

# Fisiopatologia dell'epitelio della cornea: recenti acquisizioni

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CENTRO NAZIONALE TRAPIANTI

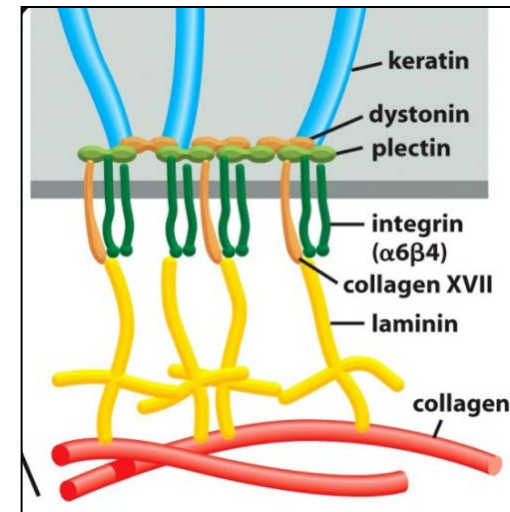
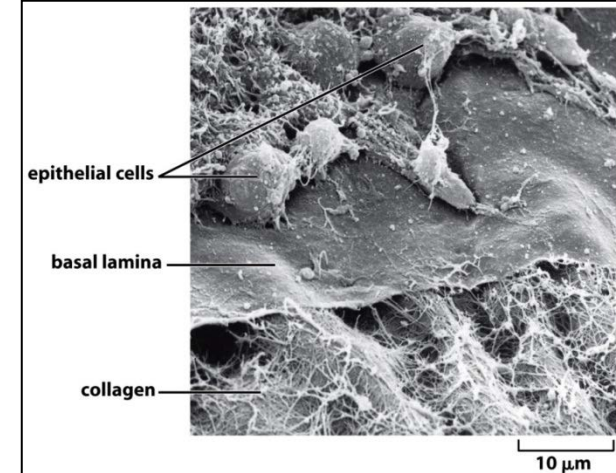
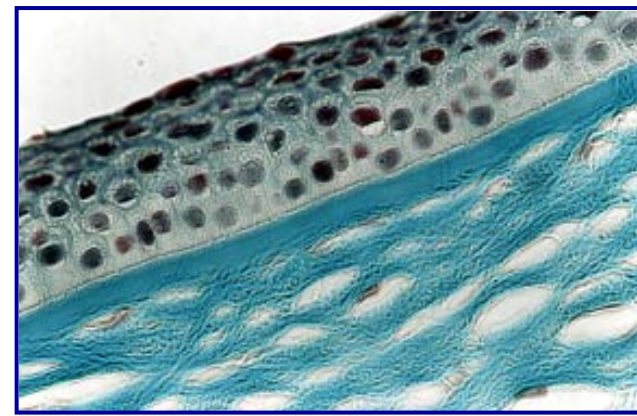


*Agence française  
de sécurité sanitaire  
des produits de santé*

Simposio **Società Italiana Banche degli Occhi**  
XVI Congresso Nazionale SITraC, Roma, 23-25 febbraio 2011

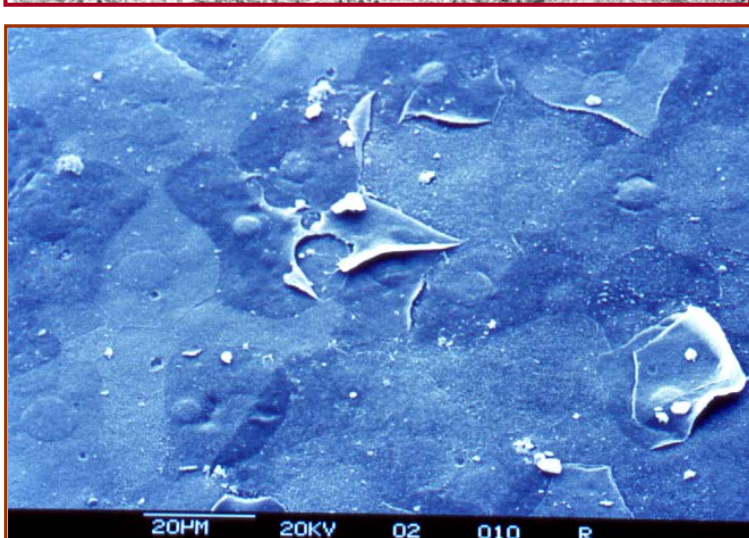
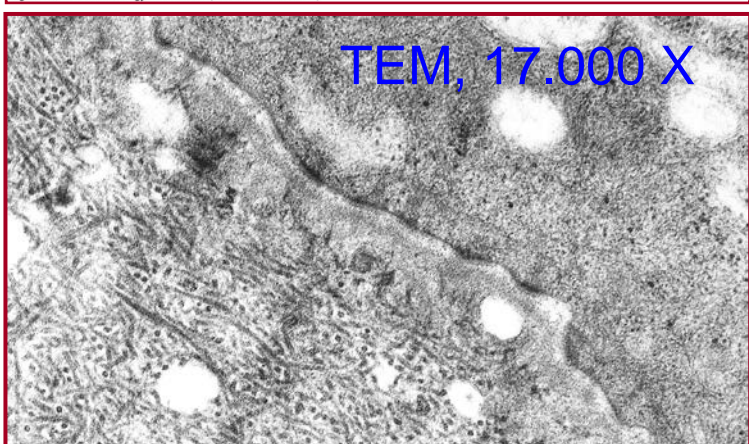
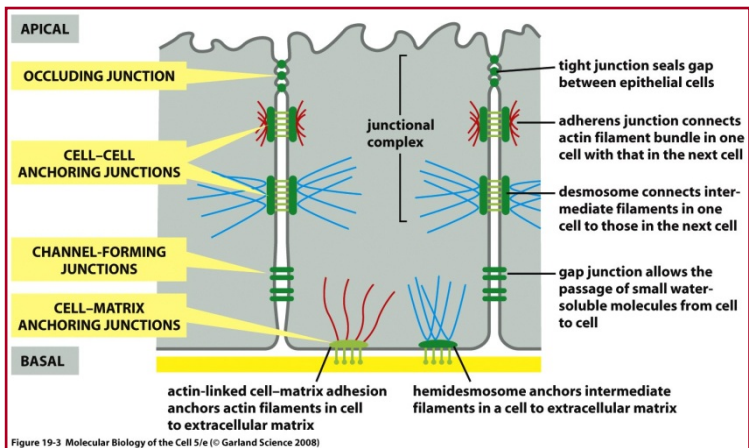
# The corneal epithelium (50 $\mu\text{m}$ )

- Superficial layer (1-2 layers), wing cells (3 layers)
- Basal layer (20 $\mu\text{m}$ )
- Epithelial nerves ( $\varnothing < 0.1\mu\text{m}$ )
- Sub-basal epithelial nerve plexus: migrate centripetally ( $\varnothing 0.1-0.5\mu\text{m}$ )
- Epithelial basal membrane (0.05 $\mu\text{m}$ )
- Bowman's layer: acellular zone, 10  $\mu\text{m}$
- Sub-epithelial nerve plexus: stationary



