Corneal Reconstruction Using Tissue-Engineered Epithelial Cell Sheets Fabricated ex vivo From Autologous Oral Mucosal Epithelium

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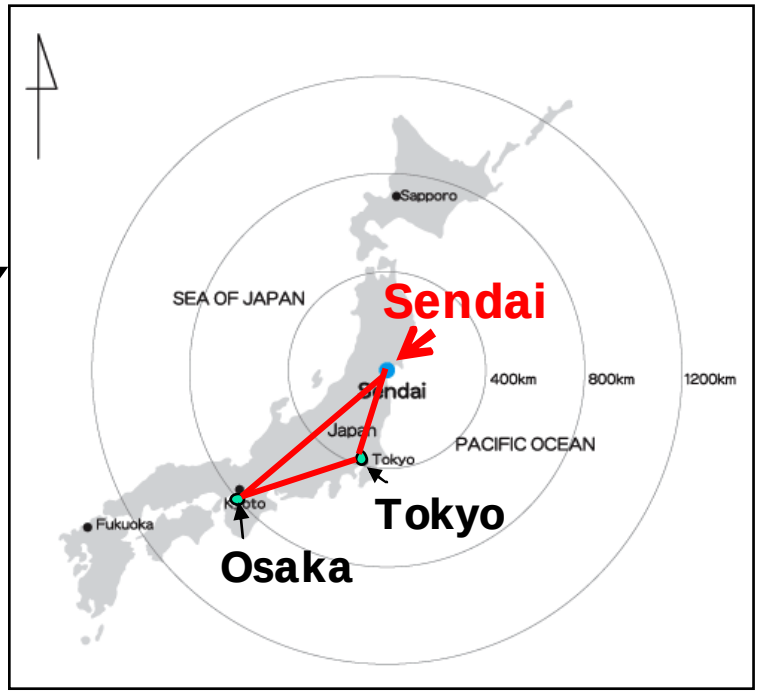
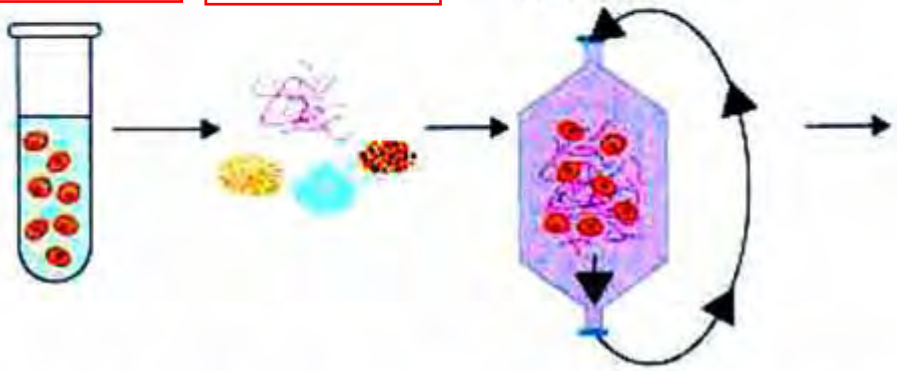


Concept of Regenerative Medicine (Tissue Engineering)

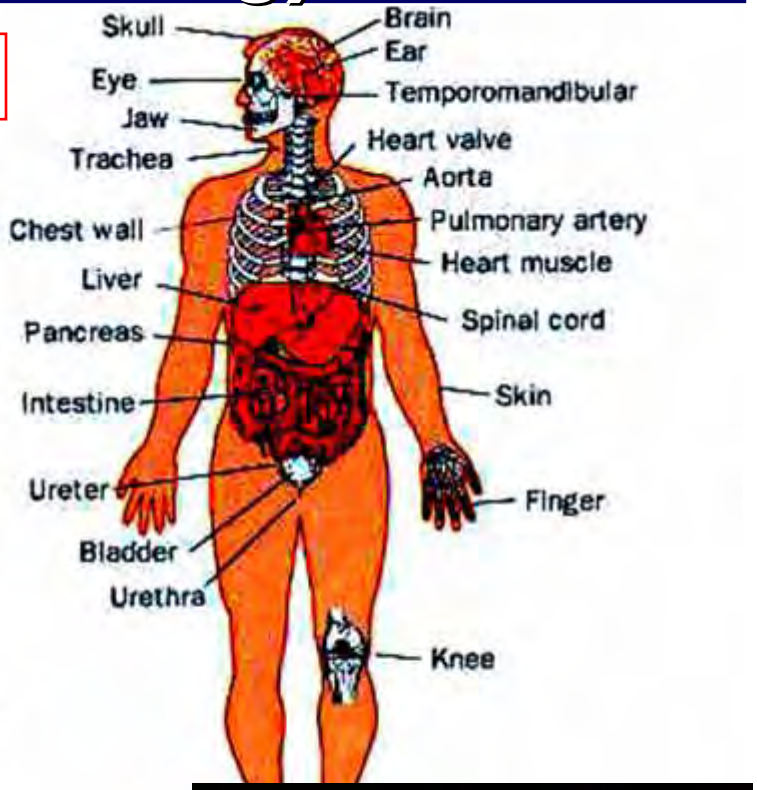
Stem Cells **Scafold** **Growth Factor**

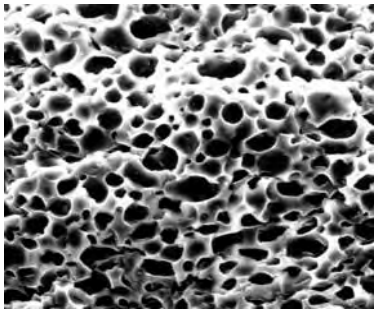


* Cells may be tissue specific, stem cells, or embryonic stem cells. They may be autologous or allogenic

† The matrix may be natural or synthetic. It may be fibrous, a foam, a hydrogel, or capsules

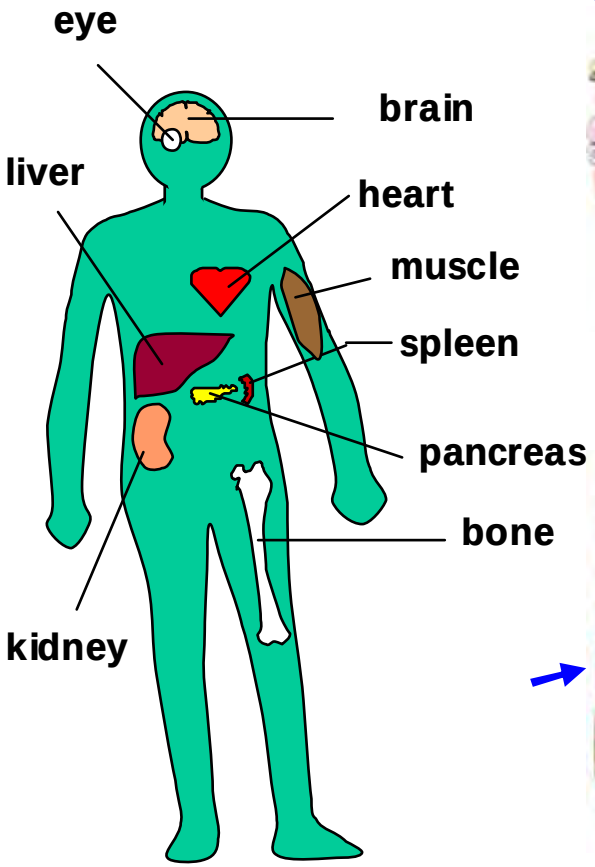
‡ In-vitro culture may be in static, stirred, or dynamic flow conditions



