Tissue Engineering in Ophthalmology

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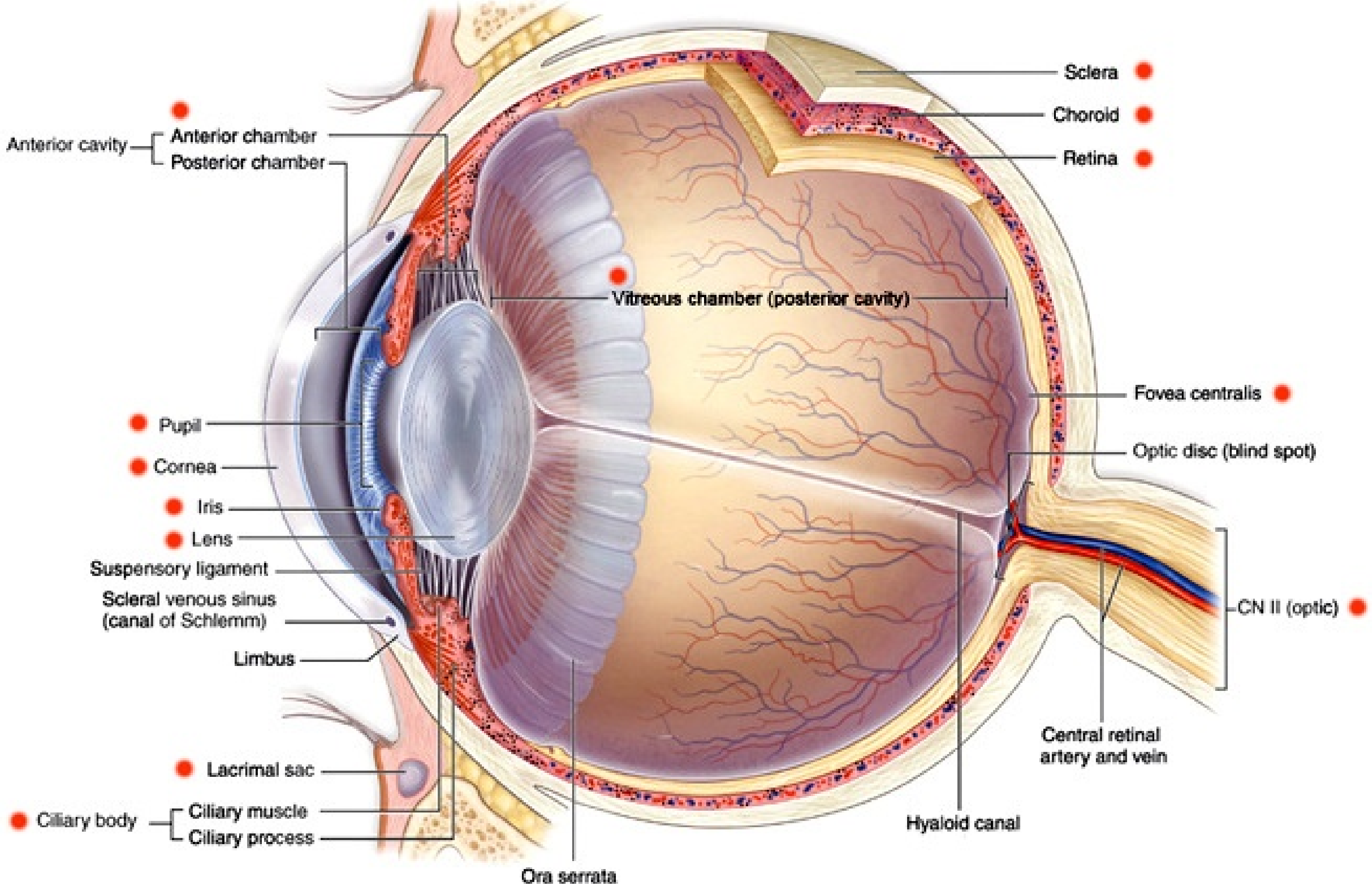
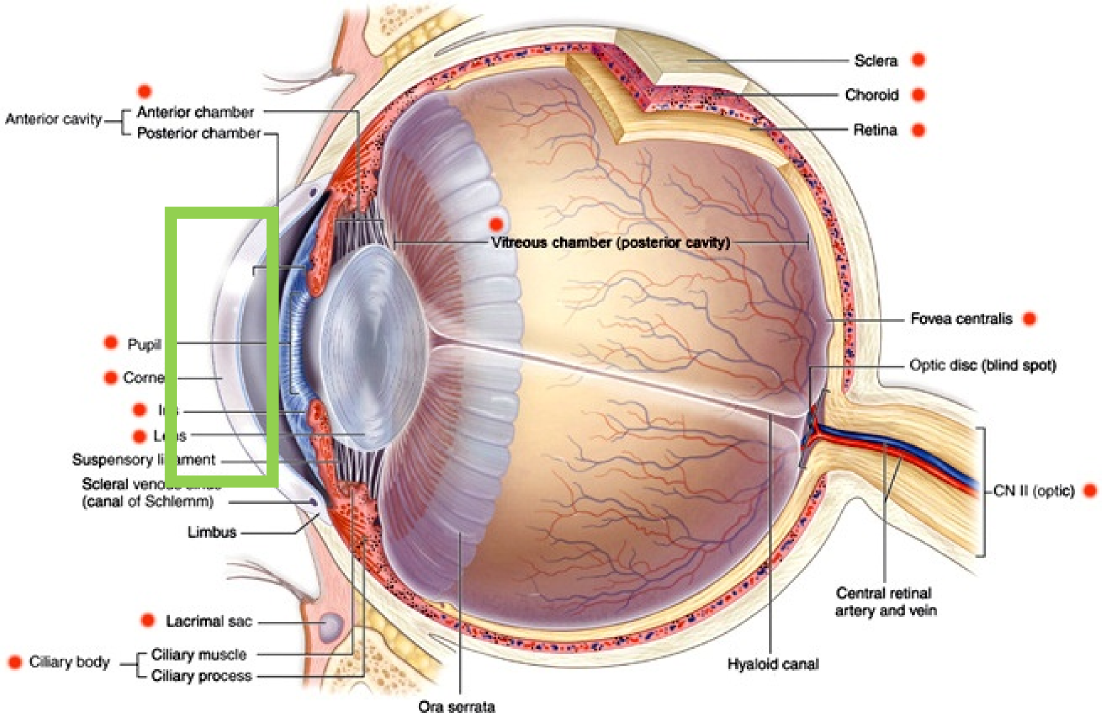
Ocular anatomy