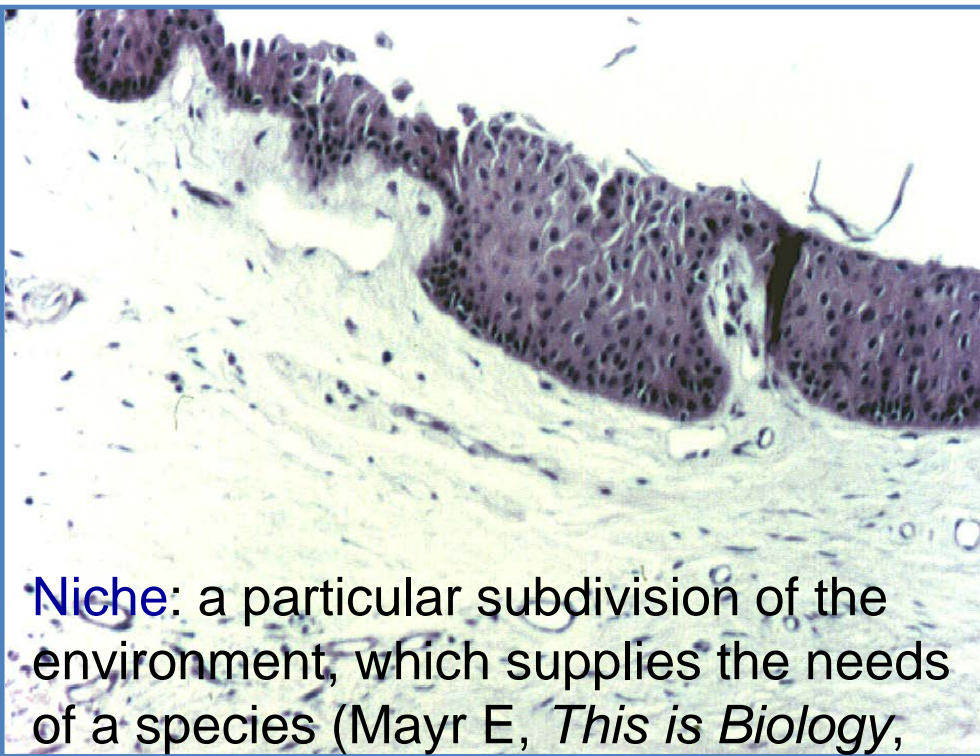
# Corneal epithelial cells

- Cell junctions typical of vertebrate epithelia: **impermeability, stability**
- Average lifespan: 7-10 days
- Routinely **involution** → **apoptosis** → **desquamation**
- Complete turnover: 1 week
- Mitosis: only **basal**, **transient amplifying** and **stem** cells



# The corneal limbus

- Basal layer:  $\approx 30\%$  epithelial stem cells
- Stem cells:
  - ✓ In self-renewing tissues
  - ✓ Low mitotic activity, high proliferative potential
  - ✓ Poorly differentiated
  - ✓ In protected anatomical sites (Vogt palisades)
  - ✓ ~ 6 Limbal Epithelial Crypts in the human limbus

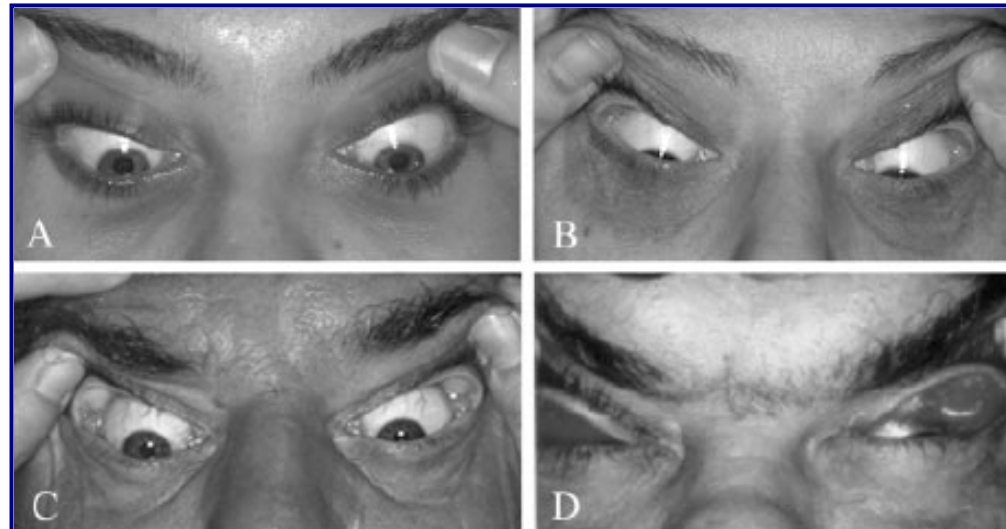


# Epiteli della superficie oculare: fisiologia

- Congruità anatomica e funzionale di palpebre e SO (occlusione, entropion, ectropion, sindrome palpebra molle)
- Film lacrimale (*tear dysfunction syndrome*)
- Innervazione sensoriale efficiente (braccio afferente per due archi riflessi):
  - ✓ **lacrimazione** (fibre parasimpatiche, VII° nervo)
  - ✓ **ammiccamento** (fibre motrici, VII° nervo)



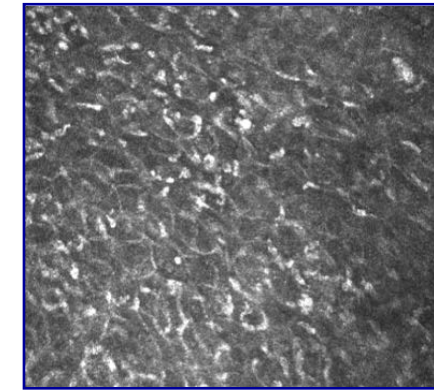
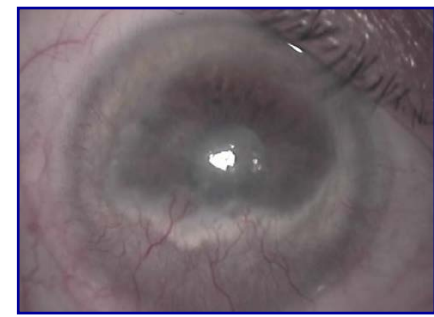
Menisco lacrimale:  
~1 mm



Tzong-Shyue Liu D et al. Tear dynamics in floppy eye lid syndrome. *IOVS* 2005;46:1188-94