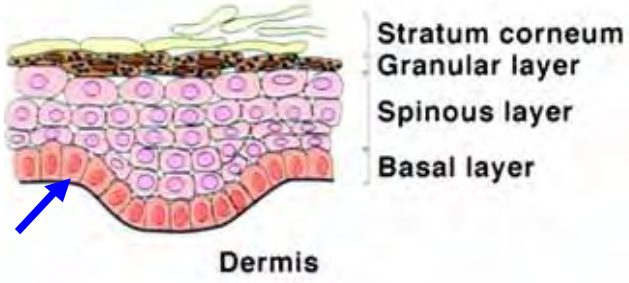


from Vacanti & Langer Science 1993

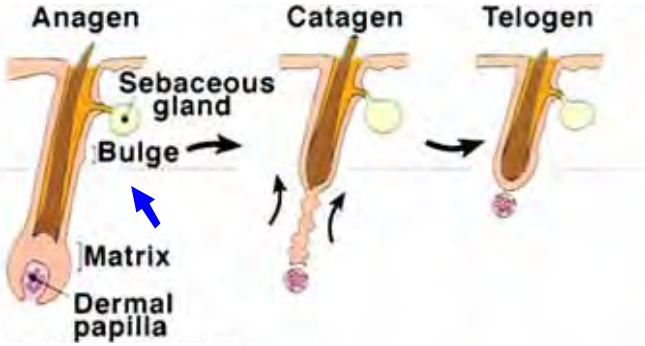
Tissue Stem Cells



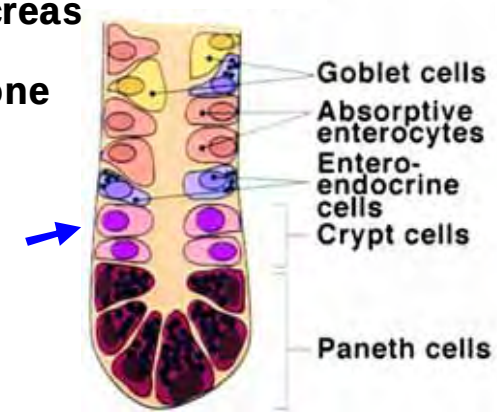
Epidermal stem cell



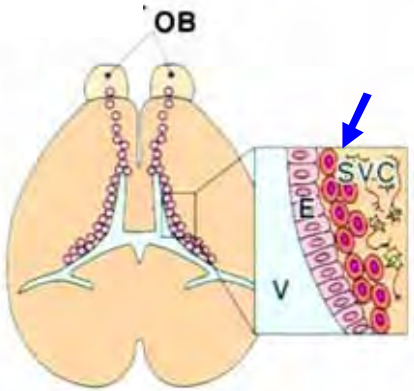
Hair follicel stem cells



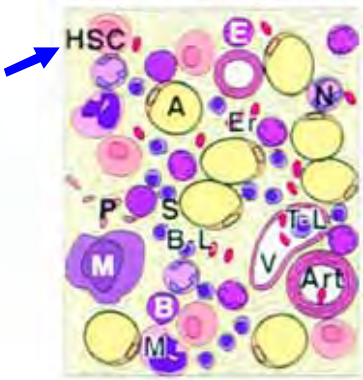
Gastrointestinal stem cells



Neural stem cells



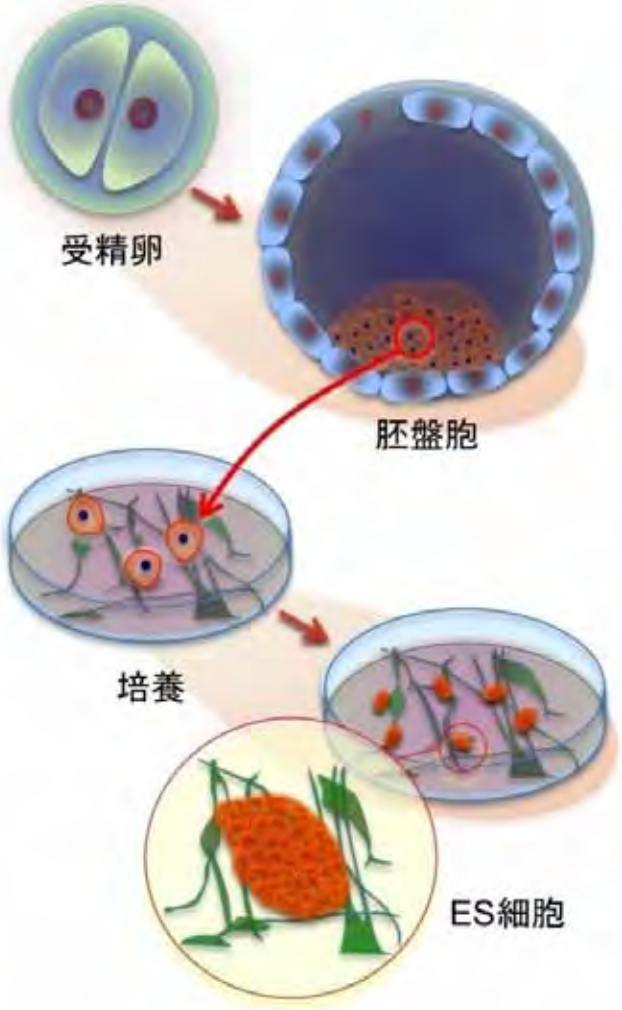
Hematopooetic stem cells



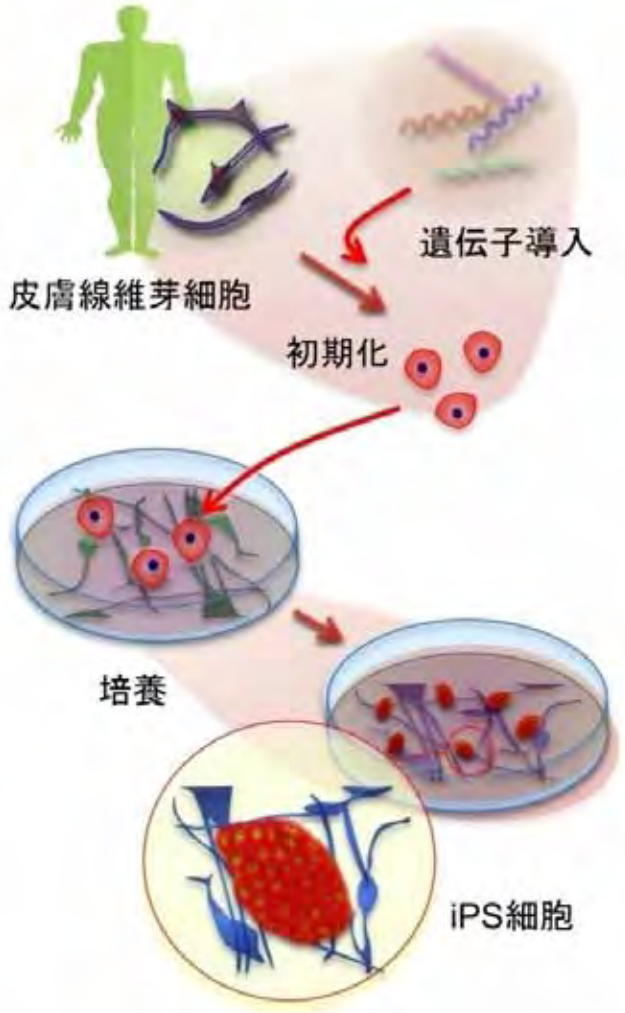
E. Fuchs, *Cell* 2000

Pluripotent Stem Cells

Embryonic stem cells



induced pluripotent stem cells

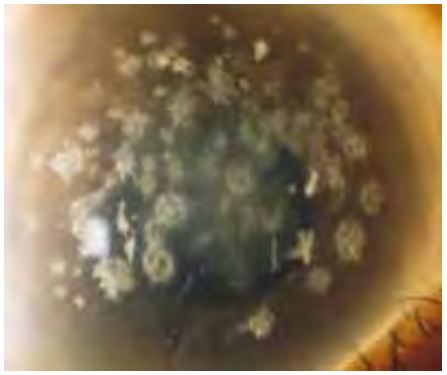


Corneal Diseases

Corneal epithelial disease

Alkali, thermal burn
Stvens-Johnson syndrome

Stromal disease



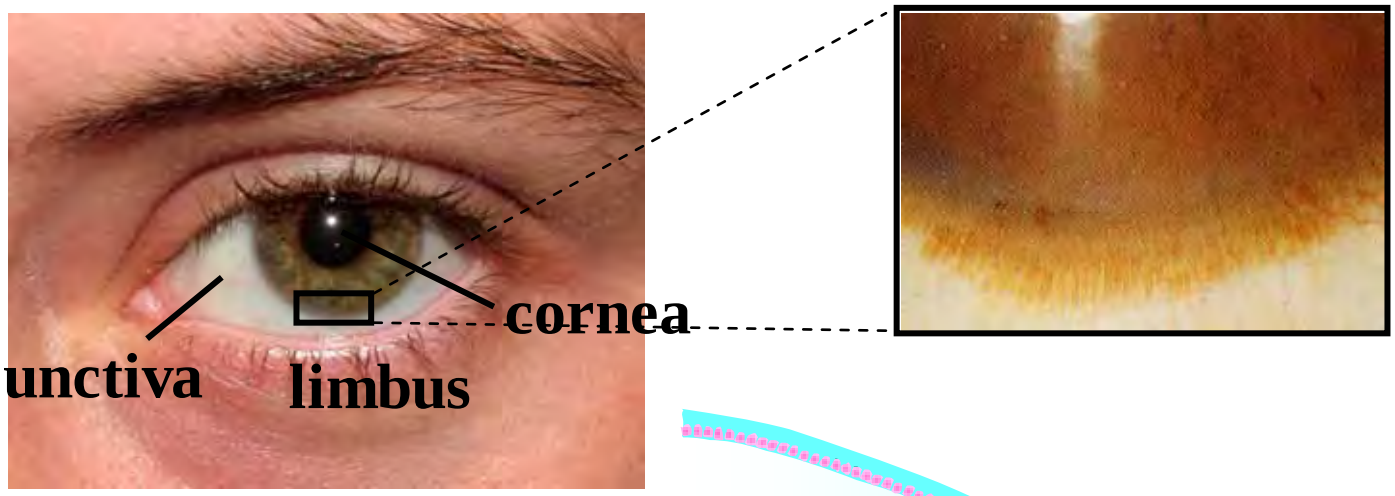
Corneal dystrophy

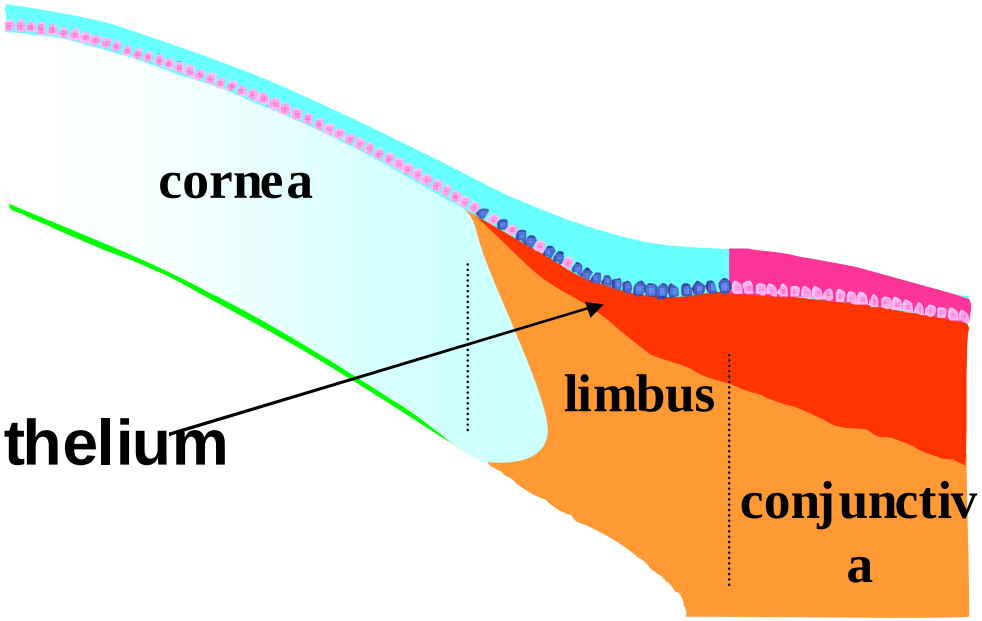
Endothelial disease

