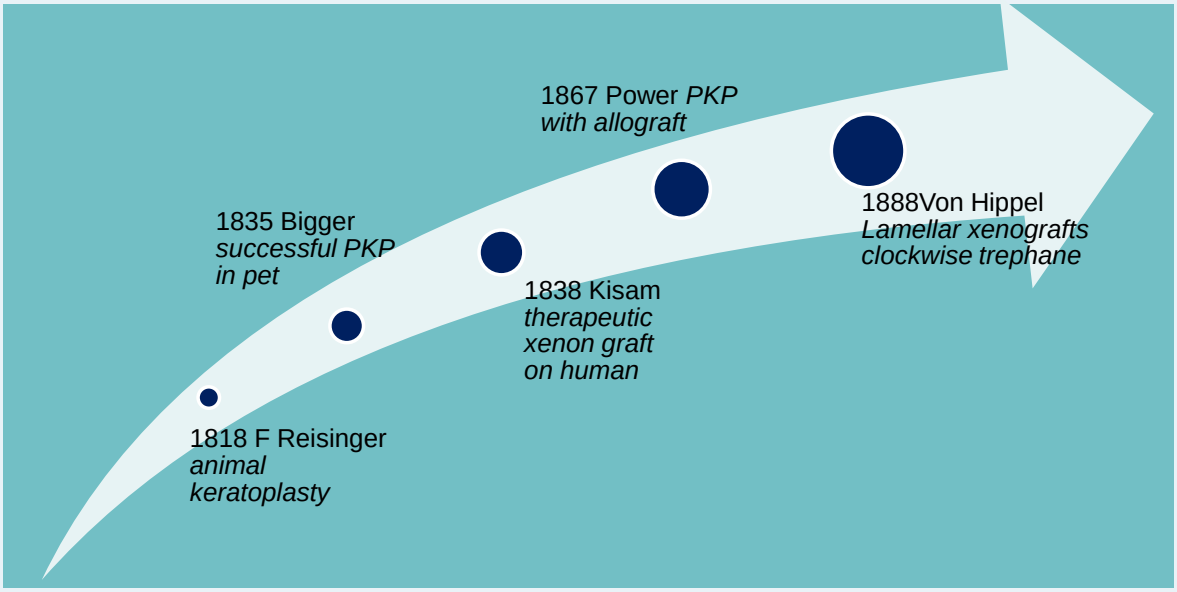
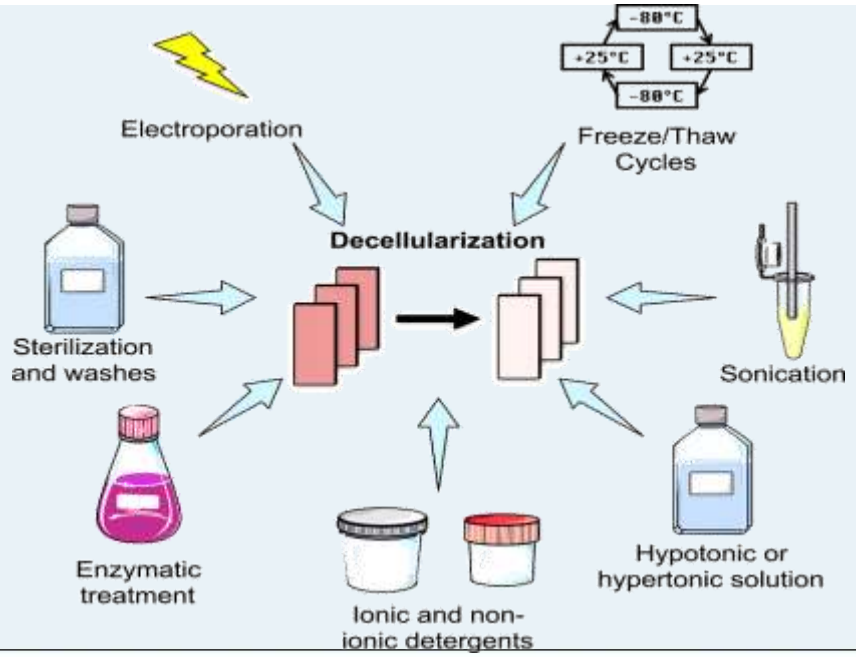
TE cornea

- Minimum risk of rejection
- Minimum need for post-surgical drugs
- Better biocompatibility and integration than the synthetic corneas permanent corneas,
- Cost-effective
- Readily available to patients.