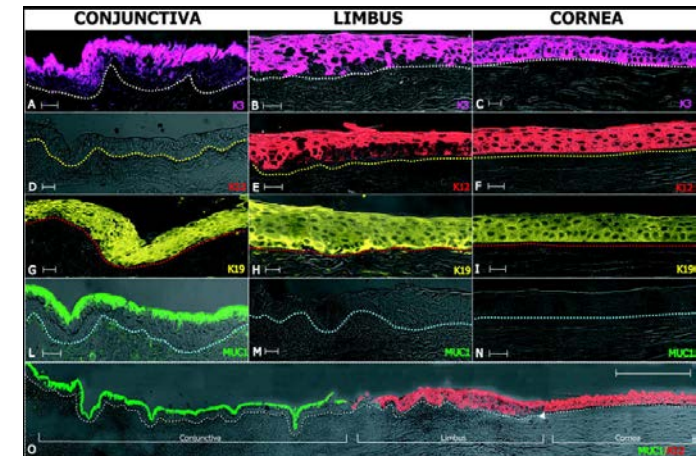
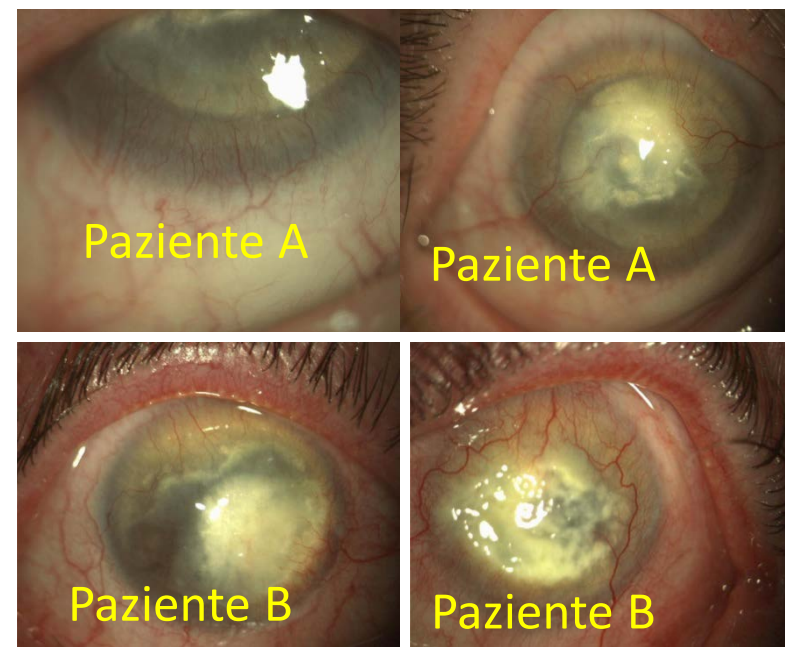
# Patologia epiteli SO: diagnosi

- Identificazione comparto anatomico
- Valutazione funzionalità del limbus
- Anamnesi (generale, oculare)
- Esame obiettivo (non solo oculare)
- Sintomi: **fastidio** [prurito, sensazione CE, sabbia negli occhi, occhio bagnato, dolore, bruciore (Mattina? Sera?), fotofobia]
- Valutazioni funzionali, test, citologia ad impressione
- Microscopia confocale
- **Obbiettività / soggettività: poca correlazione**



# LSCD

- **Danno grave:** metaplasia mucosa o squamosa, diagnosi clinica
- Patologia **lieve / moderata:** superficie corneale irregolare, difetti epiteliali persistenti o ricorrenti
- Difficile quantificare clinicamente il deficit limbare
- Utile lo studio delle cheratine dell'epitelio corneale

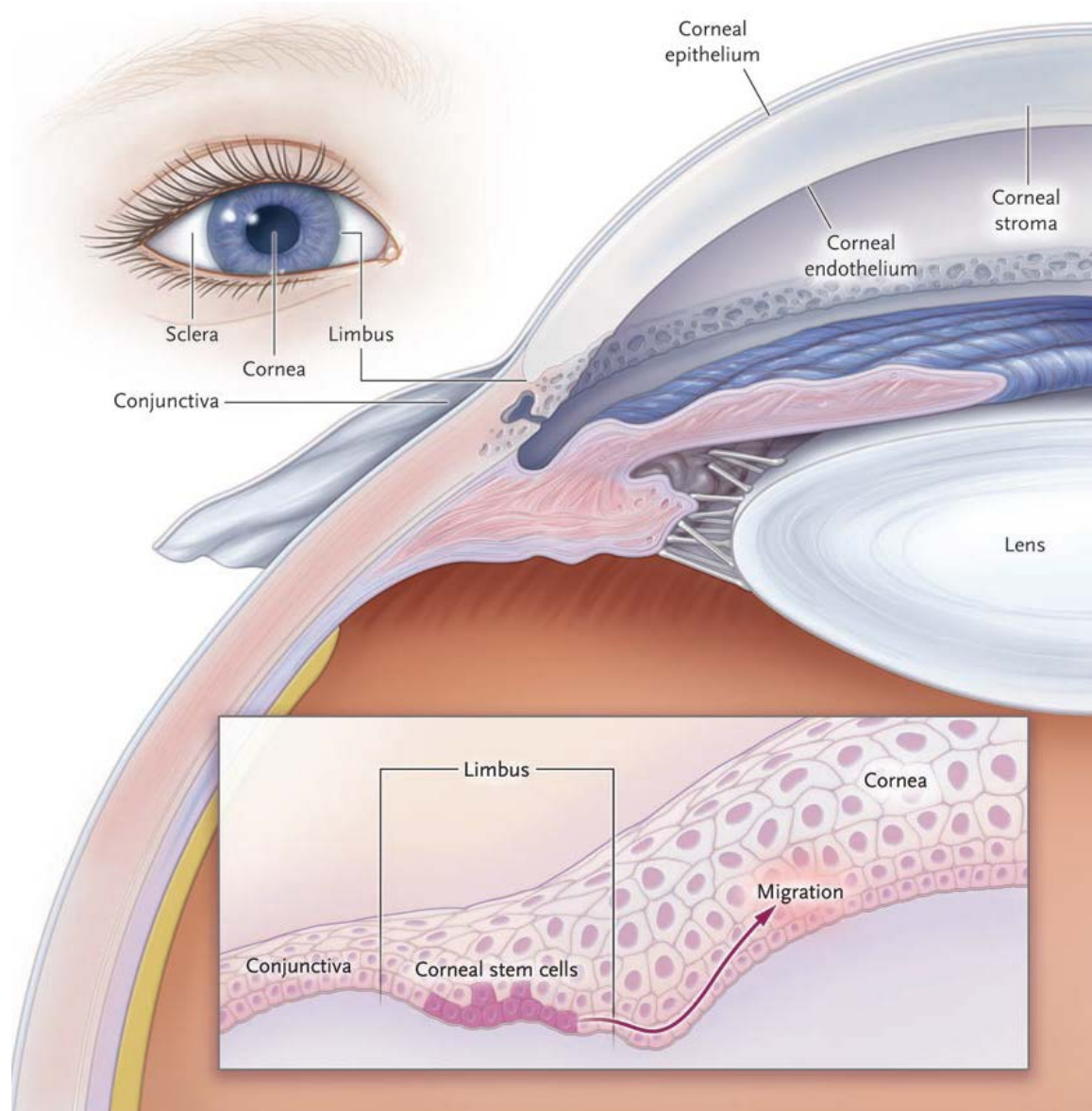


Donisi PM et al. Analysis of limbal stem cell deficiency by corneal impression cytology. *Cornea* 2003;22(6):533-8