Bullous keratopathy

Standard therapy: allogeneic corneal transplanation using eye bank cornea

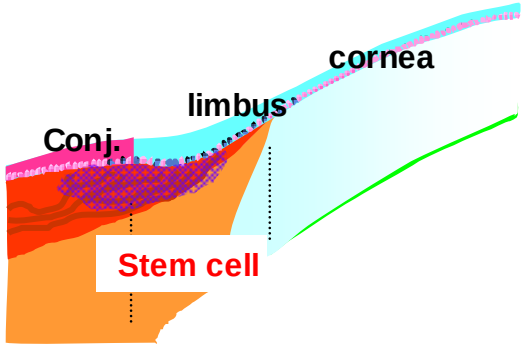
Corneal Epithelial Stem Cell



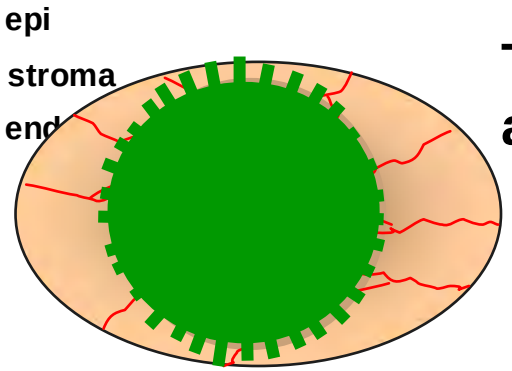


Basal cells of Limbal epithelium
=stem cells

Corneal epithelial stem cells



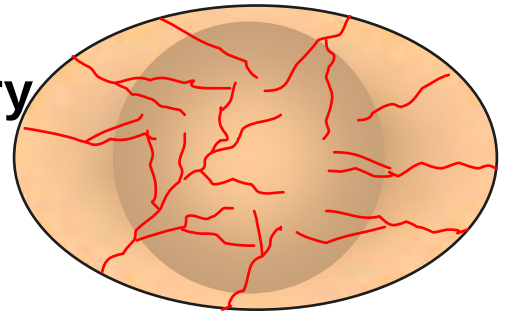
Normal cornea



Limbal stem cell deficiency

Thermal alkali injury

SJS



Limbal stem cell deficiency

Congenital

Aniridia

Secondary

Chemical burn

Thermal burn

Conjunctivalization

Idiopathic

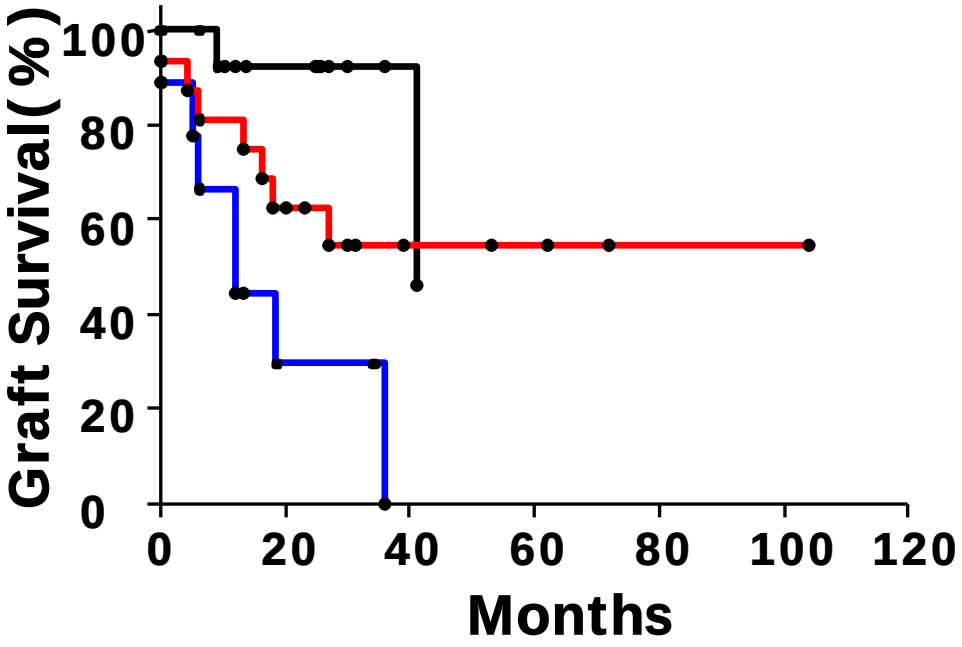
Primary

Stevens-Johnson

Ocular cicatricial pemphigoid

（Nishida K. Cornea 2003）

Long-Term Results of Corneal Transplantation for Corneal Epithelial Diseases

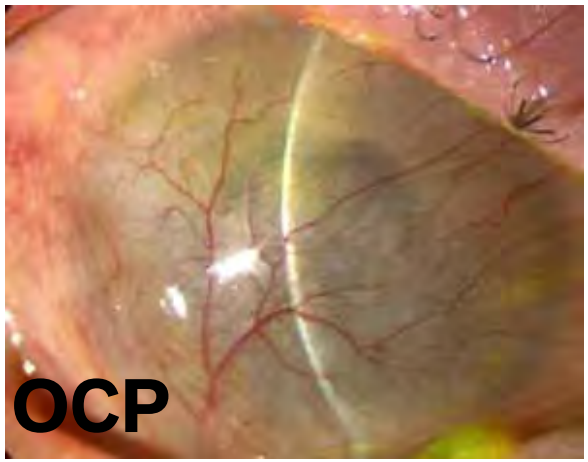


Chem Burn
SJS
Other

(Solomon et al,

Ophthalmology, 2002)