Why DC

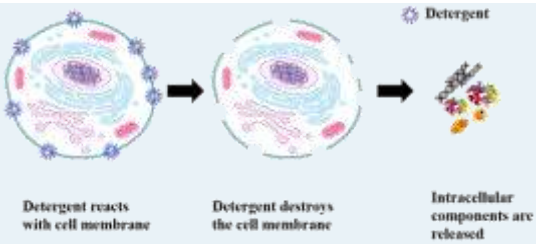
- DCs are potentially advantageous in comparison to TE corneal equivalents and KPros
  - the matrix has the ultrastructure of the native tissue.
  - Inherent biological signals may remain within the matrix, important for the maintenance of specific cellular functions and phenotype



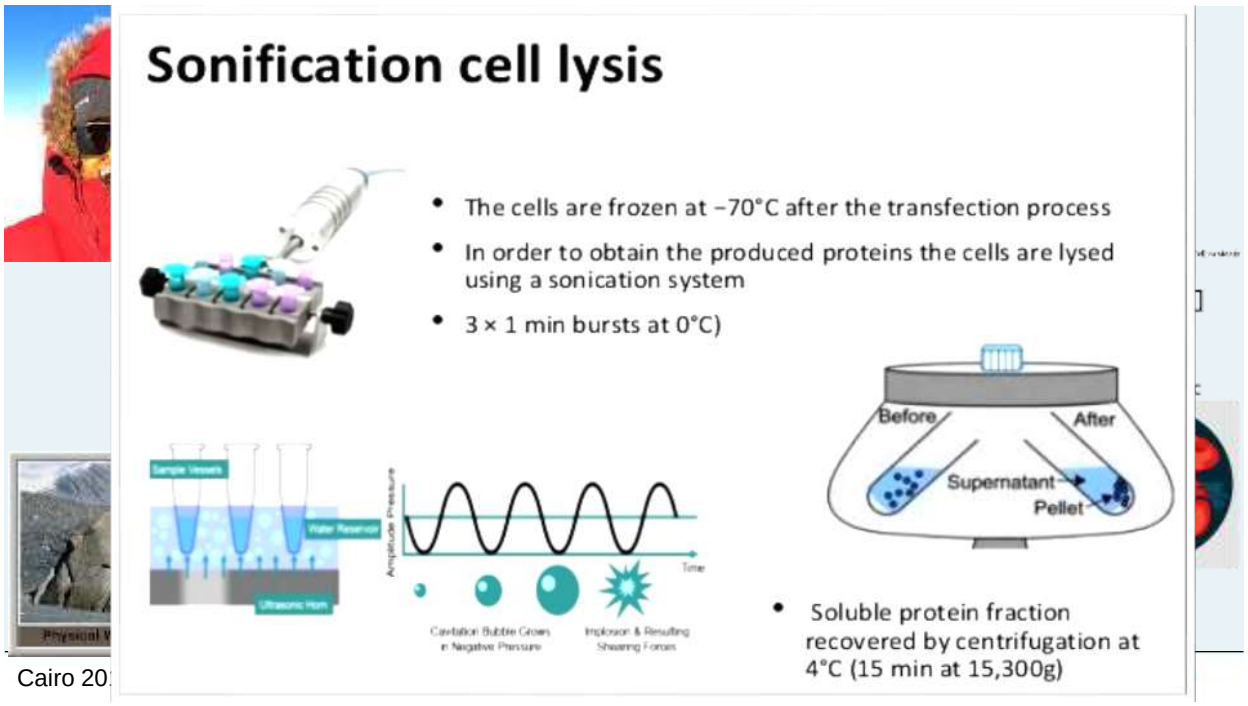
Enzymes

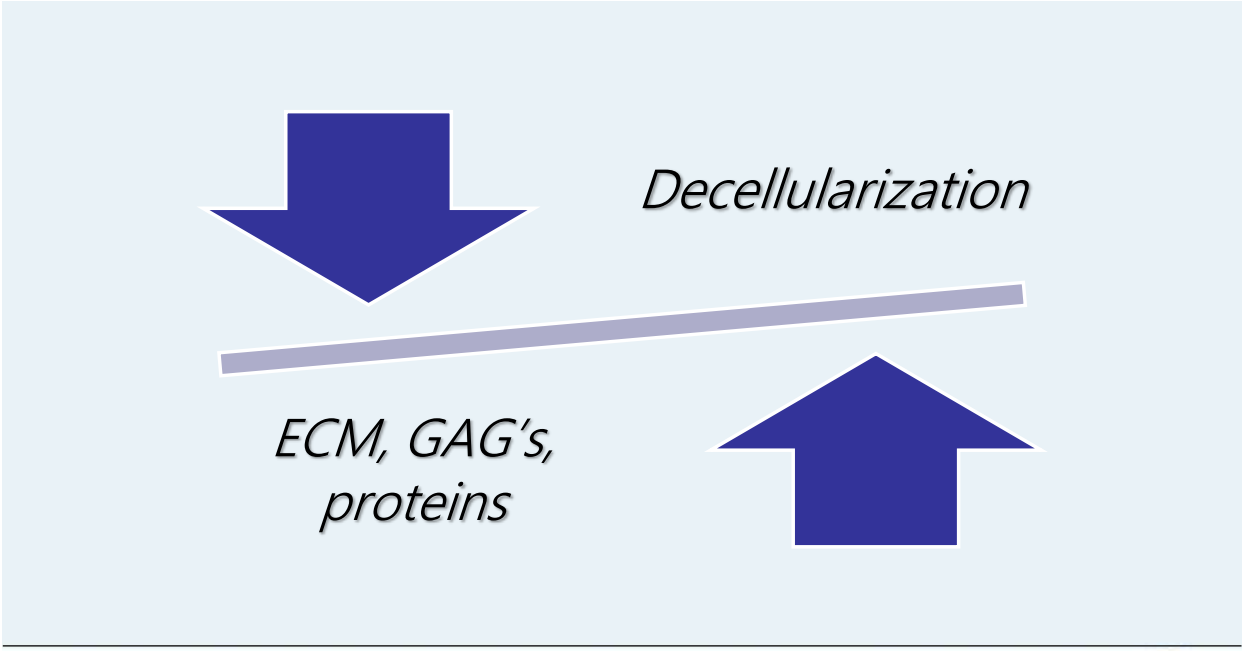
- Trypsin
- Dispase
- Phospholipidases A_2-PLA_2
- Nucleases