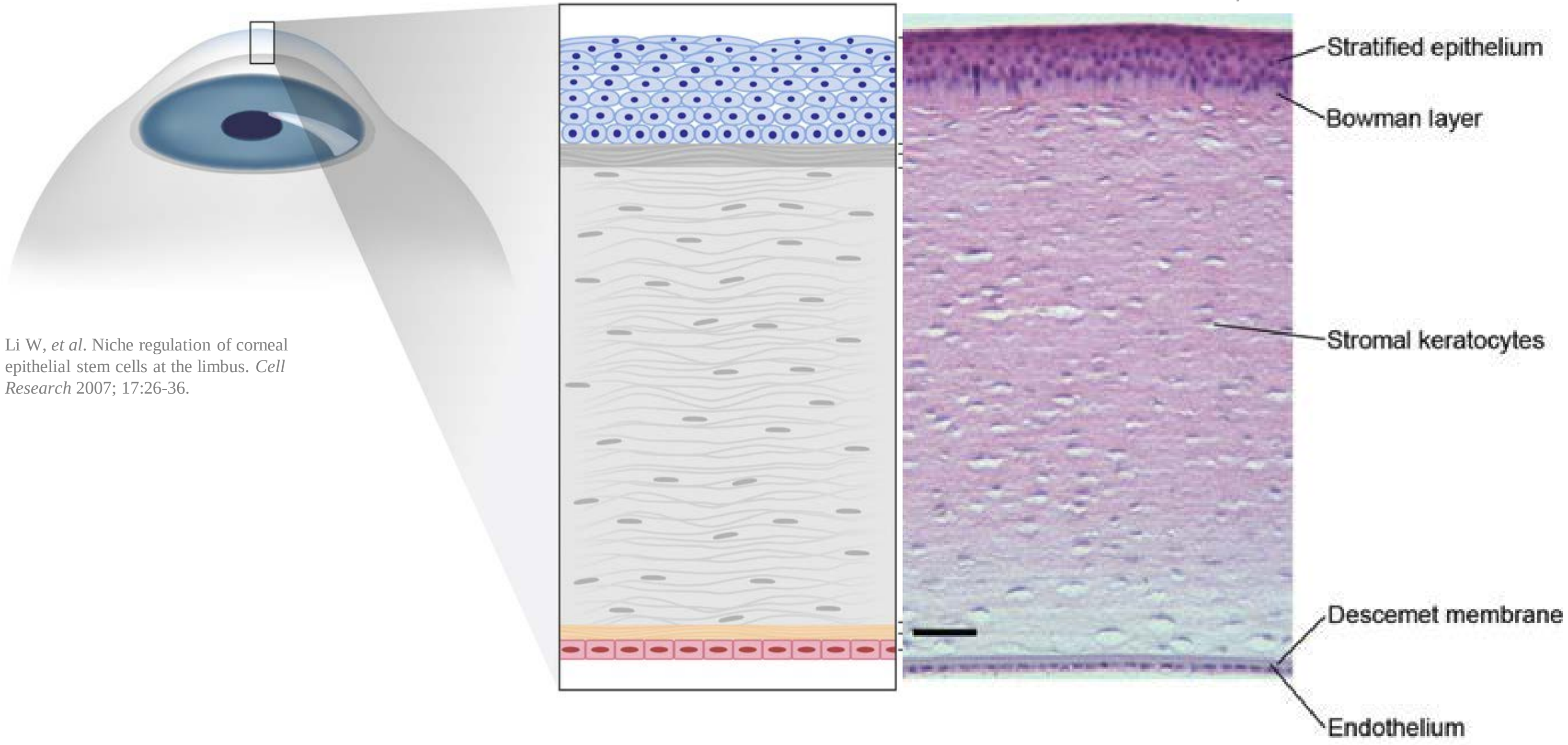


http://www.unioneyeworks.com/ocular-anatomy/

anterior segment



Corneal anatomy



doi 10.1089/ten.teb.2014.0397

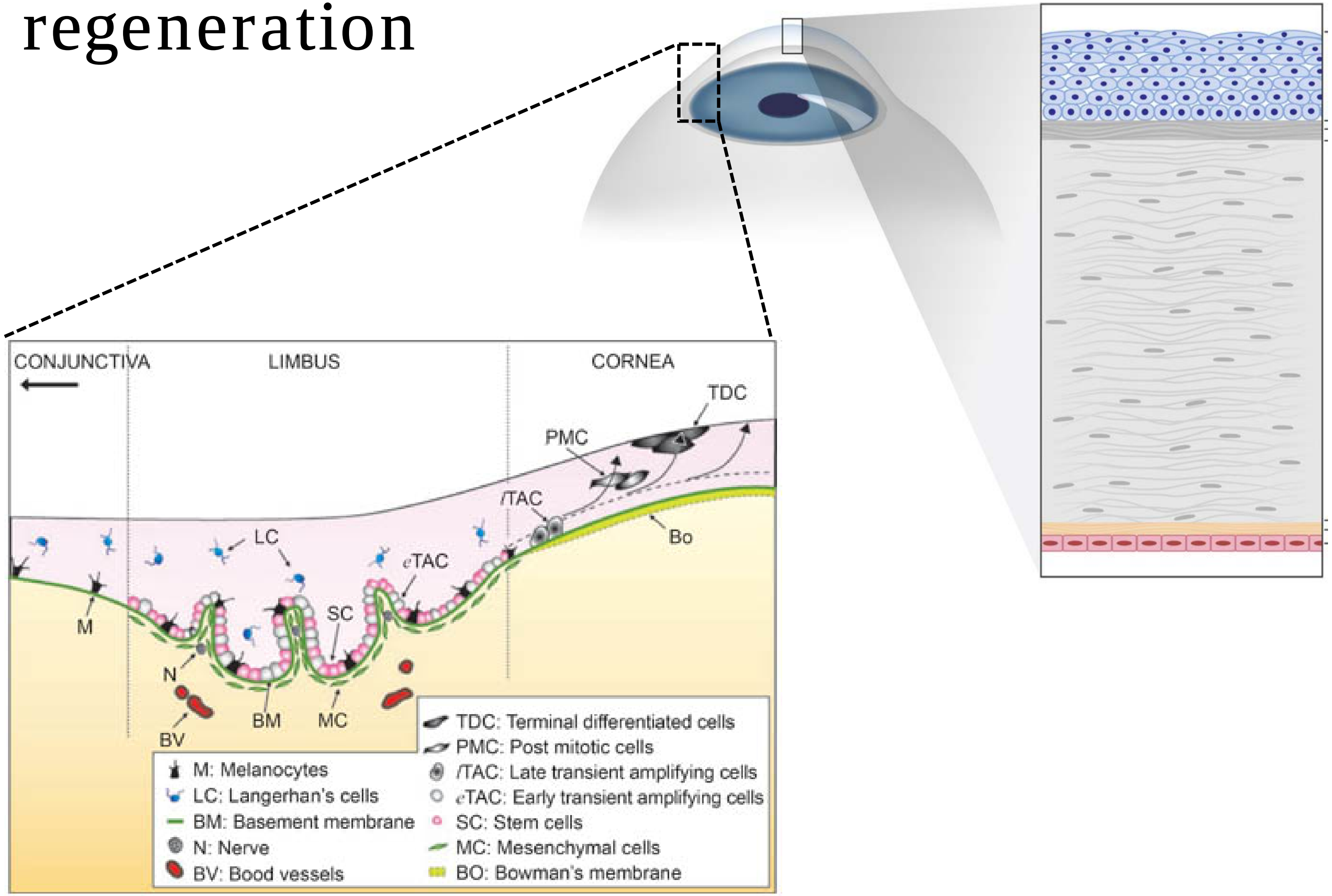
Li W, *et al*. Niche regulation of corneal epithelial stem cells at the limbus. *Cell Research* 2007; 17:26-36.

- stratified epithelium
- highly innervated, nociceptive R

- stroma: 90 % of thickness
- collagen fibrils: 200-250 lamellae, type I & V
- proteoglycans for interfibrillar spacing: transparency & hydration
- keratocytes

- DM: VIII, basement membrane
- endo: corneal deturgescence n# decline w/ age

Corneal epithelial homeostasis & regeneration



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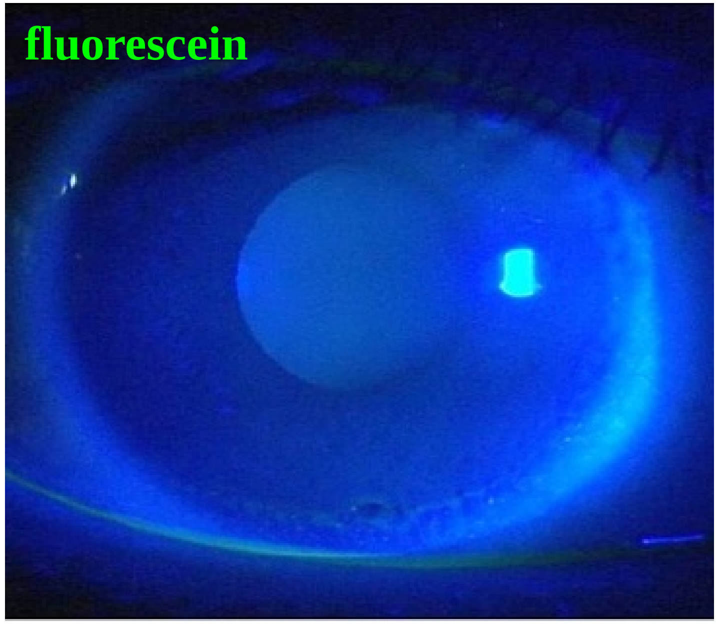
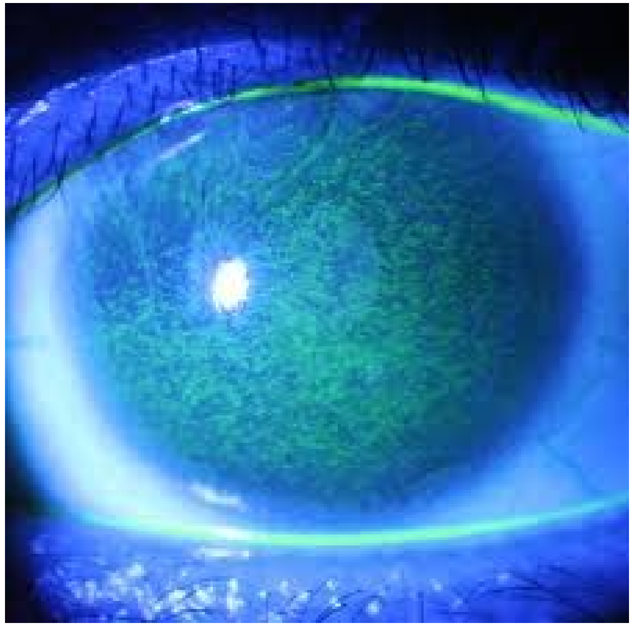
illnesses/traumas requiring corneal tissue engineering