Barbaro V et al. Evaluation of ocular surface disorders: A new diagnostic tool based on impression cytology and confocal laser scanning microscopy. *Br J Ophthalmol* 2010;94:926-32

# Trattamento patologie della SO

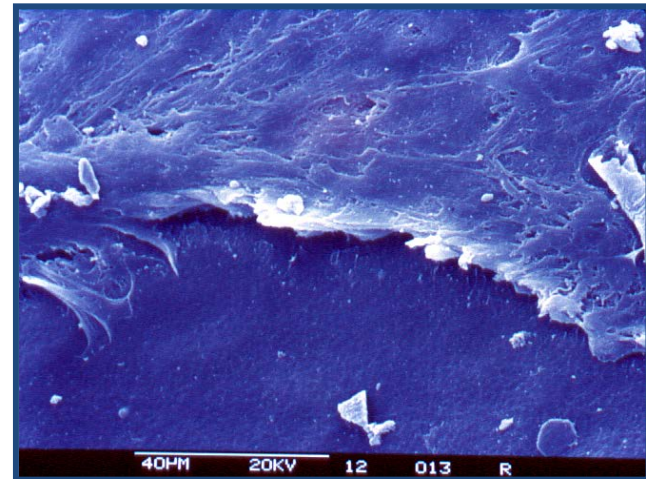
- Ricostruzione strutture
- Terapia della sindrome da disfunzione lacrimale
- Terapia dell'infiammazione
- Stimolazione dei fenomeni riparativi





# SO: lesioni

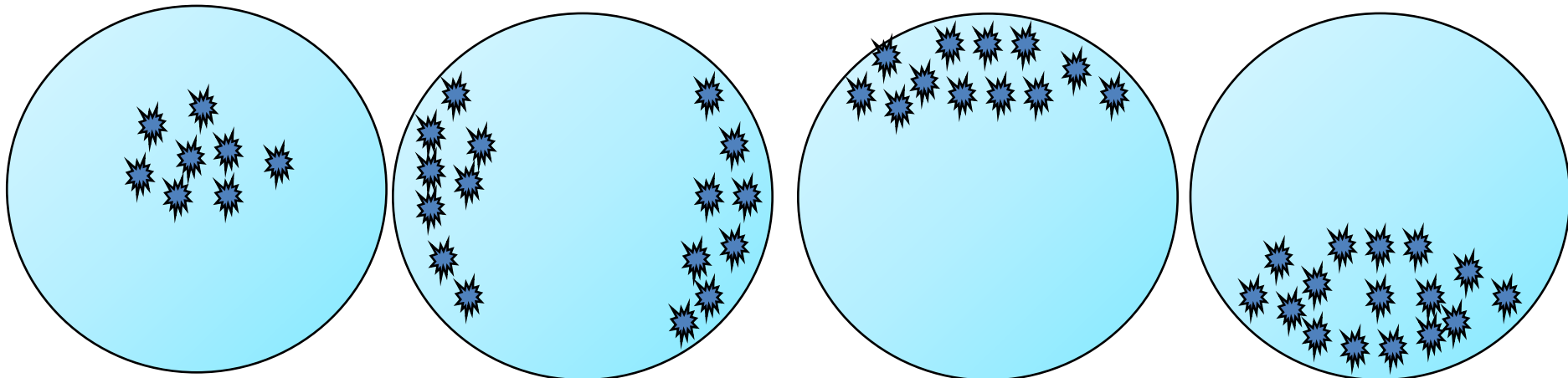
- Lesione epitelio, no danno MB: guarigione precoce
- Lesione epitelio e danno MB: guarigione precoce, ritardata adesione epitelio-stroma
- ✓ Riparazione epitelio corneale post-PRK: 72 ore (fase di latenza, migrazione, proliferazione)
- ✓ Ripristino adesione epiteliale: 2-3 mesi
- Lesione epiteliale con **LSCD** o deficit di cellule staminali congiuntiva: **metaplasia mucosa** o **squamosa**



Female, 43 years, 25 y post ibuprofen-induced toxic epidermal necrolysis

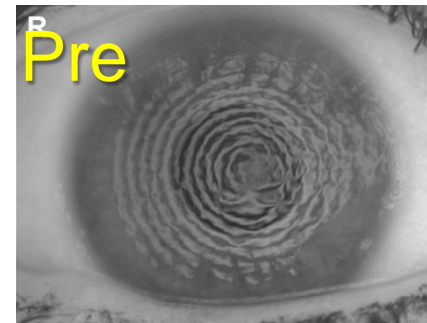
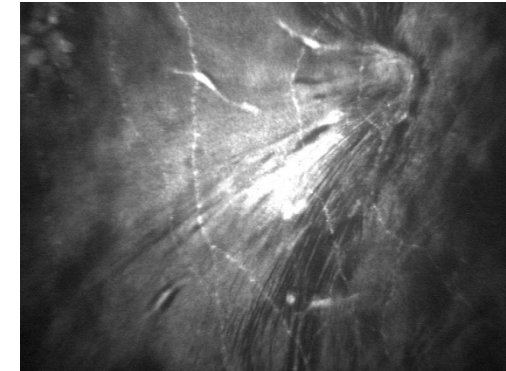
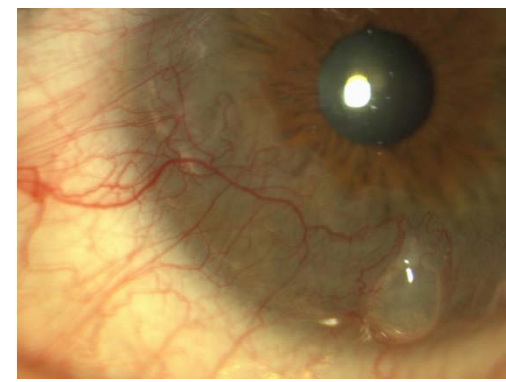
# Ulcera corneale

- Sempre preceduta da difetto epiteliale
- **Centrale**: eziologia infettiva
- **Periferica**: ipersensibilità, autoimmunità
  - ✓ **settore sup**: palpebra, cheratocongiuntivite limbica
  - ✓ **settore inf**: inoclusione, alterazioni gh Meibomio, *dry eye* (alterazioni congiuntivali precedono quelle corneali)
- Epiteliopatia puntata **diffusa**: tossicità da farmaci
- Coinvolgimento primitivo **stroma**: cheratite interstiziale