- Sera
- EDTA

Chemicals

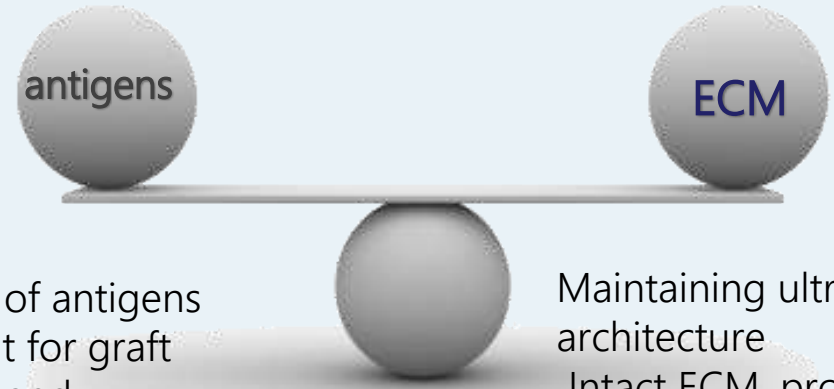


- Alcohols
- Acid-Alcali
- Ionic Detergents
  - *SDS*
  - *SD*
- Non ionic Detergents
  - *Triton X*


Sonification cell lysis
The cells are frozen at −70°C after the transfection process
In order to obtain the produced proteins the cells are lysed using a sonication system
3 × 1 min bursts at 0°C)
Before
After
Supernatant
Pellet
Soluble protein fraction recovered by centrifugation at 4°C (15 min at 15,300g)