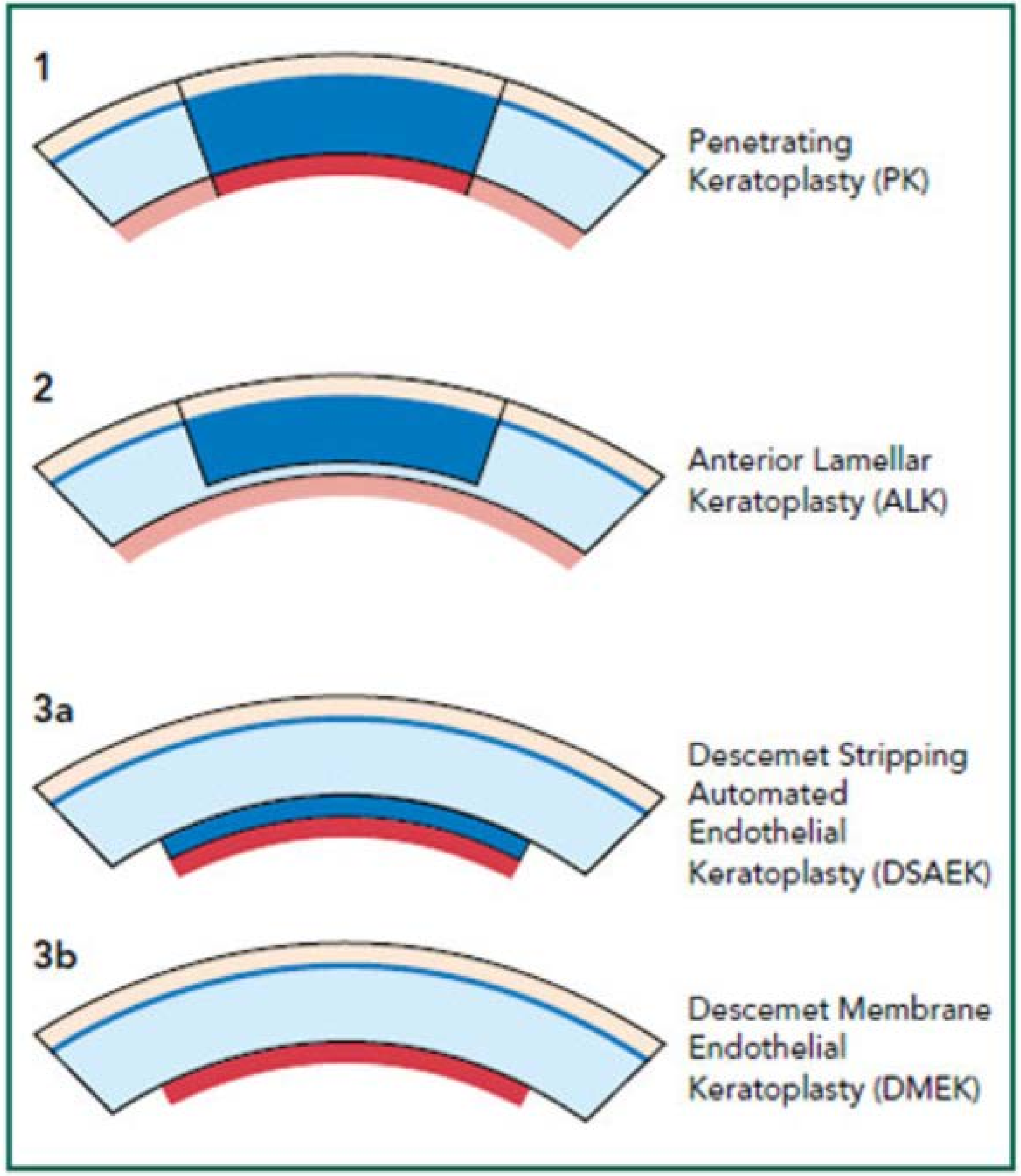
- physical / chemical injury
- photokeratitis
- Sjögren Syndrome: auto-ab α moisture-producing glands (lacrimal, salivary) (kerato-conjunctivitis sicca)
- LSC deficiency
- loss of endothelial cells

alkaline burn
LSC deficiency
NV
opacification
visual loss





surgical interventions for corneal regeneration



donor cornea required!

major risks / problems:

- low availability of donor material
- aging of the population
- graft rejection
- transmissible diseases
- refractive surgery

➢ requirement of TE

Corneal anatomy

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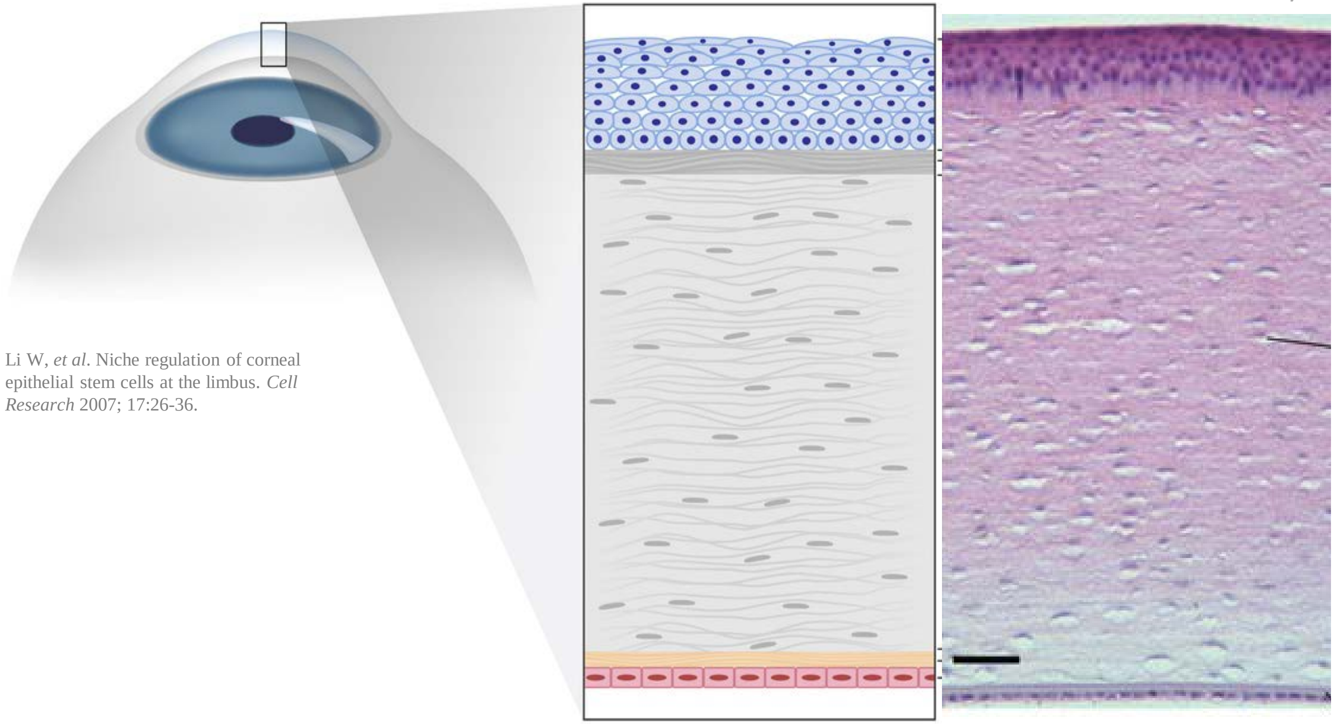

Li W, *et al*. Niche regulation of corneal epithelial stem cells at the limbus. *Cell Research* 2007; 17:26-36.

requirements of tissue-engineered corneas:

- protection:
  physical or chemical injury
  damaging UV light

- transparency
- refractive power

} corneal deturgescence

- withstand IOP & tensile forces

TE approaches:

- purely cell-based
- decellularized
- synthetic and natural polymer-based constructs

Table 5.1 Scaffold Materials Used in Corneal Tissue Engineering (Wang et al., 2013)

Scaffold Materials Used in Corneal Tissue Engineering	
Acellular Cornea Stroma	Derived from the acellular allogeneic or autologous graft
Amniotic Membrane	Situated in the inner membrane of fetal membranes, including the monolayer of epithelial cells, thick basement membrane, and avascular stroma
Collagen	Natural protein material commonly used in TE; Type I collagen is the major composition of human cornea;Collagen fibrils provide physical support to tissues; Can promote cell adhesion and proliferation better than synthetic polymers
Fibrin	Commonly used protein; Human fibrin is low in price, readily available, and has good tolerance to cells
Chitin	Chitin is a linear polysaccharide; Nontoxic; Promotes growth factor production and acts as a carrier for the release of growth factors
Silk fibroin	Extracted from natural silk protein polymer fibers; Transparent, easy to handle, free from disease transmission

cell types for tissue-engineered corneal replacements

Primary animal-derived corneal epithelial cells
Primary animal-derived corneal stromal cells
Primary animal-derived corneal endothelial cells
Primary animal-derived dorsal root ganglion (DRG) cells
Immortalized human corneal epithelial cells
Immortalized human corneal stromal cells
Immortalized human corneal endothelial cells
Primary human corneal epithelial cells
Primary human corneal fibroblast cells
Primary human corneal endothelial cells
Primary human corneal stromal stem cells