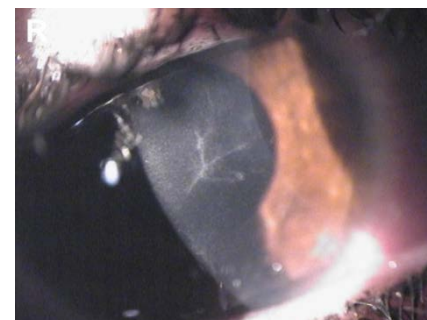


# SO: rigenerazione epiteli

- Presenza di cellule staminali sane
- Ripristino di una membrana basale normale
- Fattori di crescita
- Normale fisiologia film lacrimale e annessi
- Funzionalità cellule staminali: **limitata** dall'infiammazione



Epithelial and topographic alterations in **chronic blepharitis**



# OS epithelia and growth factors

## Fibronectin:

- Expressed at the site of corneal epithelial defects
- Provisional matrix for the migration of epithelial cells
- Stimulates epithelial wound healing *in vitro* and *in vivo*
- Autologous plasma fibronectin eyedrops treat corneal PED

## Substance P, insulin-like GF-1:

- Stimulate corneal epithelial repair *in vitro* and *in vivo*
- Substance P (FGLM-amide) and insulin-like growth factor-1 (SSSR)-derived peptides eyedrops: treat corneal PED

Nishida T. Translational research in corneal epithelial wound healing. Eye Contact Lens 2010;36(5):300-4

Nishida T, Yanai R. Advances in treatment for neurotrophic keratopathy. Curr Opin Ophthalmol 2009;20(4):276-81

# OS epithelia and growth factors

- Agonist and antagonist drugs of **epidermal growth factor receptors (EGFR)**: possible treatment of some skin and corneal disorders
- EGFR activation: promote corneal reepithelialization
- EGFR inhibition: delays epithelial cell proliferation and stratification during corneal regeneration
- **hrEGF** eye drops: could be a treatment for promoting regeneration in epithelial defects

# Neurotrophic factors (NTF)

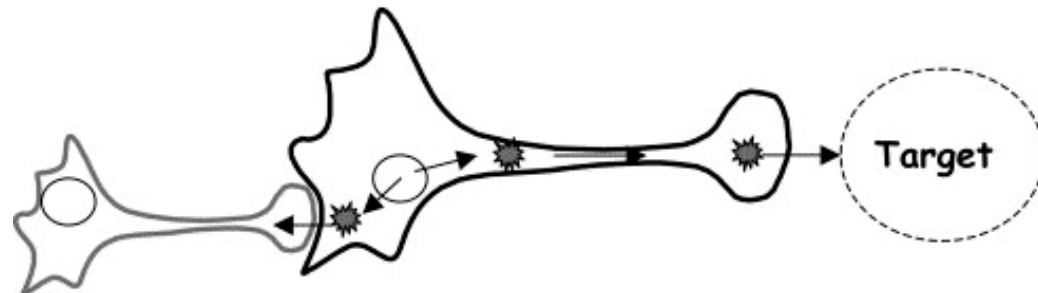
- Family of polypeptides derived from neuron's target cells
- Promote survival of peripheral and central neurons by protecting them from apoptosis
- Possess a range of biological effects in non-neural cells
- Autocrine or paracrine action on stem cells outside CNS

A Retrograde neurotrophin signalling



B

Anterograde neurotrophin signalling



Qi H et al. Patterned expression of neurotrophic factors and receptors in human limbal and corneal regions. *Mol Vis* 2007;16(13):1934-41

# Neurotrophic factors (NTF)

## 1. Neurotrophins (best characterized family):

- ✓ nerve growth factor (NGF)
- ✓ brain-derived neurotrophic factor (BDNF)
- ✓ neurotrophin (NT)-3
- ✓ NT-4/5
- ✓ NT-6

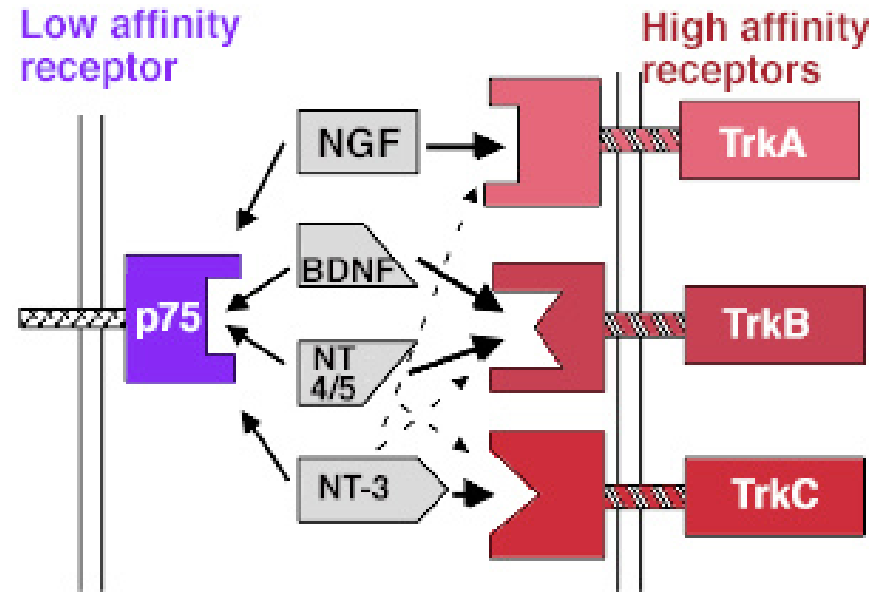
## 2. Glial cell line-derived neurotrophic factor (GDNF)-family

## 3. Ciliary neurotrophic factor (CNTF)-family

# Neurotrophins

- Same low-affinity receptor, **p75NTR**
- Different **Trk receptor tyrosine kinase** for high-affinity binding and signal transduction