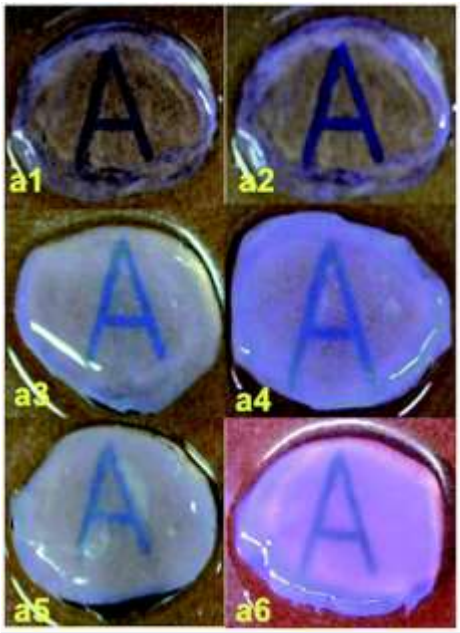

Decellularization
ECM, GAG's, proteins

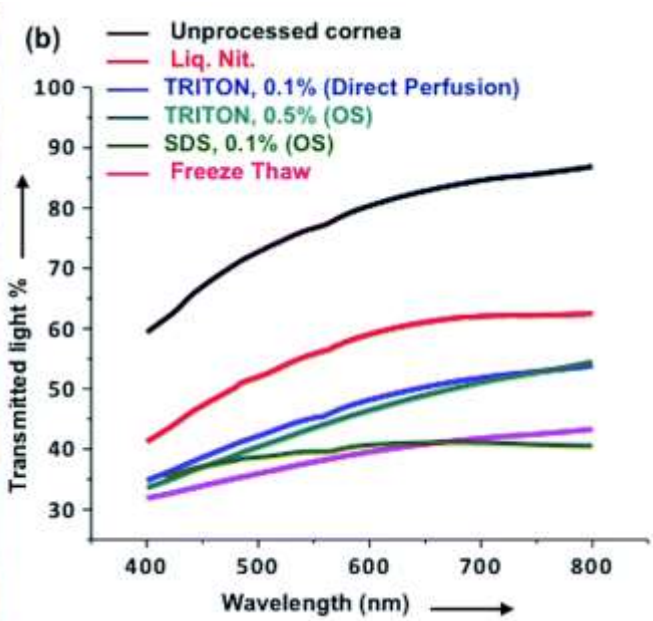
Get the balance right

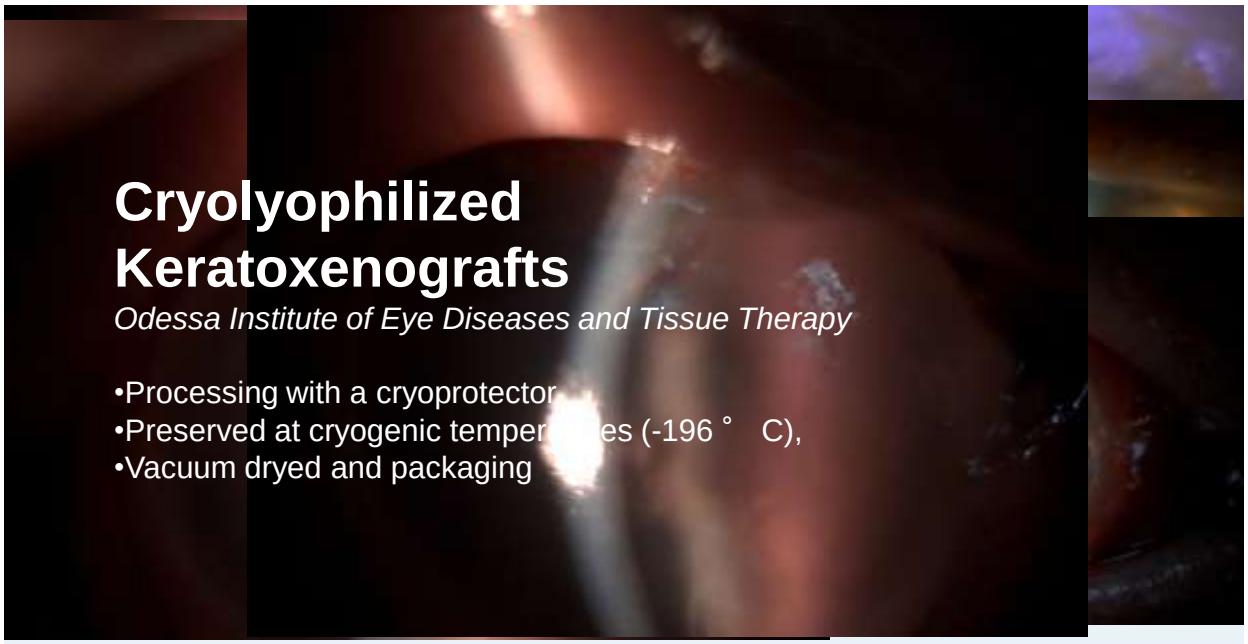


Removal of antigens important for graft rejection and inflammation

Maintaining ultrastructural architecture
Intact ECM, proteins, GAG's, and biological signals for keratocytes