High Risk of Rejection



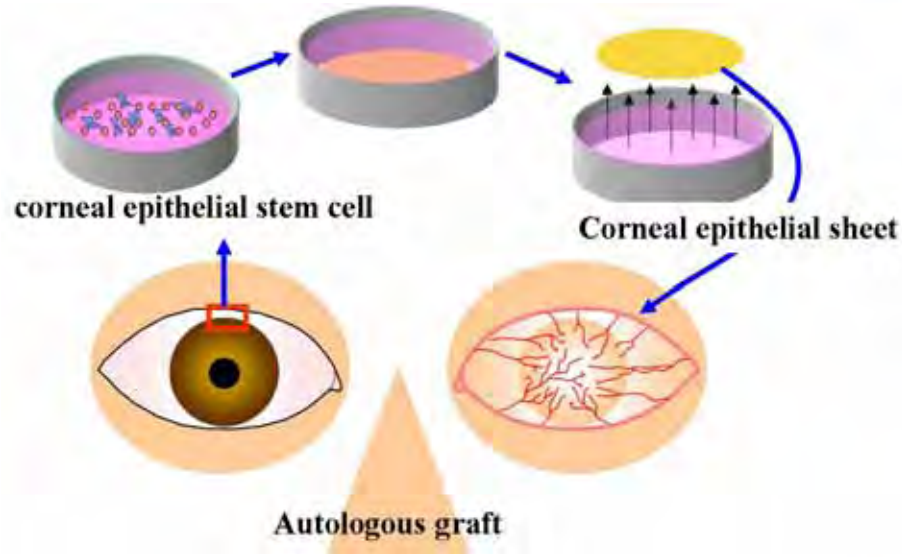
Organ / tissue Transplant → **Regenerative Medicine**

Corneal transplant → **Autologous epithelial cell sheet transplantation**

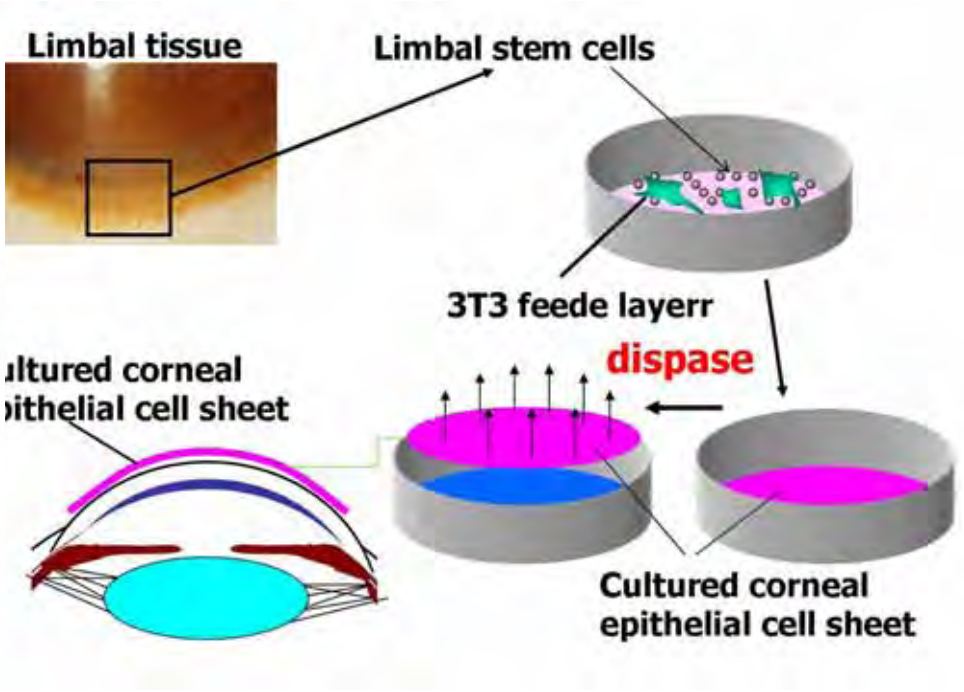
Rejection
Limited number of Donor cornea

Initial Report

Cell source: Corneal epithelial stem cell



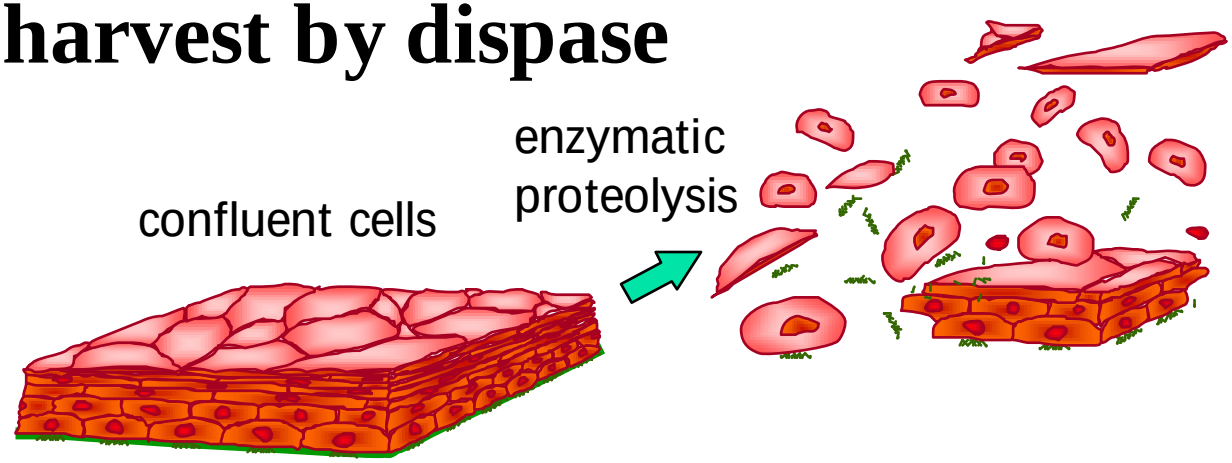
Cultured corneal epithelial sheet harvested by dispase treatment



Pellegrini et al. Lancet 1997

The problems of Previous Methods

- **Cell sheet harvest by dispase**



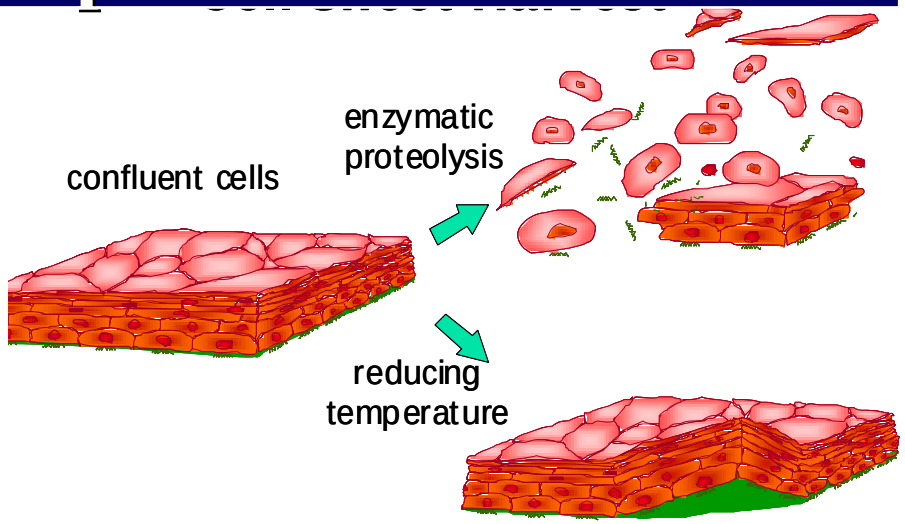
marked degradation of cell-to-cell junction and ECM ⇒*fragile cell sheet*