- NGF → TrkA
- BDNF, NT-4/5 → TrkB
- NT-3 → TrkC



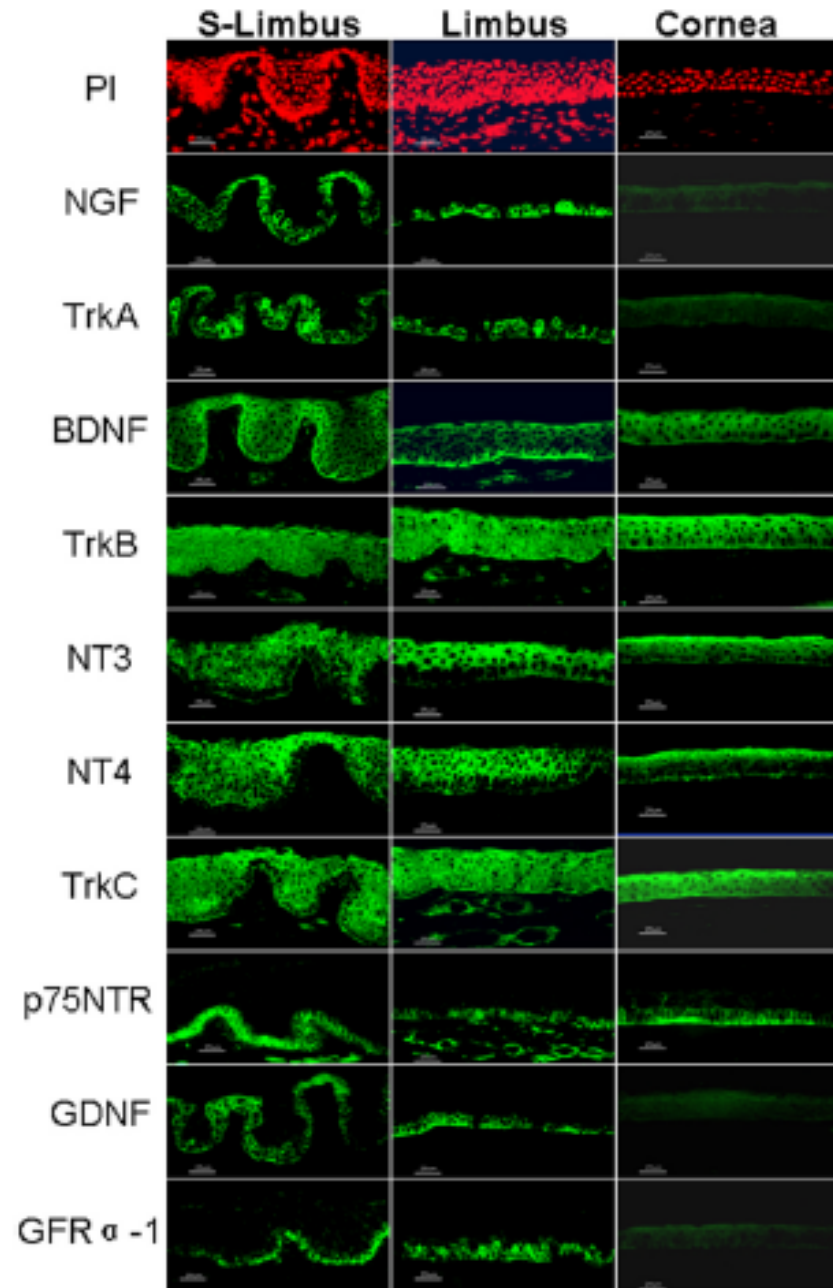


# OS epithelia and NTF

Three patterns of NTF expression potentially involved in epithelial-mesenchymal interaction on the OS:

1. **epithelial type**: NGF, GDNF
2. **paracrine type**: neurotrophin (NT)-3, NT-4/5
3. **reciprocal type**: BDNF

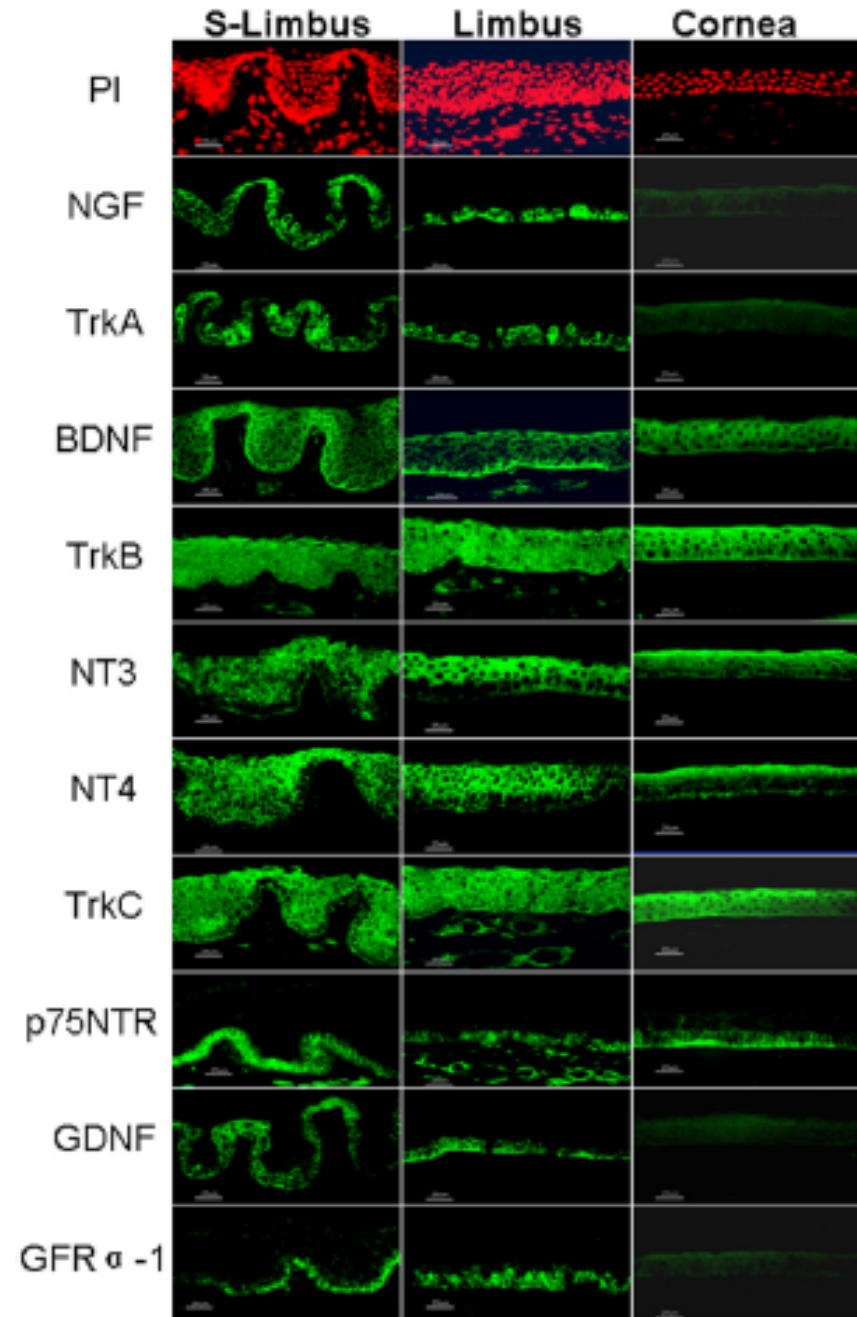
Qi H et al. Patterned expression of neurotrophic factors and receptors in human limbal and corneal regions. *Mol Vis* 2007;16 (13):1934-41



# OS epithelia and NTF

Limbal basal cells express three staining patterns for NTFs:

1. positive for NGF, GDNF, and their receptors, TrkA and GDNF family receptor alpha (GFRalpha)-1
2. relatively high levels of BDNF
3. negative for NT-3 and NT-4



# OS epithelia and neurotrophic factors (NTF)

- **p75NTR**: expressed by the basal layer of the entire corneal and limbal epithelia
- **TrkB, TrkC**: expressed by corneal and limbal epithelia
- **BDNF, p75NTR, TrkB, and TrkC**: expressed by limbal stroma cells
- No immunoreactivity to ciliary neurotrophic factor (CNTF) and its receptor, CNTFRalpha in cornea tissue *in situ*
- NTFs and their receptors may play a vital role in maintaining corneal epithelial stem cells in the limbus
- NGF, GDNF, GFRalpha-1, TrkA, BDNF: may define the corneal epithelial stem cell phenotype

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

## Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration

Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D.,  
Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D.