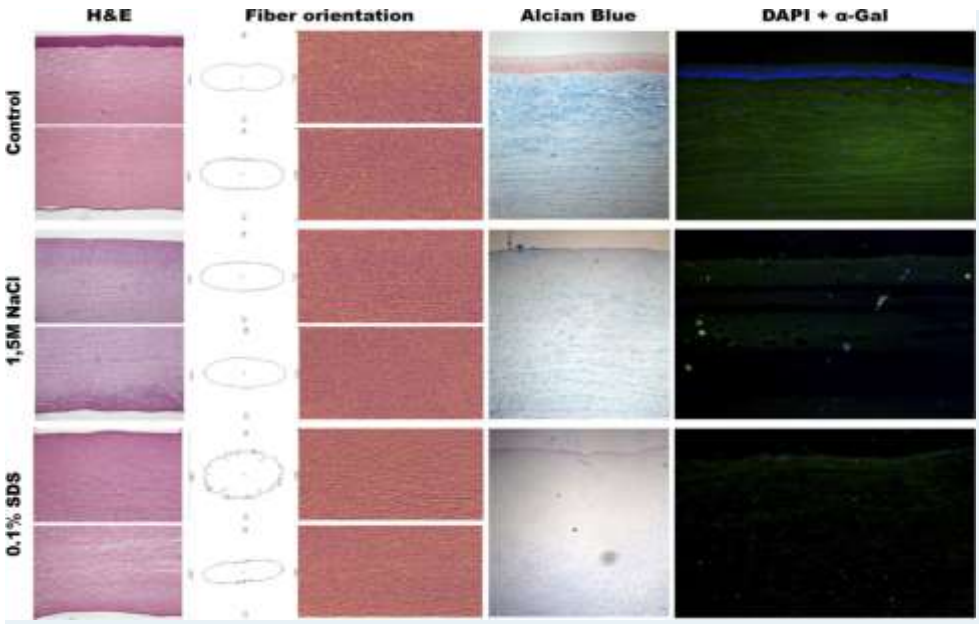






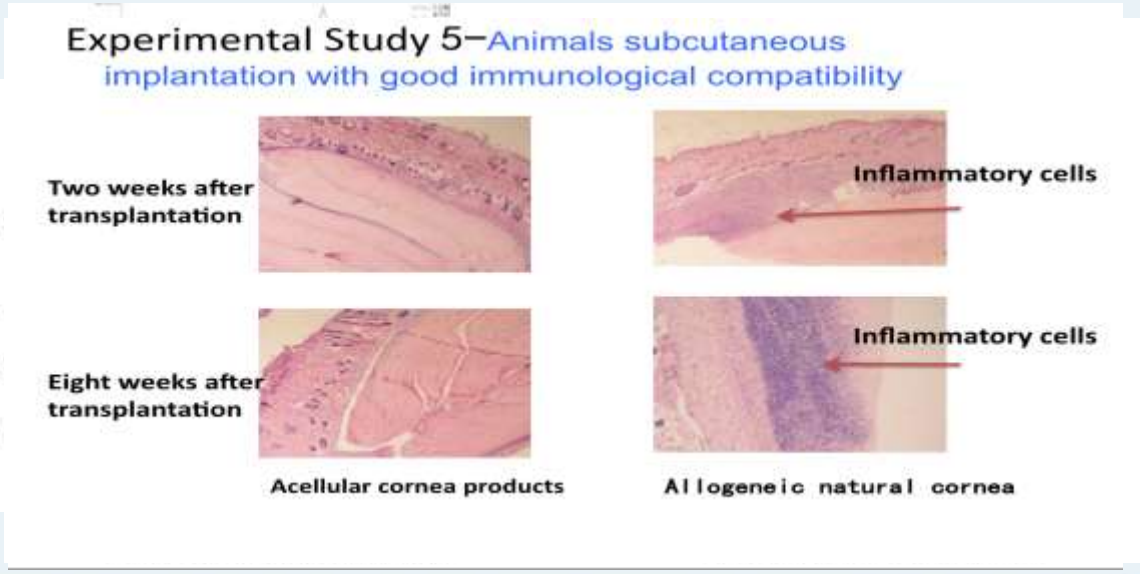
Acornea

- The Acornea is a heterogeneous cornea.
- Stripped of cells, hybrid proteins and other antigens, maintaining its natural collagen structure.
- Able to integrate with the surrounding tissue and foster the regeneration of its own cells.



Analysis of NPC (top row) and corneas decellularized with 1.5 M NaCl (middle row) and 0.1% SDS (bottom row). First column: H&Eof anterior and posterior corneas; second column: fiber orientation analysis with polar plot images; third column: azimuthal images; fourth column: Alcian blue staining of the different samples; fifth column: fluorescence immunohistochemistry against α-gal epitope.