- **Cell source: corneal epithelial stem cells**

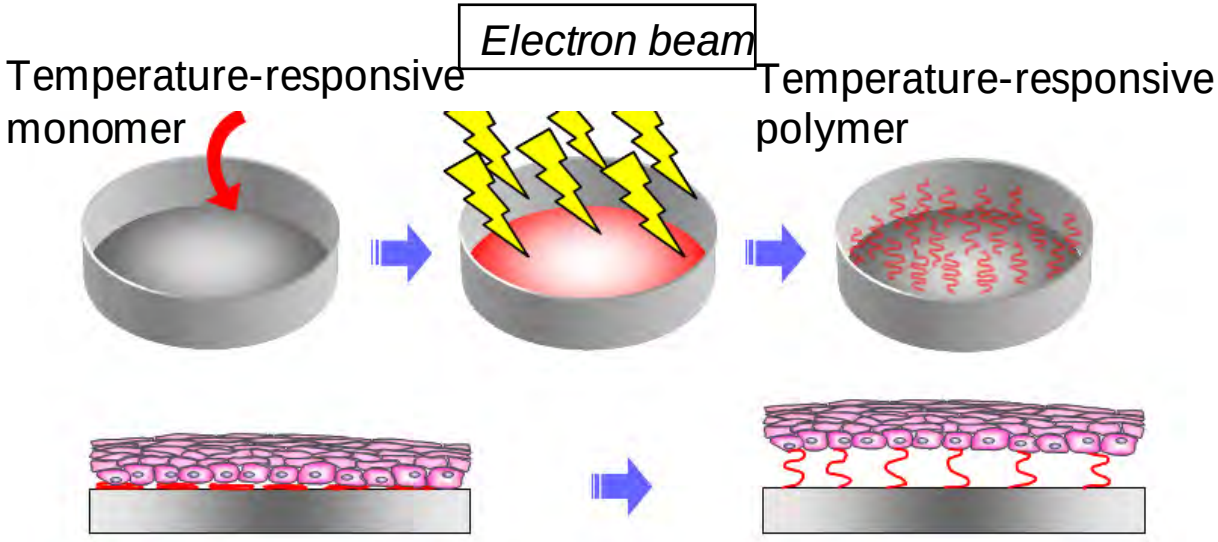
  Applied only to unilateral disease

Cell sheet harvest Issue
Use of Temperature responsive culture dish

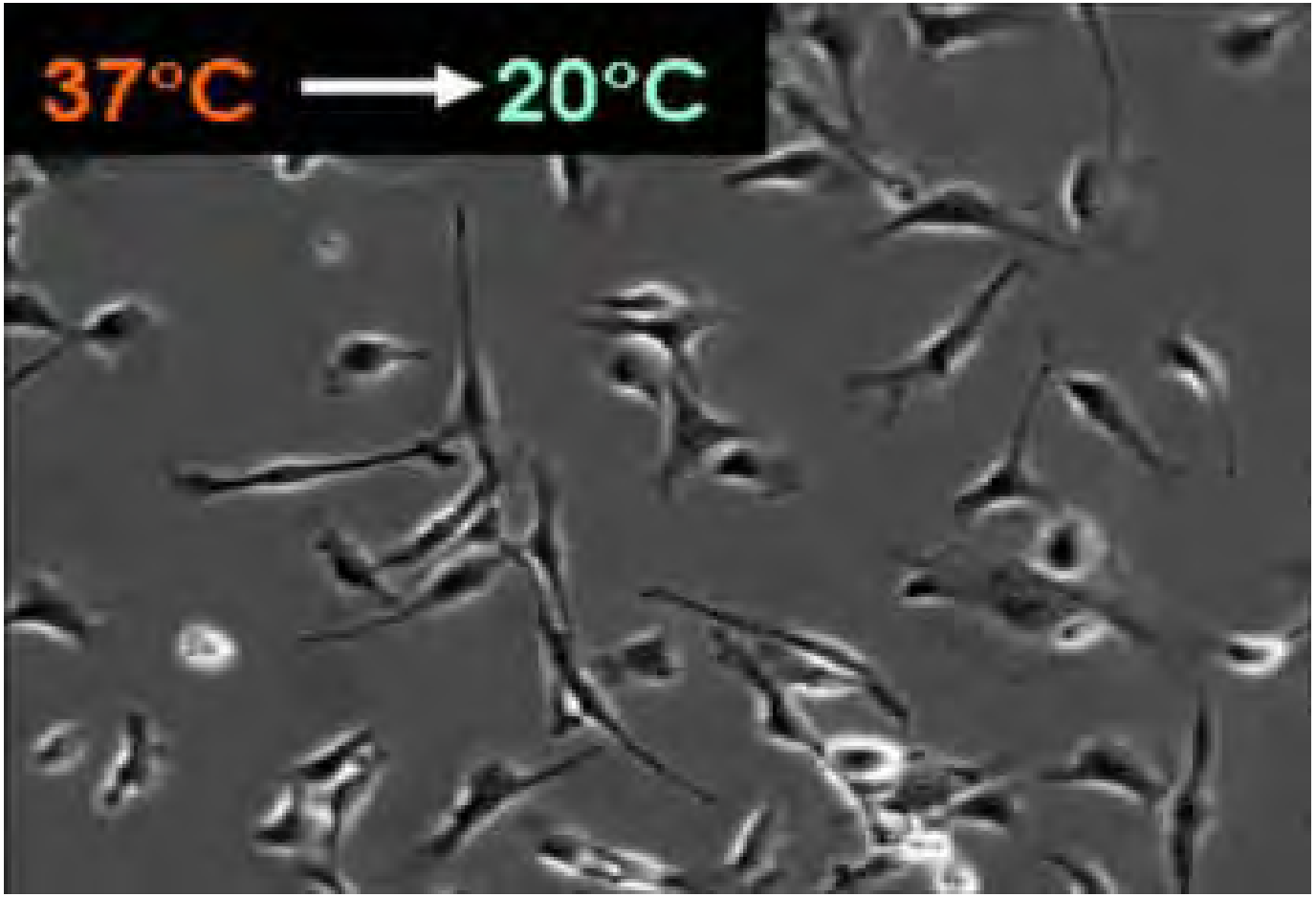
- **37℃ hydrophobic**
  ⇒ cell attachment
- **below 32℃ hydrophilic**
  ⇒ cell detachment



✓ Retain cell-cell junctions as well as deposited extracellular matrix below the lower cell membranes

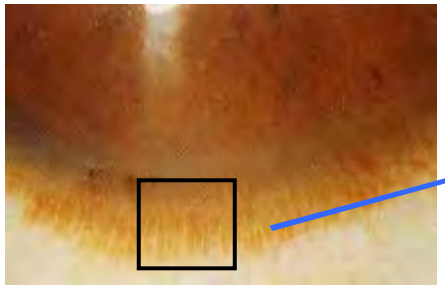


Cell attachment and detachment by temperature change

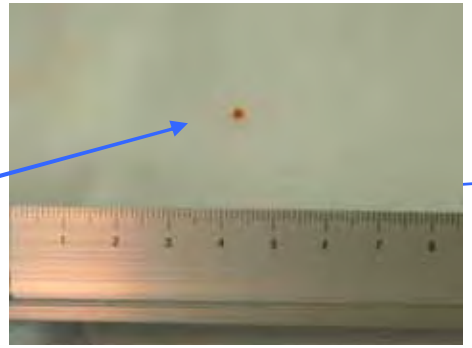


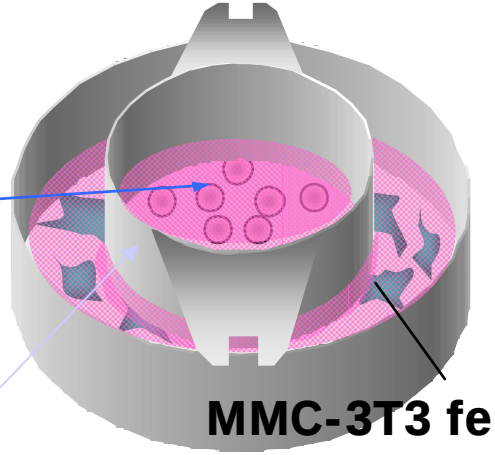
NEW TECHNOLOGY: tissue-engineered epithelial cell sheet tranplantation