### ABSTRACT

#### BACKGROUND

Corneal renewal and repair are mediated by stem cells of the limbus, the narrow zone between the cornea and the bulbar conjunctiva. Ocular burns may destroy the limbus, causing limbal stem-cell deficiency. We investigated the long-term clinical results of cell therapy in patients with burn-related corneal destruction associated with limbal stem-cell deficiency, a highly disabling ocular disease.

#### METHODS

We used autologous limbal stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency. Clinical results were assessed by means of Kaplan–Meier, Kruskal–Wallis, and univariate and multivariate logistic-regression analyses. We also assessed the clinical outcome according to the percentage of holoclone-forming stem cells, detected as cells that stain intensely (p63-bright cells) in the cultures.

#### RESULTS

Permanent restoration of a transparent, renewing corneal epithelium was attained in 76.6% of eyes. The failures occurred within the first year. Restored eyes remained stable over time, with up to 10 years of follow-up (mean, 2.91±1.99; median, 1.93). In post hoc analyses, success — that is, the generation of normal epithelium on donor stroma — was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Cultures in which p63-bright cells constituted more than 3% of the total number of clonogenic cells were associated with successful transplantation in 78% of patients. In contrast, cultures in which such cells made up 3% or less of the total number of cells were associated with successful transplantation in only 11% of patients. Graft failure was also associated with the type of initial ocular damage and postoperative complications.

#### CONCLUSIONS

Cultures of limbal stem cells represent a source of cells for transplantation in the treatment of destruction of the human cornea due to burns.

From San Raffaele Scientific Institute, Ophthalmology Unit, Milan (P.R., S.M., G.P., A.S.); and the Center for Regenerative Medicine Stefano Ferrari, University of Modena and Reggio Emilia, Modena, Italy (M.D.L., G.P.). Address reprint requests to Professor Pellegrini at the Center for Regenerative Medicine Stefano Ferrari, University of Modena and Reggio Emilia, Via Glauco Gottardi 100, Modena, Italy, or at graziella.pellegrini@unimore.it.

This article (10.1056/NEJMoa0905955) was published on June 23, 2010, at NEJM.org.

N Engl J Med 2010.

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Graziella Pell

### Summary

**Background** Corneal damage due to ocular burns leads to re-epithelialization failure. Since conjunctival stem cells represent two-thirds of the corneal epithelium, limbal epithelial transplantation of autologous stem cells. However, this approach is limited in bilateral lesions. We investigated restoring the corneal epithelium by transplantation of limbal stem cells.

**Methods** We used autologous limbal stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency.

## Ex Vivo Expansion of Autologous Cultivated Corneal Epithelium

Martin Grueter, MD  
A Contact Lens Transplantation for Limbal Stem-Cell Deficiency

<sup>1</sup>Department of Ophthalmology  
Education Foundation

**Abstract.** Corneal damage due to ocular burns leads to re-epithelialization failure. Since conjunctival stem cells represent two-thirds of the corneal epithelium, limbal epithelial transplantation of autologous stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency.

**Background.** Corneal damage due to ocular burns leads to re-epithelialization failure. Since conjunctival stem cells represent two-thirds of the corneal epithelium, limbal epithelial transplantation of autologous stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency.

Nick Di Girolamo

# The Role of Limbal Stem Cells in Corneal Epithelial Maintenance

Testing the Dogma

Harminder S. Dua, MD, PhD,<sup>1</sup> Ammar Miri, MD,<sup>1</sup> Thaer Alomar, MD,<sup>1</sup> Aaron M. Yeung, MD,<sup>1</sup> Dalia G. Said, MD<sup>1,2</sup>

**Objective:** To study and characterize the epithelial cells in patients with a central "island" of normal epithelial cells surrounded with 360° of clinically apparent limbal stem cell (SC) deficiency with conjunctivalization of the limbus and peripheral cornea.

**Design:** Observational, prospective, consecutive case series.

**Participants:** Five human subjects (8 eyes) who presented with total limbal SC deficiency in 1 or both eyes with a central area of normal corneal epithelial cells.

**Methods:** Clinical slit-lamp examination, aided with fluorescein staining, for evidence of conjunctivalization and in vivo confocal microscopy (IVCM) of the conjunctivalized limbus and peripheral cornea and the normal central corneal epithelium.

**Main Outcome Measure:** Long term survival of normal stratified corneal epithelial cell sheet in the presence of total limbal SC deficiency.

**Results:** In all 8 eyes the diagnosis of limbal SC deficiency was confirmed by clinical and IVCM examination. The conjunctivalized area extended circumferentially along the entire limbus, seen clinically by the presence of fluorescein staining cells, epithelial irregularity, and vascularization and by IVCM showing bright conjunctival epithelial cells, superficial and deep blood vessels, and goblet cells. The central corneal epithelial cells had a normal appearance with polygonal superficial cells, well-defined wing cells, and smaller basal cells. The central "islands" of normal epithelial cells remained unchanged over the mean follow-up period of 60 months (range, 8–12 years).

**Conclusions:** The existence and survival of a healthy sheet of corneal epithelial cells over the follow-up period, in the presence of clinically apparent total limbal SC deficiency, suggests a limited role of limbal epithelial SC in physiologic homeostasis of the corneal epithelium.

**Financial Disclosure(s):** The authors have no proprietary or commercial interest in any materials discussed on this article. *Ophthalmology* 2009;116:856–863 © 2009 by the American Academy of Ophthalmology.

## MUC1 BIOSYNTHESIS IN HUMAN CORNEAL AND CONJUNCTIVAL EPITHELIA

Flavio Mantelli<sup>1</sup> and Pablo Argüeso<sup>2</sup>

<sup>1</sup>Department of Ophthalmology, Campus Bio-Medico University of Rome, Italy

<sup>2</sup>Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA

# Survival of Donor Epithelial Cells after Limbal Stem Cell Transplantation

Ali R. Djalilian,<sup>1</sup> Sankaranarayana P. Mabesh,<sup>1</sup> Christian A. Koch,<sup>2</sup> Robert B. Nussenblatt,<sup>1</sup> DeFen Shen,<sup>1</sup> Zbengpin Zhuang,<sup>3</sup> Edward J. Holland,<sup>4</sup> and Chi-Chao Chan<sup>1</sup>

*Invest Ophthalmol Vis Sci.* 2005; 46: 803–7

In summary, in view of the experimental limitations of this manuscript and based on previous research showing expression of MUC1 throughout the entire human ocular surface, MUC1 is not an appropriate marker to discriminate between corneal and conjunctival epithelia.