Generation of Bioengineered Corneas with Decellularized Xenografts and Human KeratocytesInvest. Ophthalmol. Vis. Sci.. 2011;52(1):215-222. doi:10.1167/iovs.09-4773



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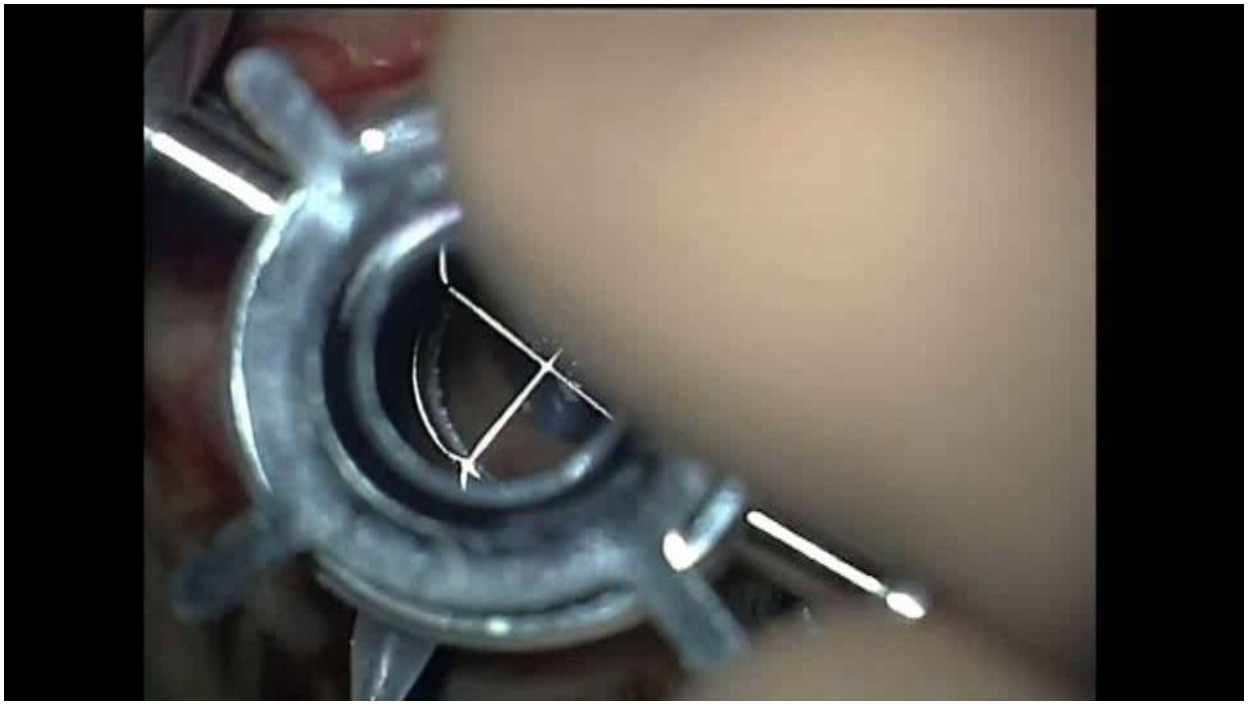