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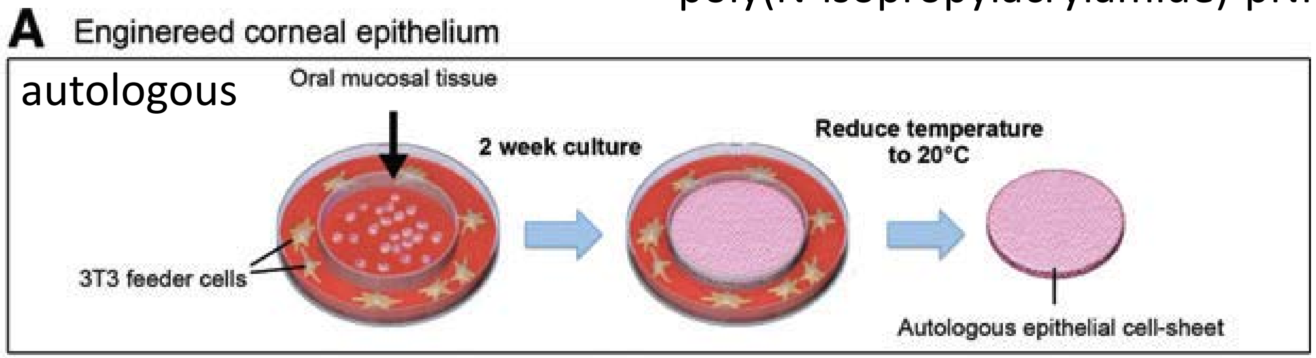
corneal epithelium

corneal epithelium scaffolds

poly(N-isopropylacrylamide)-pNIPAAM surface



autologous

feeder cells mimic SC niche,
metabolically active,
mitotically inactive

elastic hydrogel

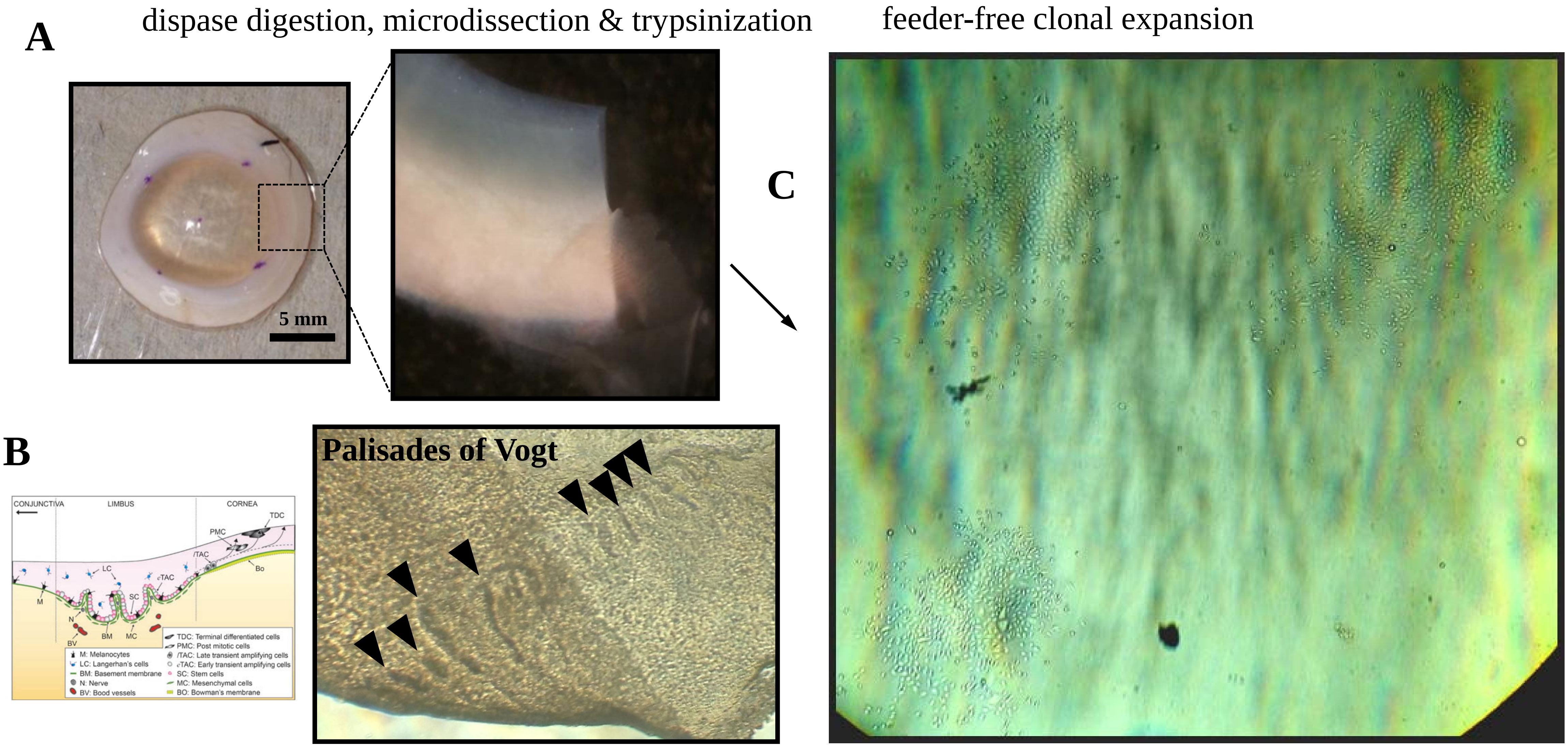
doi 10.1089/ten.teb.2014.0397

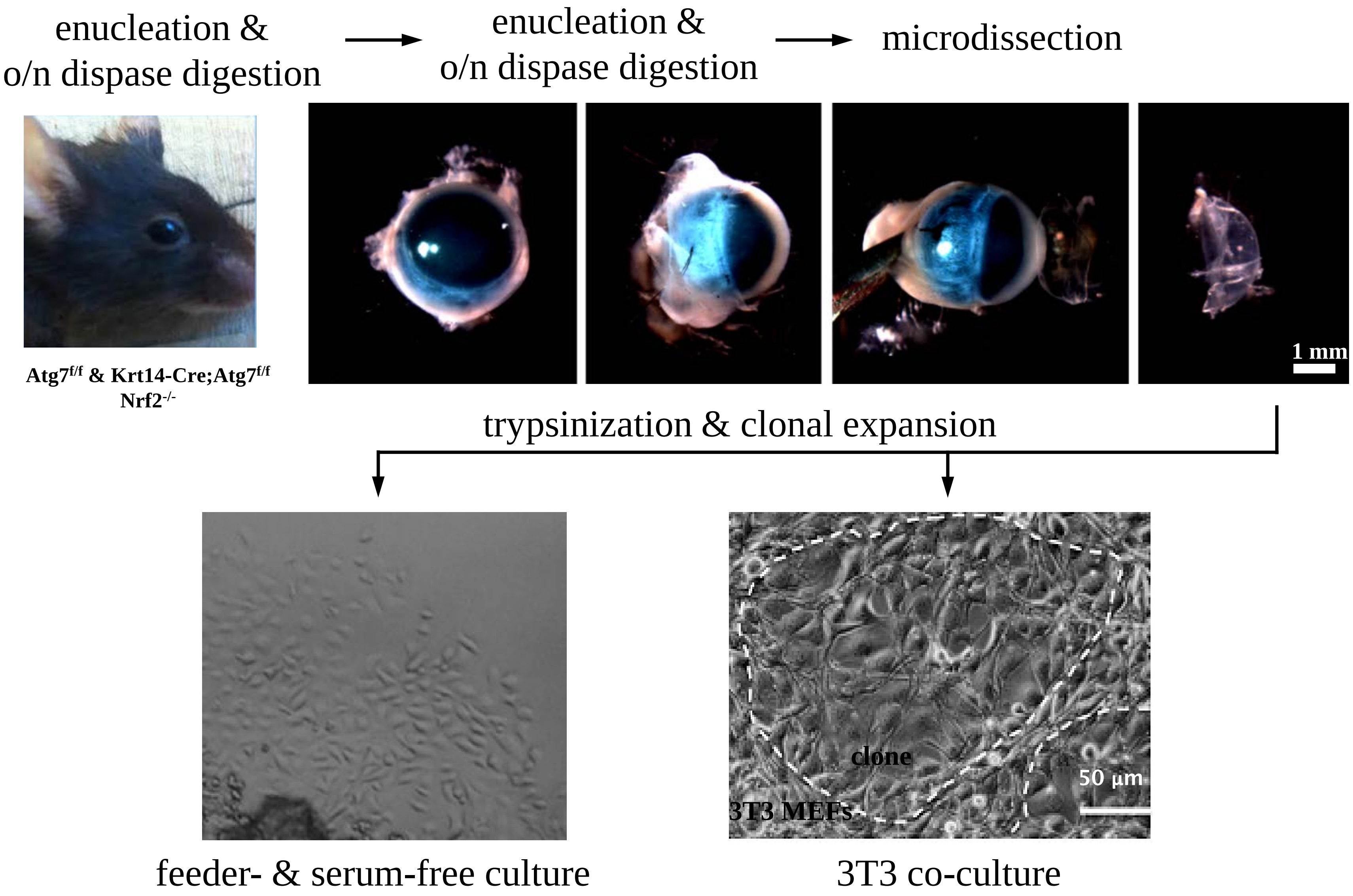
<u>human amniotic membranes</u> in several animal models
(e.g., rat, rabbit, and goat)
high inter- and intra-tissue variability in morphological, chemical, and optical properties limit the use of the human amniotic membrane in clinical settings