Limbal epithelium



2mmX2mm



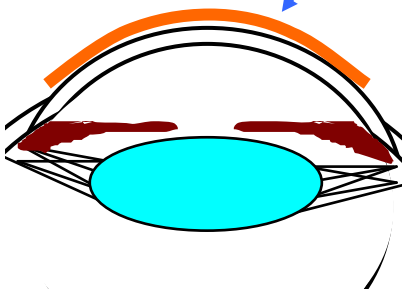


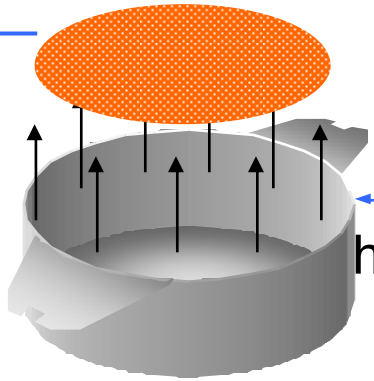
MMC-3T3 feeder

Temperature-responsive culture dish

37°C culture
2 weeks

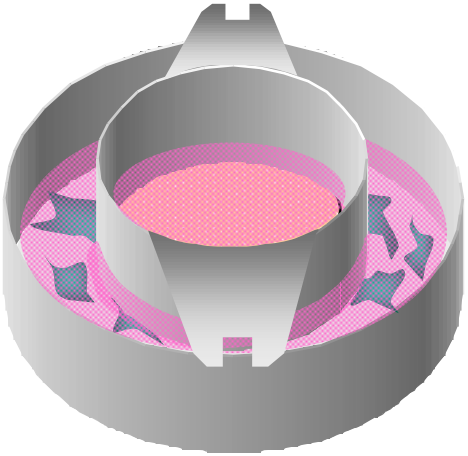
Tissue-engineered epithelial cell sheet





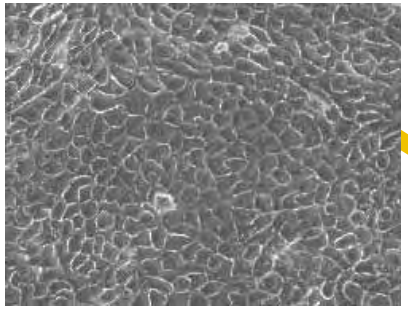
20°C

harvest

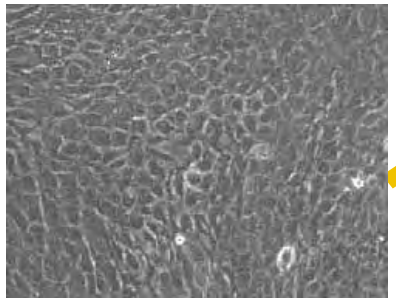


(Nishida K, Transplantation 2004)

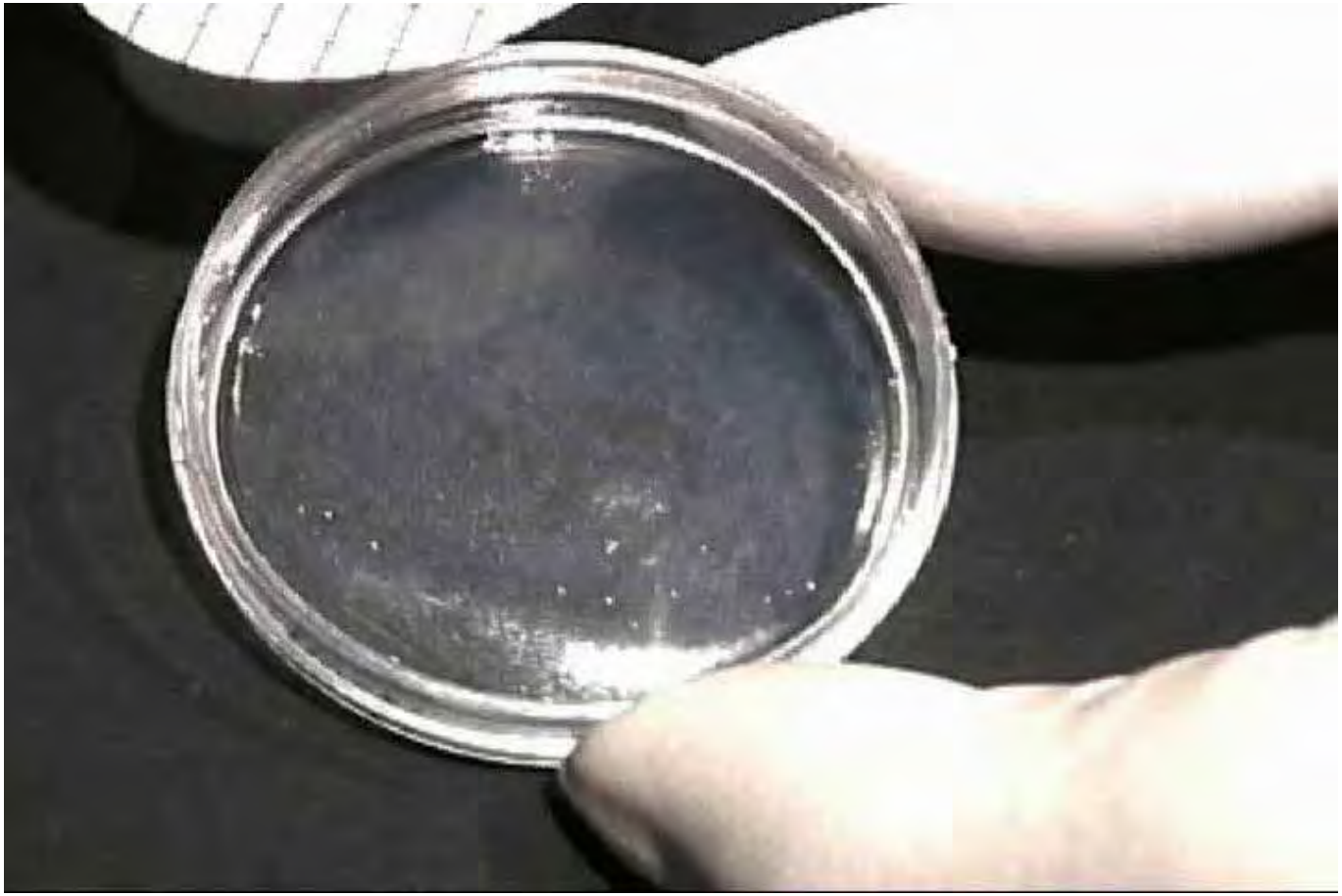
Cell sheet harvest



Cultivated corneal sheet



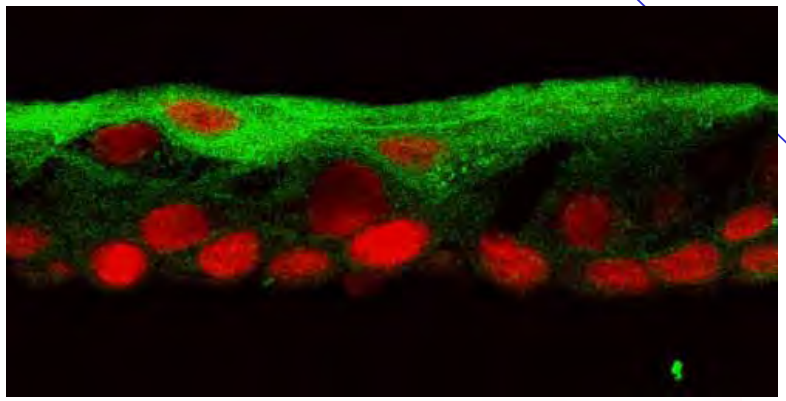
Cultivated oral mucosal sheet



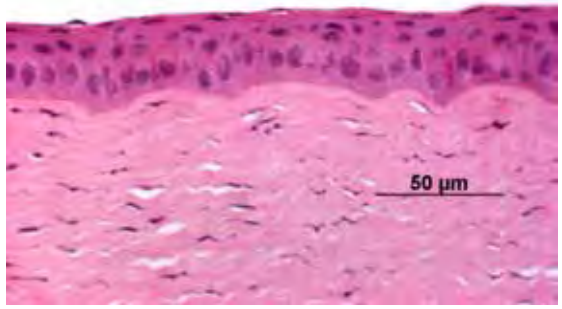
Characteristics of cultivated human corneal epithelial sheet