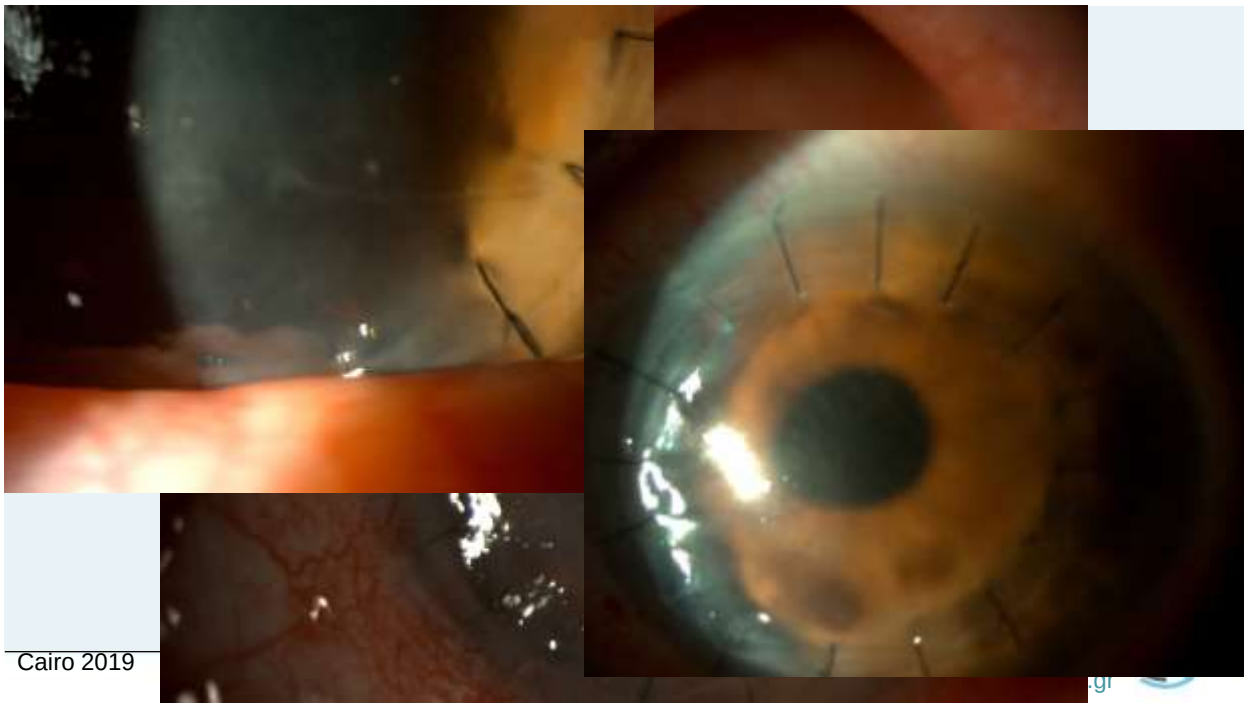

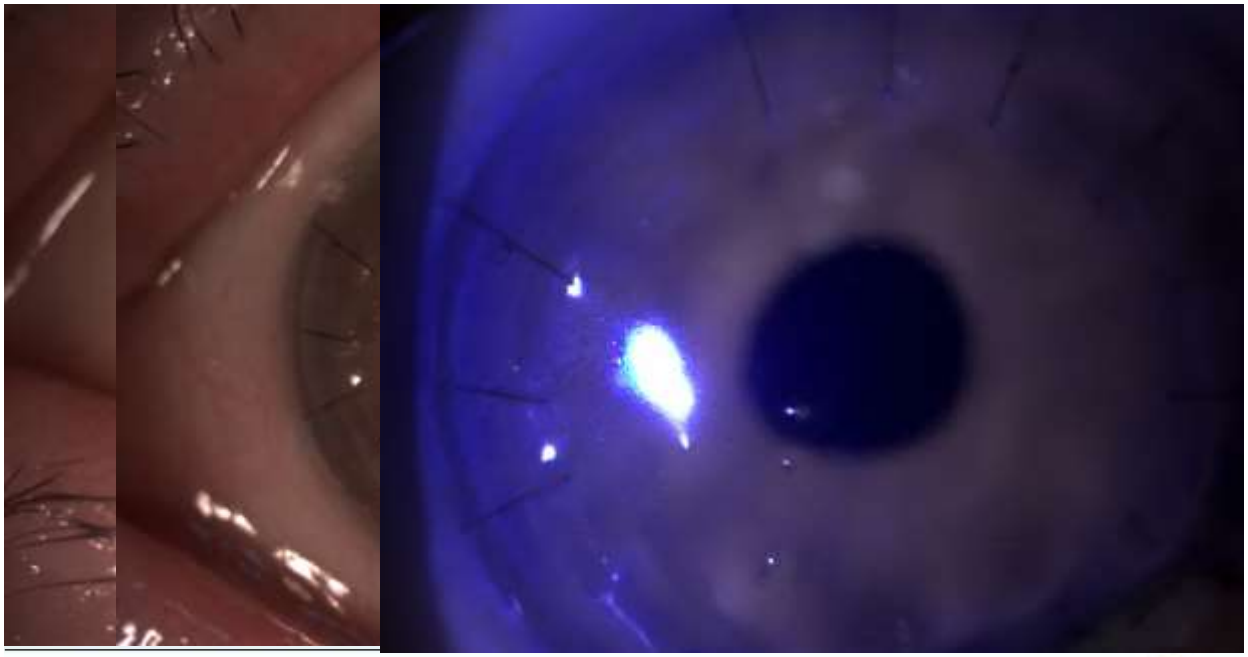

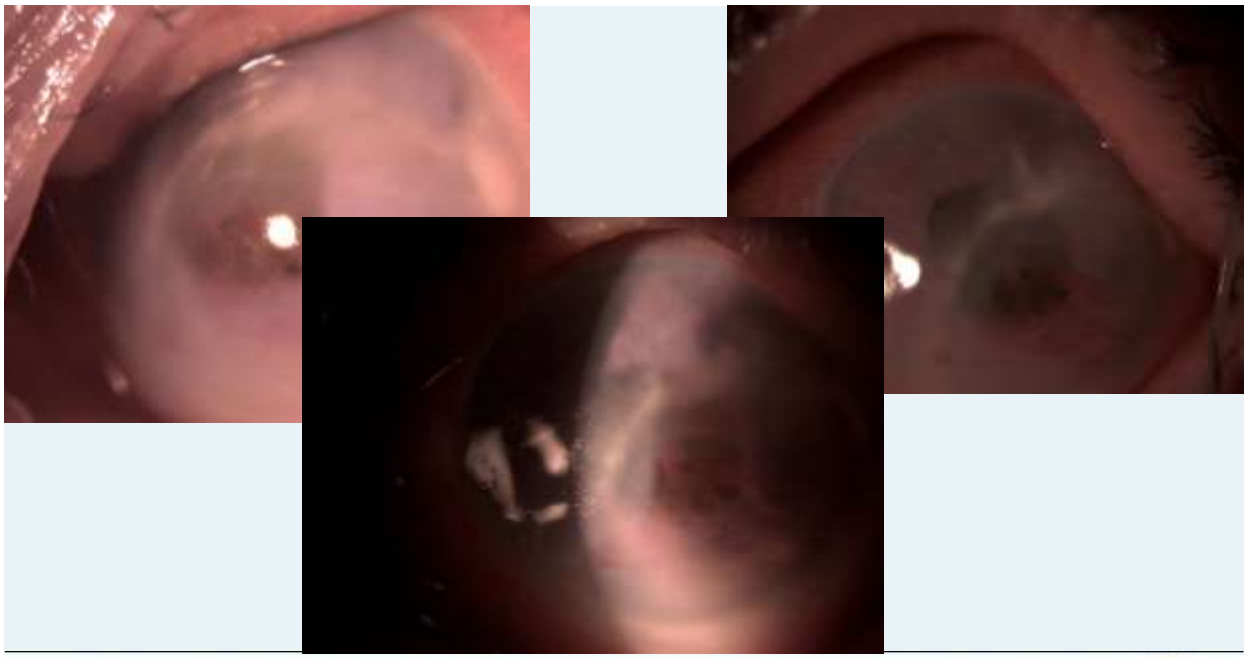