<u>human donor corneal stromal tissues</u>
lack of corneal tissue donor availability, transmissible diseases

type I collagen <u>hydrogels</u>
microcontact printing to generate 3D in vitro human corneal limbal crypts

<u>silk</u>
optical properties, mechanical robustness, & versatile processability


A
dispase digestion, microdissection & trypsinization
feeder-free clonal expansion
5 mm
C
B
CONJUNCTIVA
LIMBUS
CORNEA
TDC
PMC
lTAC
LC
eTAC
Bo
M
SC
N
BV
BM
MC
TDC: Terminal differentiated cells
PMC: Post mitotic cells
M: Melanocytes
lTAC: Late transient amplifying cells
LC: Langerhan's cells
eTAC: Early transient amplifying cells
BM: Basement membrane
SC: Stem cells
N: Nerve
MC: Mesenchymal cells
BV: Bood vessels
BO: Bowman's membrane
Palisades of Vogt

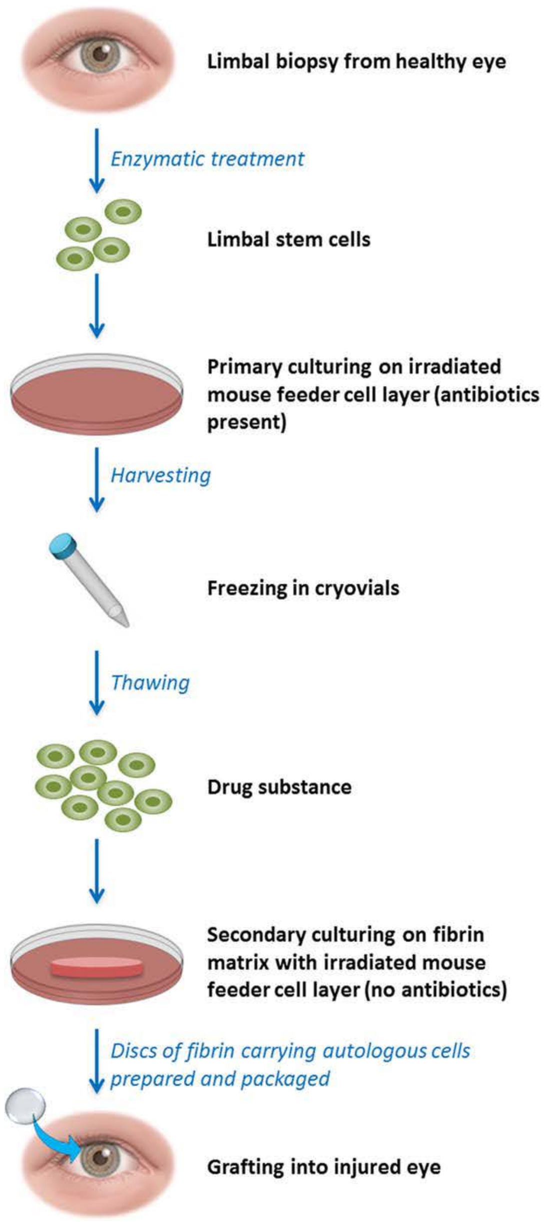

enucleation &
o/n dispase digestion
enucleation &
o/n dispase digestion
microdissection
Atg7f/f & Krt14-Cre;Atg7f/f
Nrf2-/-
1 mm
trypsinization & clonal expansion
clone
3T3 MEFs
50 µm
feeder- & serum-free culture
3T3 co-culture

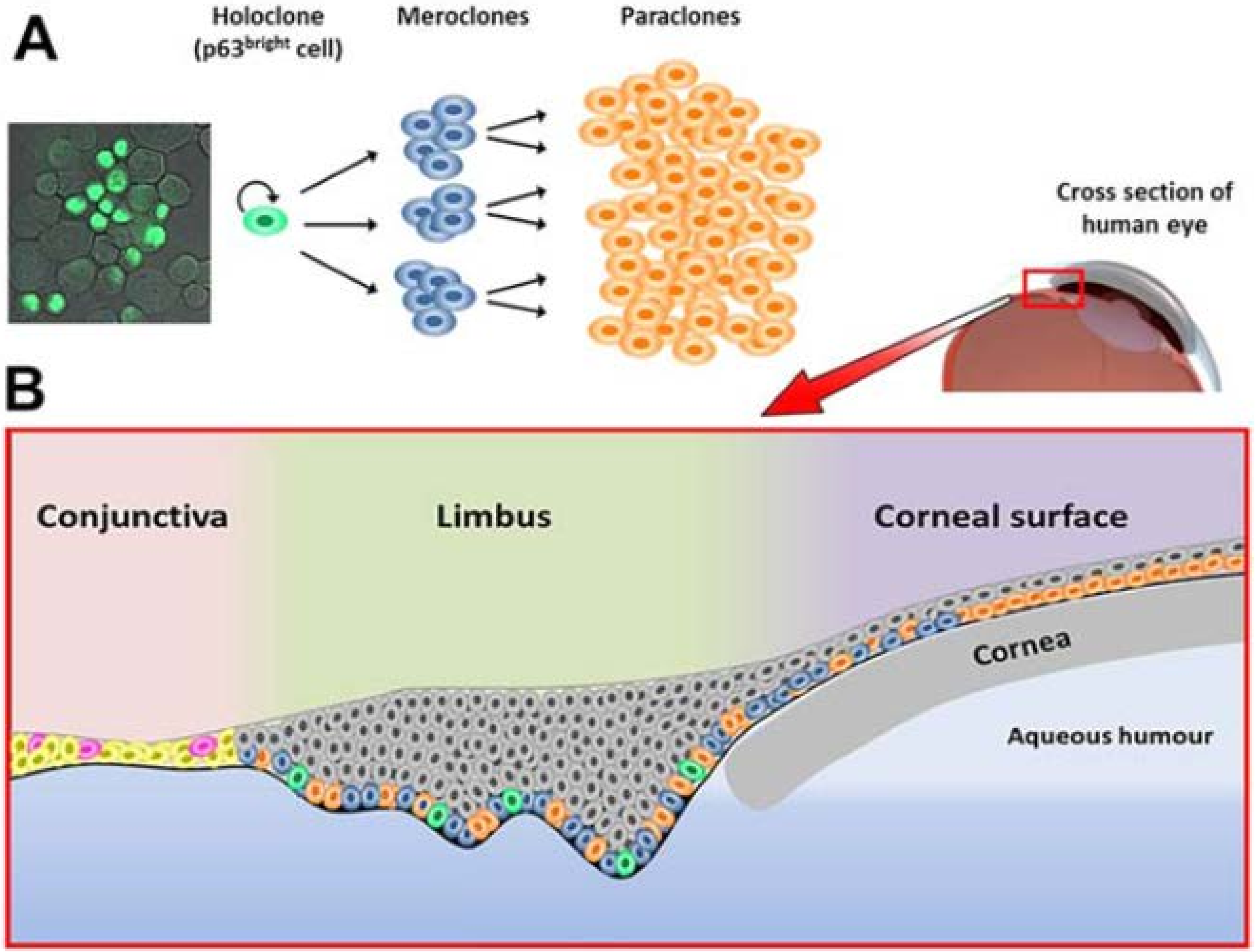
first stem cell-based medicinal product (ATMP) approved in the Western world

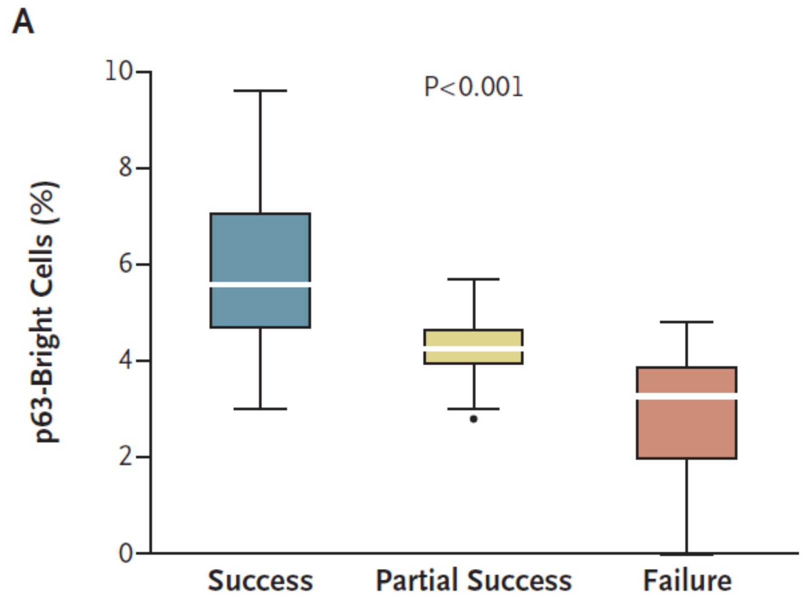
Tx of moderate to severe LSC deficiency due to e.g. acid/alkali burns, solvent burns, abrasive agents, chemical agents