*Br J Ophthalmol*, 2010

Nature

Vol 456 | 13 November 2008 | doi:10.1038/nature07406

LETTERS

## Oligopotent stem cells are distributed throughout the mammalian ocular surface

François Majo<sup>1,3,†</sup>, Ariane Rochat<sup>1,3</sup>, Michael Nicolas<sup>1,3</sup>, Georges Abou Jaoudé<sup>2</sup> & Yann Barrandon<sup>1,3</sup>

# TOPICAL TREATMENT WITH NERVE GROWTH FACTOR FOR CORNEAL NEUROTROPHIC ULCERS

ALESSANDRO LAMBIASE, M.D., PAOLO RAMA, M.D., STEFANO BONINI, M.D., GIANCARLO CAPRIOGLIO, M.D., AND LUIGI ALOE, PH.D.

**Background** Corneal neurotrophic ulcers associated with impairment of sensory innervation of the cornea may lead to loss of vision, and there is no effective treatment for these ulcers. We evaluated the effects of nerve growth factor in patients with this disorder.

**Methods** Twelve patients (14 eyes) with severe corneal neurotrophic ulcers associated with corneal anesthesia were treated with topical nerve growth factor 10 times daily for two days and then 6 times daily until the ulcers healed. Treatment continued for 2 weeks after the ulcers healed, and the patients were then followed for up to 15 months. The evolution of the corneal disease during treatment and follow-up was evaluated by slit-lamp examination, photography, fluorescein-dye testing, and tests of corneal sensitivity and best corrected visual acuity.

**Results** Corneal healing began 2 to 14 days after the initiation of treatment with nerve growth factor, and all patients had complete healing of their corneal ulcers after 10 days to 6 weeks of treatment. Corneal sensitivity improved in 13 eyes, and returned to normal in 2 of the 13 eyes. Corneal integrity and sensitivity were maintained during the follow-up period (range, 3 to 15 months). Best corrected visual acuity increased progressively during treatment and follow-up in all patients. There were no systemic or local side effects of treatment.

**Conclusions** In this preliminary, uncontrolled study, topically applied exogenous nerve growth factor restored corneal integrity in patients with corneal neurotrophic ulcers. (N Engl J Med 1998;338:1174-80.)

## Amniotic membrane grafts, “fresh” or frozen? A clinical and in vitro comparison

Philip J Addis, Charles J Hunt, John K G Dart



**Background/claims**—The use of “fresh” (hypothermically stored) and frozen amniotic membrane (AM) was compared in a patient with cicatricial pemphigoid with stem cell failure. The viability of both “fresh” and frozen AM epithelial cells was assessed after storage.

**Methods**—AM was stored at either +4°C (“fresh”) or at –80°C (frozen). A “fresh” graft was applied to the cornea following superficial keratectomy. Subsequently, a further frozen graft was applied to the same eye. Viability of the stored AM epithelium was assessed by investigating membrane integrity and mitochondrial activity.

**Results**—In both cases the cornea re-epithelialised and visual acuity improved. Improvement, however, was not sustained.

**Conclusion**—Although both procedures led to an improvement in visual acuity, “fresh” tissue performed no better than frozen in promoting re-epithelialisation. The authors suggest that logistical, safety, and cost considerations outweigh any benefits of using “fresh” as opposed to frozen graft material.

(Br J Ophthalmol 2001;85:905–907)

# Autologous serum eye drops for ocular surface disorders

G Geerling, S MacLennan, D Hartwig

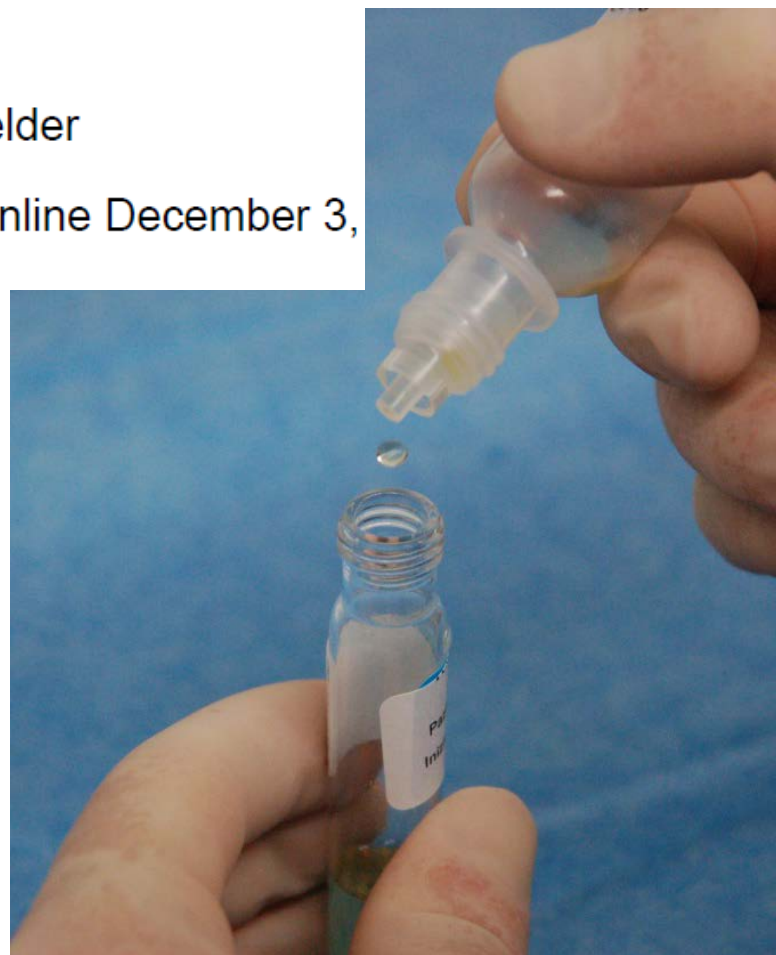
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*Br J Ophthalmol* 2004;88:1467-1474. doi: 10.1136/bjo.2004.044347

## Corneal nerve regeneration in neurotrophic keratopathy following autologous plasma therapy

Kavita Rao, Christopher Leveque and Stephen C Pflugfelder

*Br J Ophthalmol* 2010 94: 584-591 originally published online December 3, 2009



# Amniotic membrane extract



Clinical and Experimental Ophthalmology 2009; 37: 855–863 doi: 10.1111/j.1442-9071.2009.02159.x

## Original Article

### Amniotic membrane extraction solution for ocular chemical burns

Lingyi Liang PhD,<sup>1</sup> Wei Li MD,<sup>2</sup> Shiqi Ling PhD,<sup>3</sup> Hosam Sheha PhD,<sup>4</sup> Weiqiang Qiu PhD,<sup>1</sup> Chaoyang Li MD<sup>1</sup> and Zuguo Liu PhD<sup>1,2</sup>

Published in final edited form as:

*J Cell Physiol.* 2008 June ; 215(3): 657–664. doi:10.1002/jcp.21345.

### Reversal of Myofibroblasts by Amniotic Membrane Stromal Extract

Wei Li<sup>1,2,3</sup>, Hua He<sup>1</sup>, Ying-Ting Chen<sup>1,2</sup>, Yasutaka Hayashida<sup>1,2</sup>, and Scheffer C.G. Tseng<sup>1,2,\*</sup>