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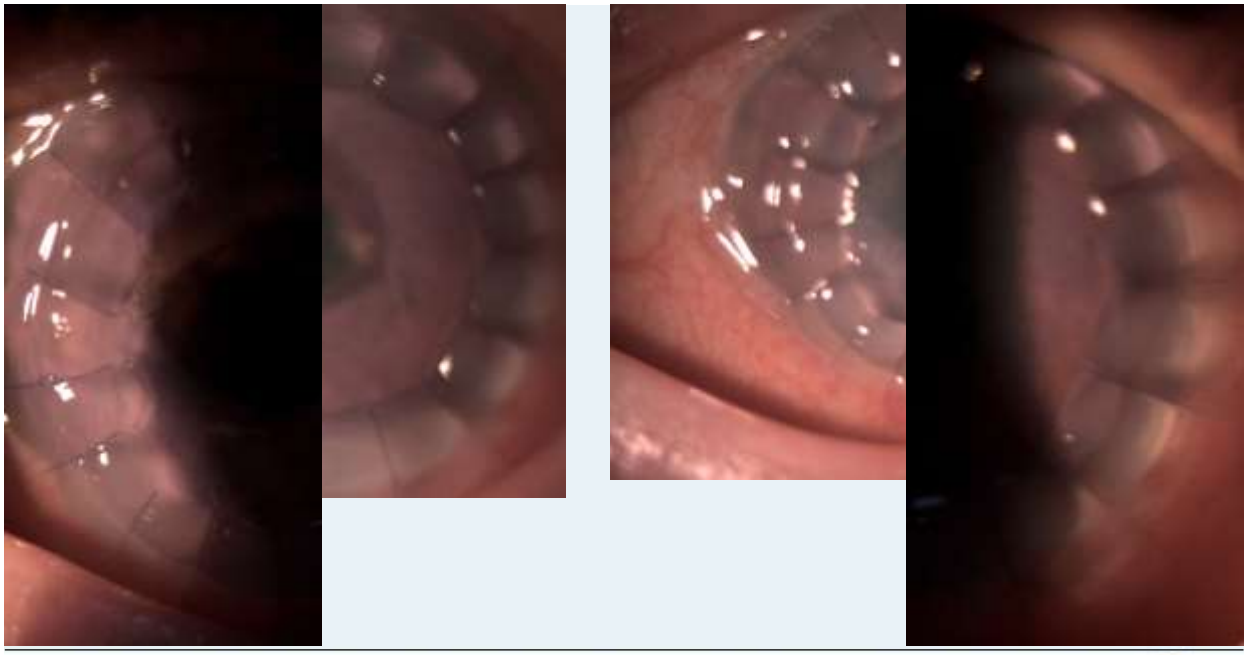

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