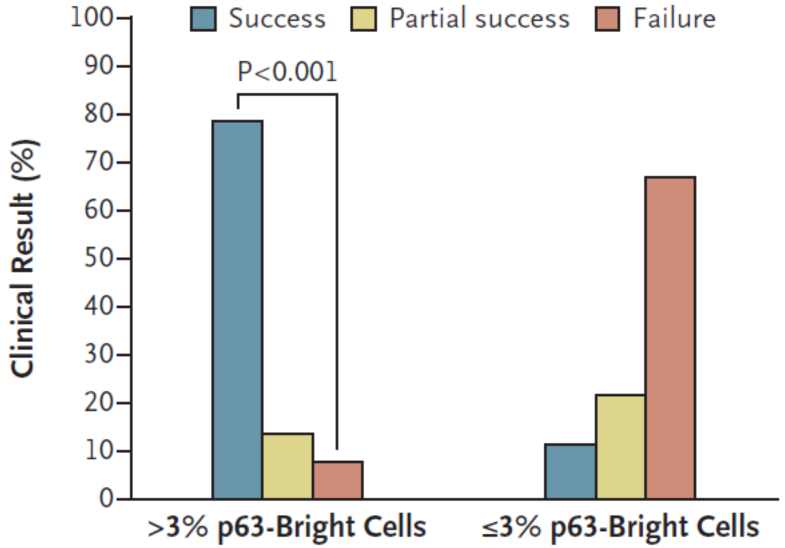
requirements:
1-2 mm^2 limbal biopsy
no severe corneal stromal defects

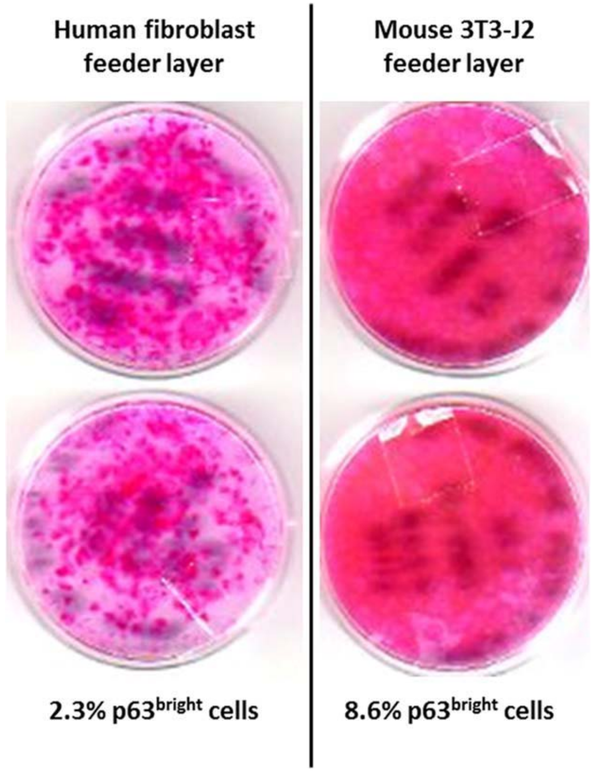
no graft rejection

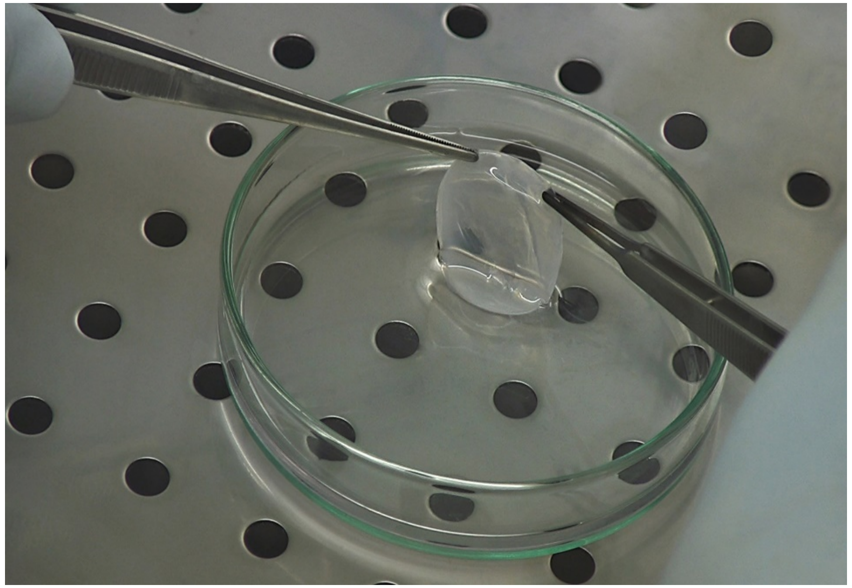


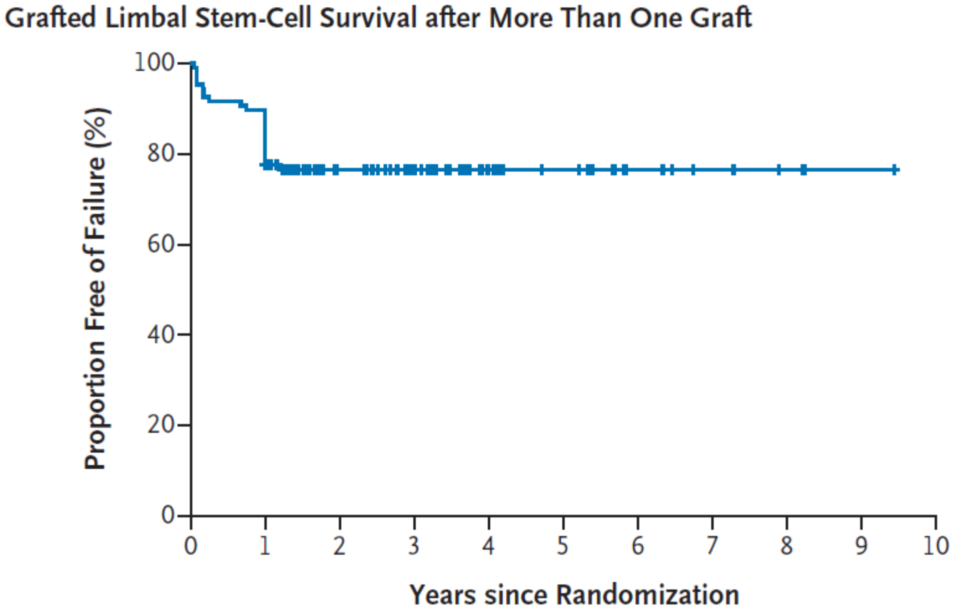

A
Holoclone
(p63bright cell)
Meroclones
Paraclones
Cross section of
human eye
B
Conjunctiva
Limbus
Corneal surface
Cornea
Aqueous humour


A
P<0.001
p63-Bright Cells (%)
10
8
6
4
2
0
Success
Partial Success
Failure


Success
Partial success
Failure
P<0.001
Clinical Result (%)
100
90
80
70
60
50
40
30
20
10
0
>3% p63-Bright Cells
≤3% p63-Bright Cells