H&E

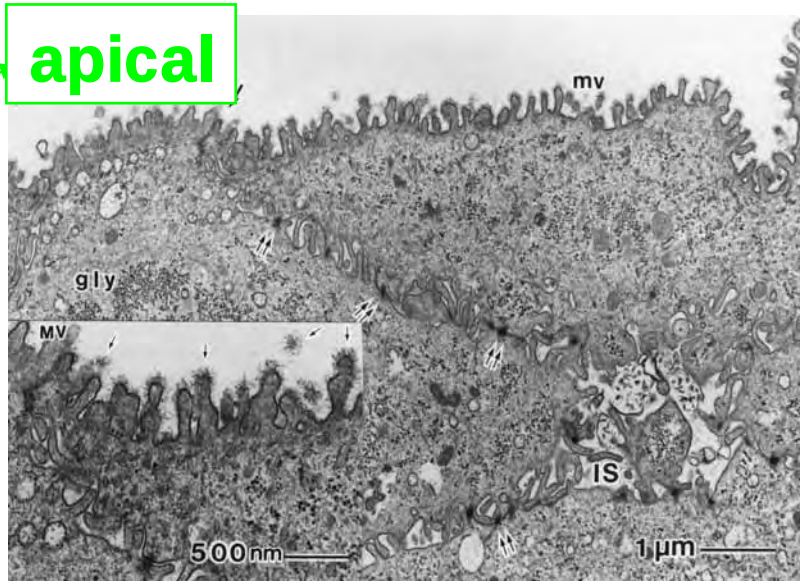
K3



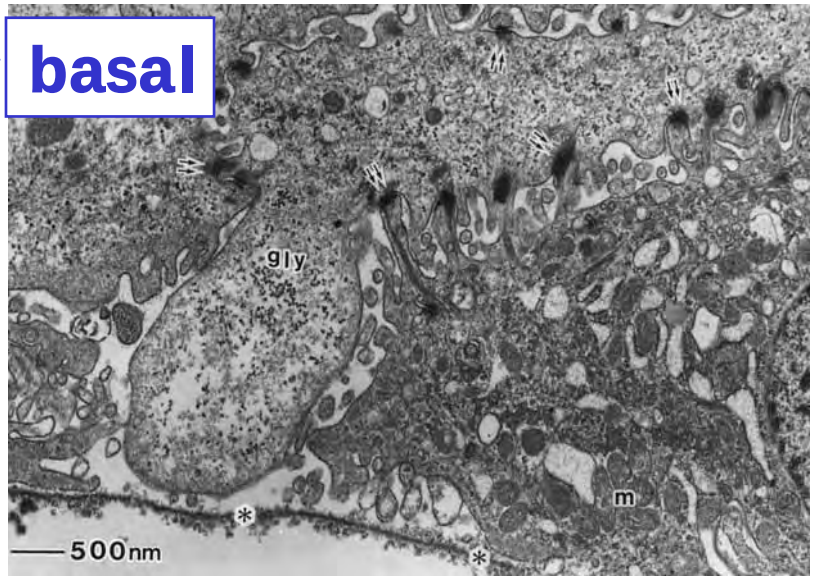
Normal cornea



apical



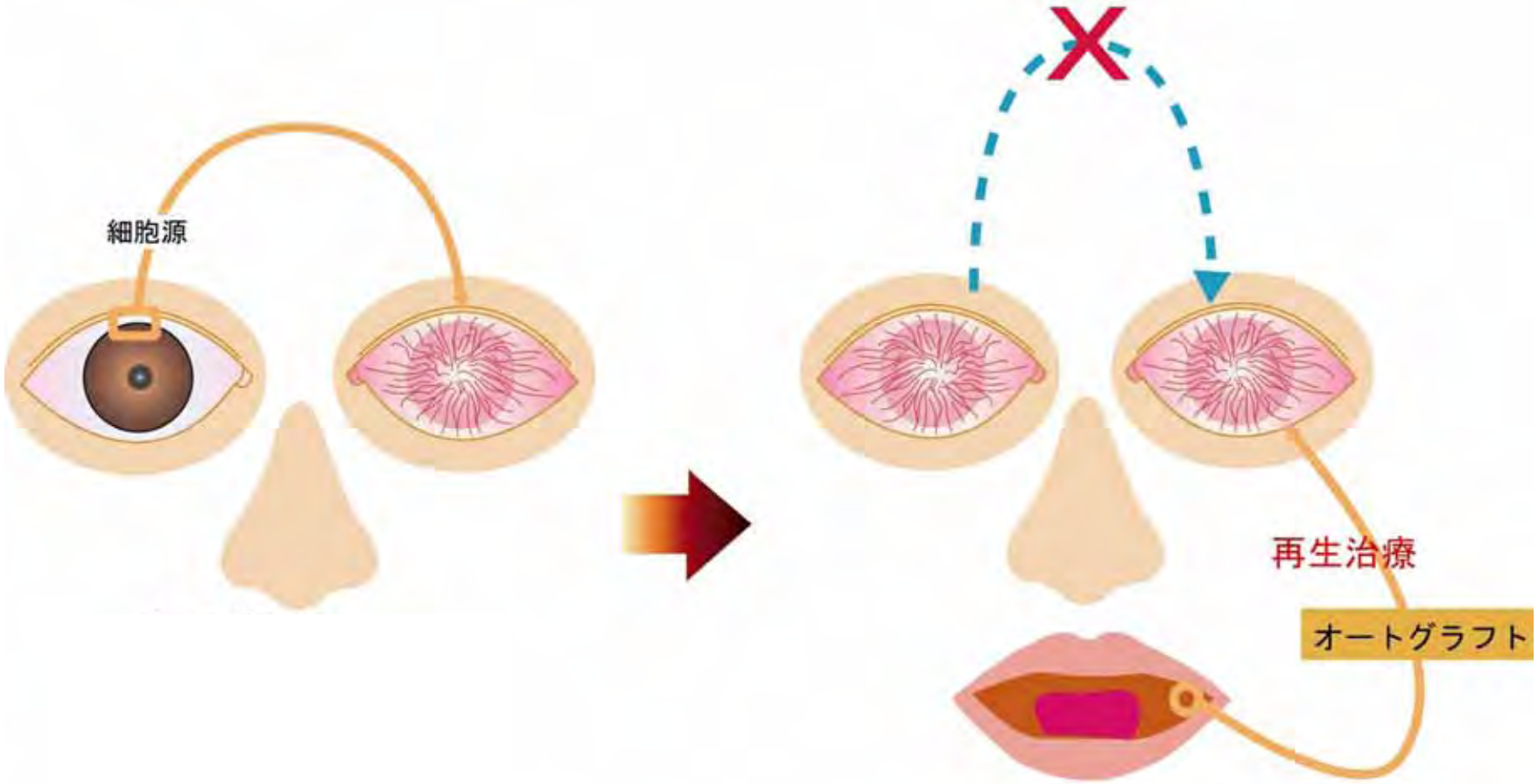
basal



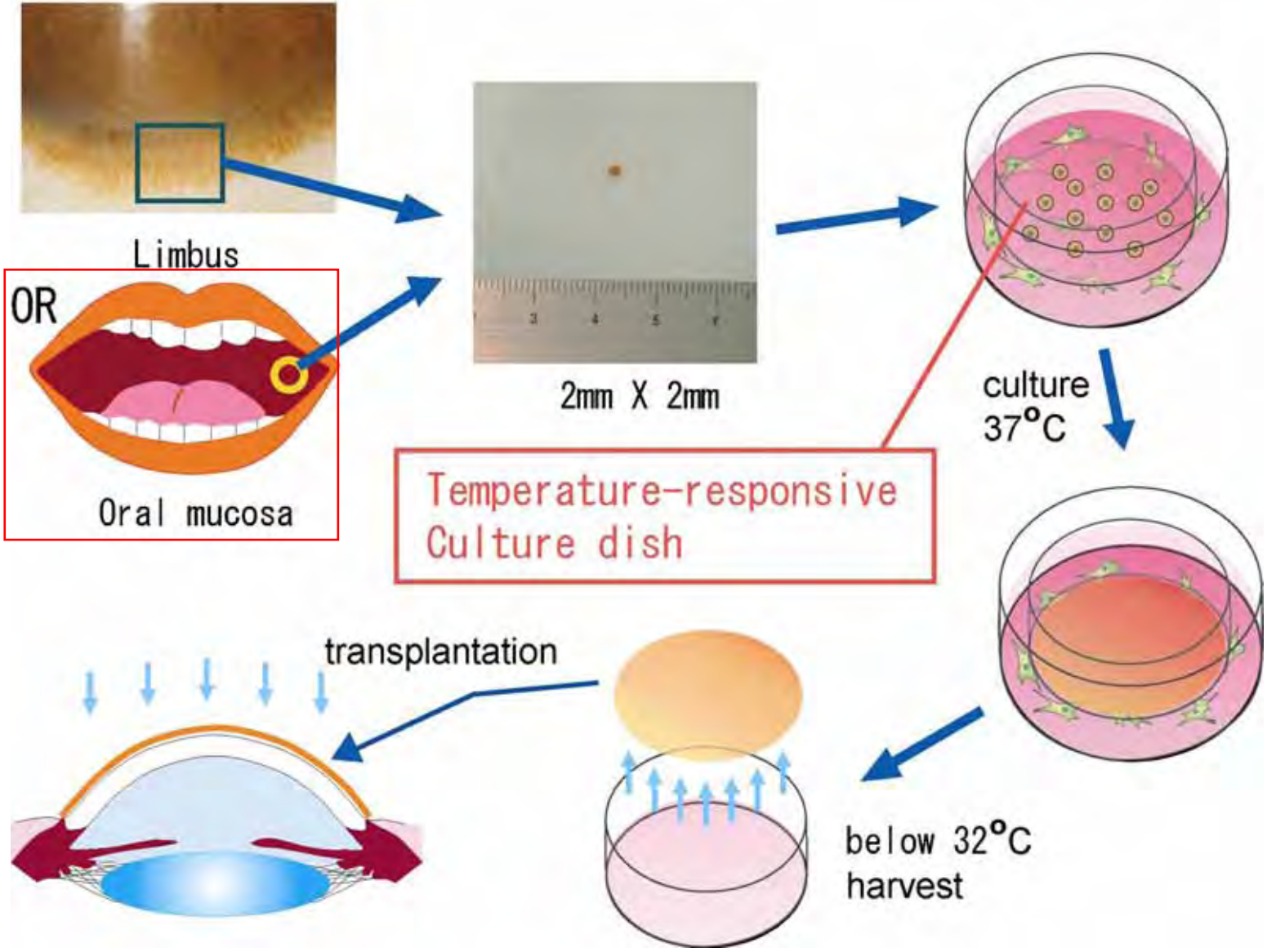
(Nishida K, Transplantation 2004)

Autologous cell soruce

Unilateral diseases **Bilateral diseases**



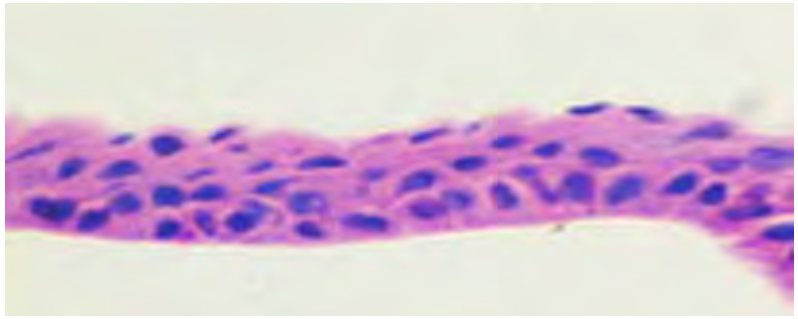
Transplantation of tissue-engineered epithelial cell sheet for corneal diseases