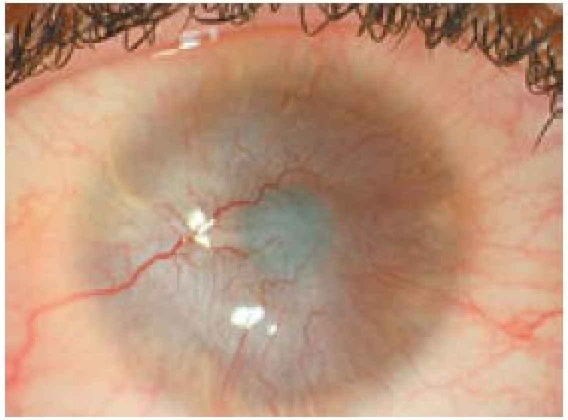




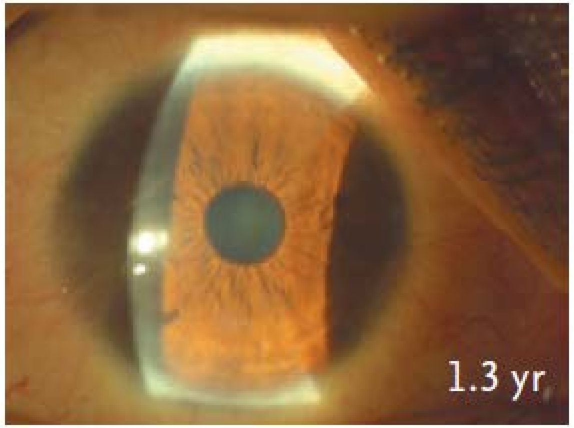


acid burn

Before



After



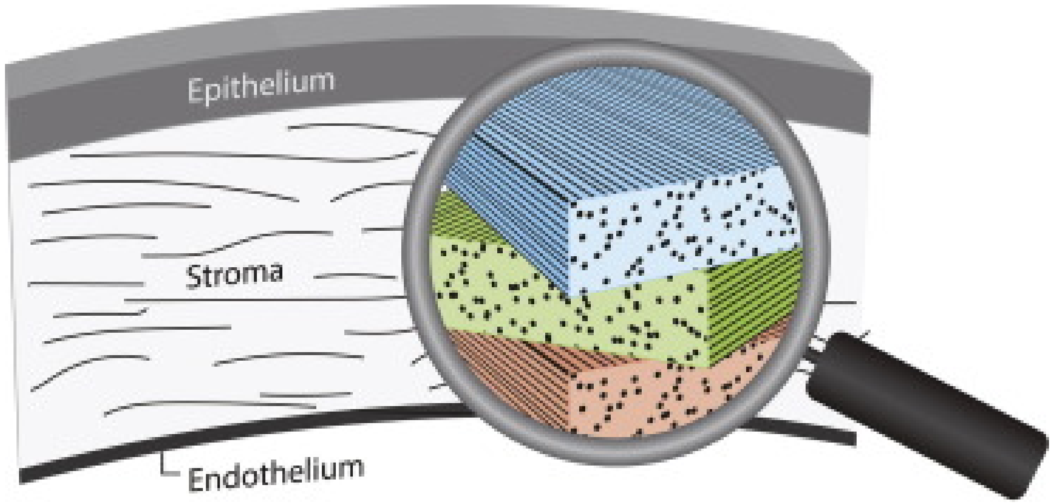
corneal stroma

corneal stroma

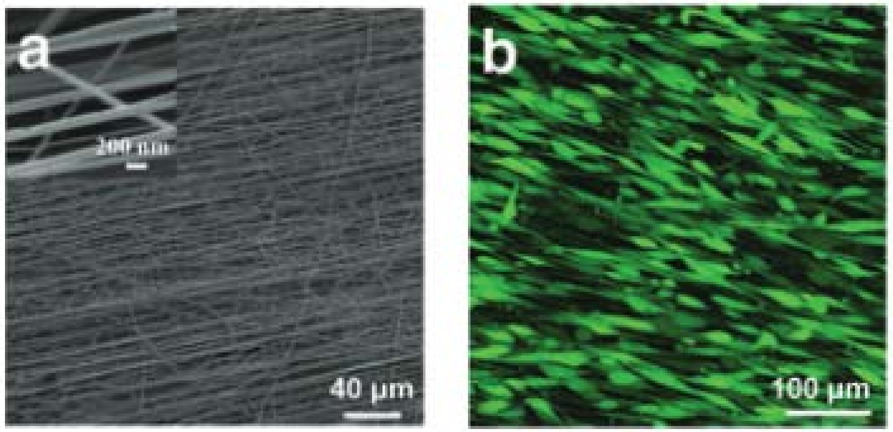
Scaffold properties:
- Biocompatibility
- Optical transparency
- mechanical strength
- Strong enough to withstand handling during surgery

synthetic materials:
tunable properties
imitate collagen fibril lamellae

cell-based approach
poly(esterurethane) urea substrate + human stromal SCs -> keratocytes



https://doi.org/10.1016/j.actbio.2018.01.023



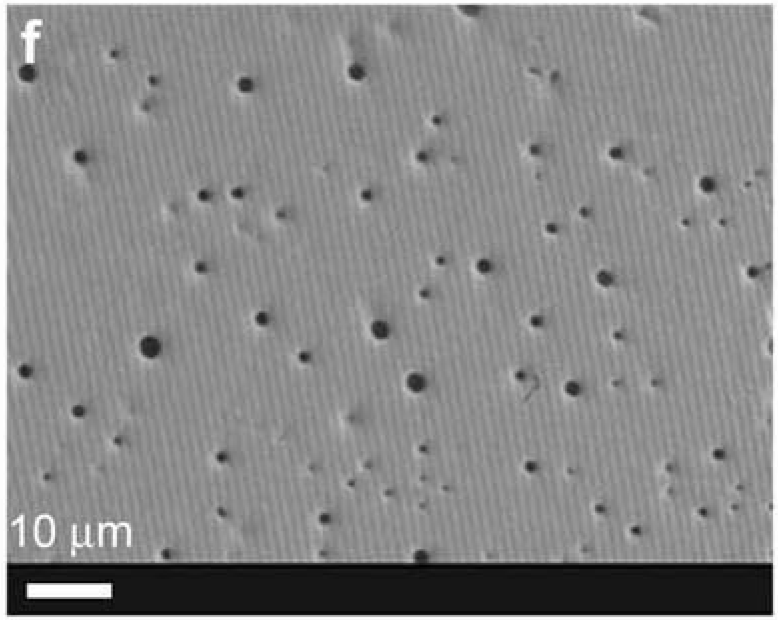
https://doi.org/10.1089/ten.tea.2012.0545

corneal stroma

hydrogels from reconstituted type I collagen + living cells
susceptibility to cell-mediated remodeling & degradation
chemical cross-linkers

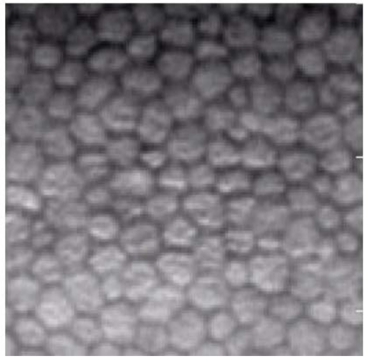
silk
thin films of optically clear silk proteins
mechanically robust, porous

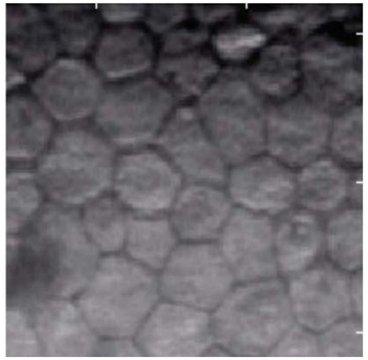
in combination with appropriate cell types

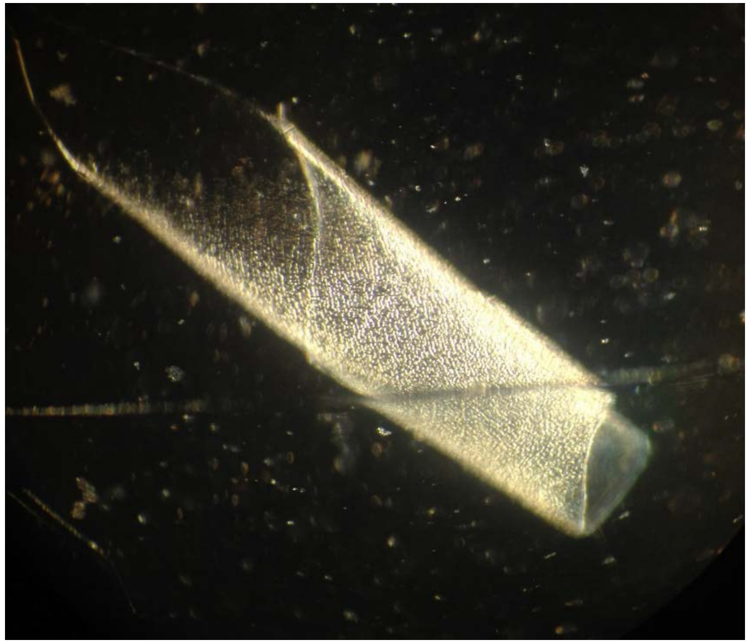


corneal endothelium & nerves

corneal endothelium





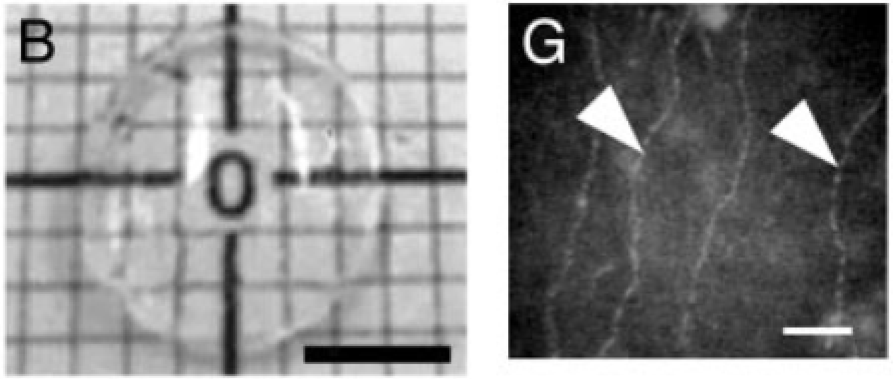


natural polymer substrates:
type I collagen, gelatin, decellularized tissues, chitosan, and chondroitin sulfate

Decellularized aminiotic membrane in combination with human corneal endothelial cells in lamellar keratoplasty: functionned as a corneal endothelium equivalent

corneal innervation

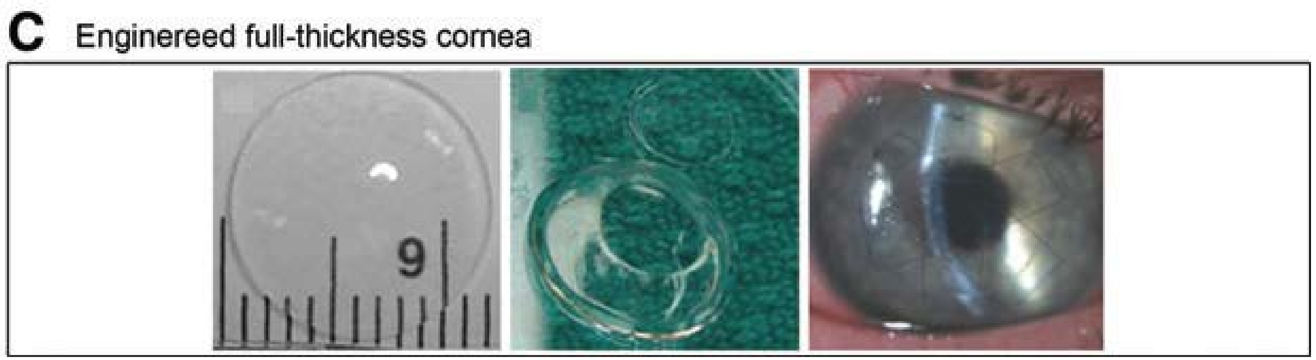
nociceptive nerve protrusions, which end in the epithelial layer
mechanical and thermal sensors
maintain the overall cornea health
lack in innervation -> dry eye
reduced corneal sensitivity, diffuse corneal ulcers



10.1073/pnas.2536767100

promote neural growth & regeneration:
decoration of substrate with laminin-derived peptides
CXL collagen substitutes, lamellar keratoplasty in a porcine model, recovering preoperative nerve density at 1-year postsurgery

full thickness cornea



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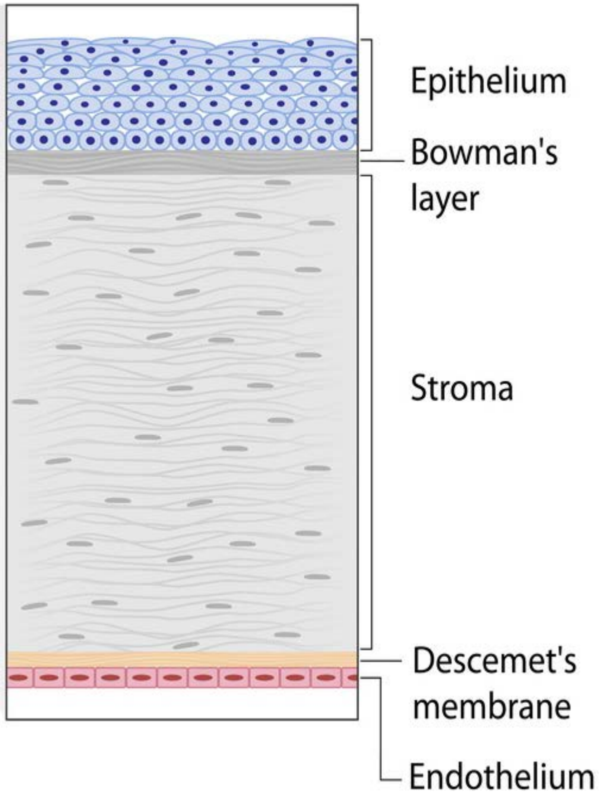
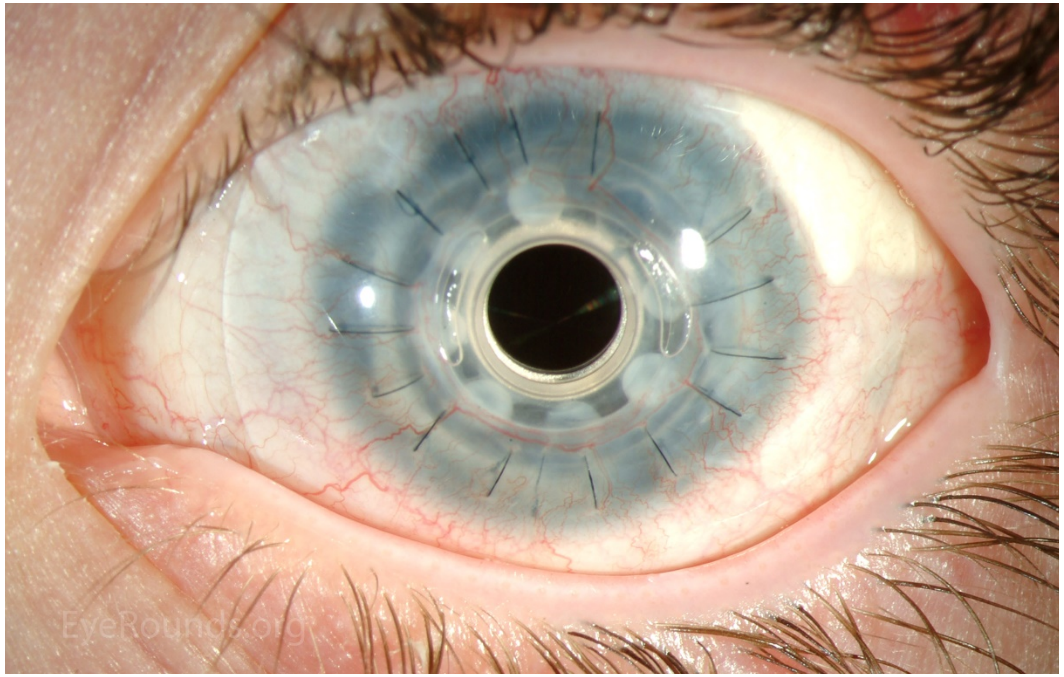


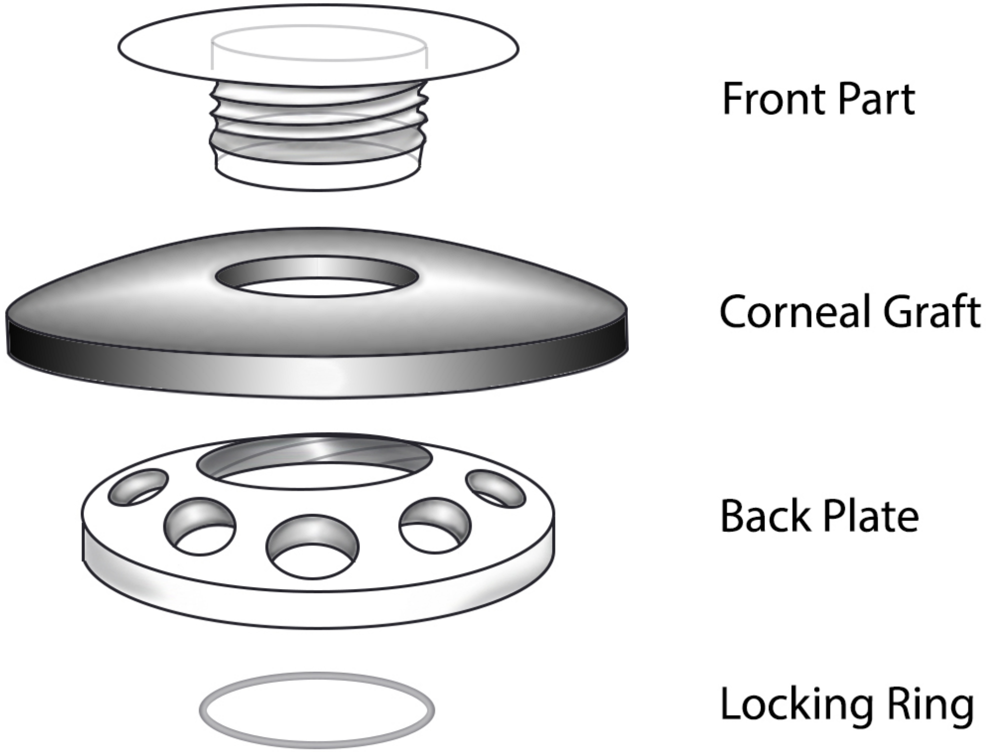
acellular porcine cornea + amniotic epithelial cells
rabbit lamellar keratoplasty ->
rejection & degradation of the tissue-engineered cornea

cross-linked recombinant human collagen type III
anterior partial keratoplasty

2a post surgery: stably integrated, innervated, avascularized, but delayed epithelial closure

boston type I kerato prosthesis





ocular surface in good condition
intact tear film & lids

repeated graft failure
herpetic keratitis

thank you for your attention