(Nishida K et al. Transplantaion, 2004)　(Nishida K, et al. N Engl J Med 2004)

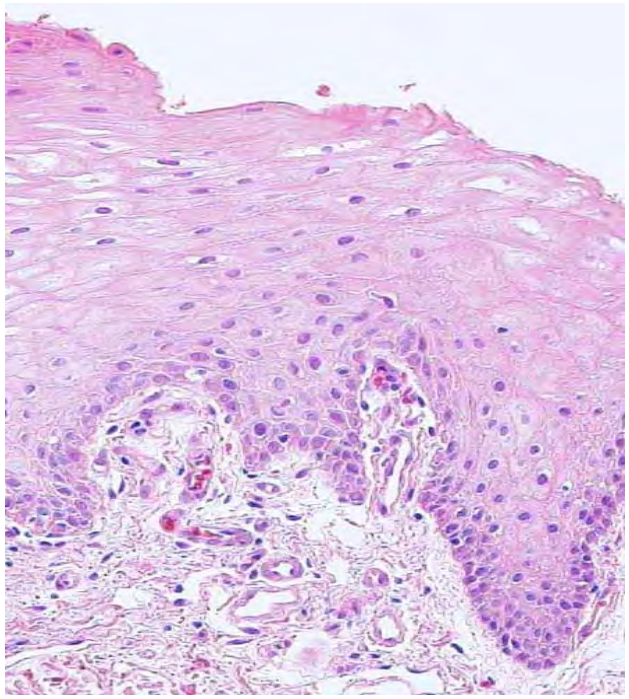
Histology

Normal cornea



Corneal epithelial cell sheet

(Nishida K et al. Transplantaion, 2004)



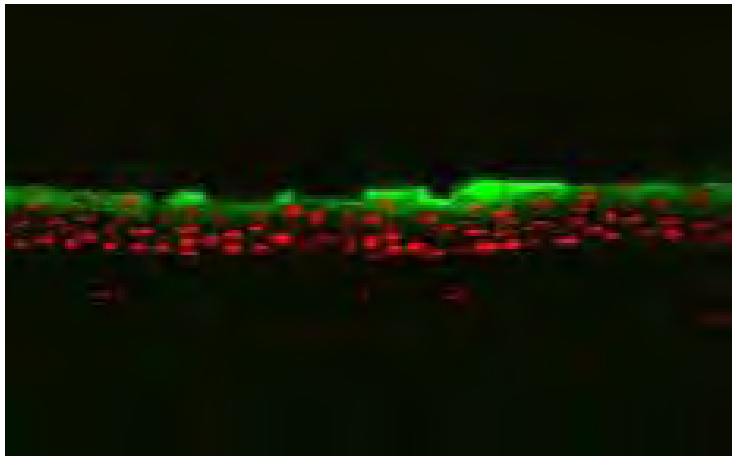
Normal oral mucosa

Oral mucosal epithelial cell sheet

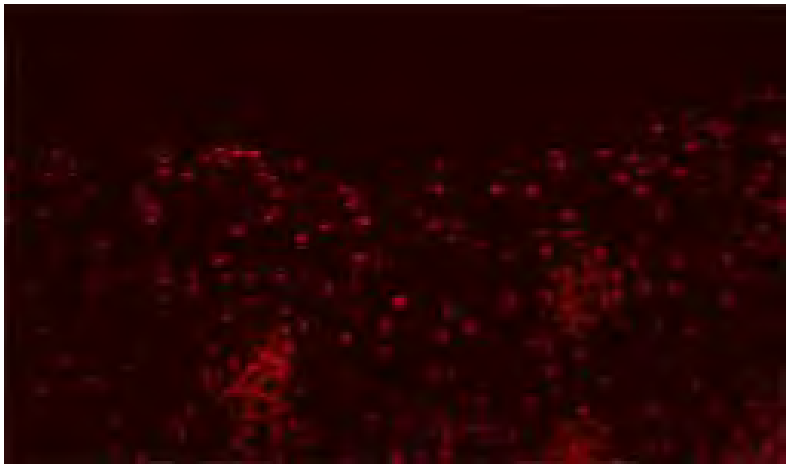
(Nishida K, et al. N Engl J Med 2004)

Immunostaining for MUC 16

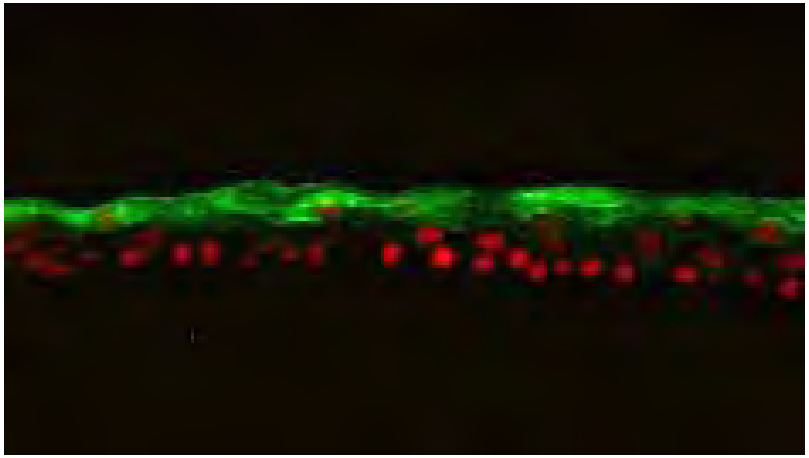
Corneal epithelium (in vivo)



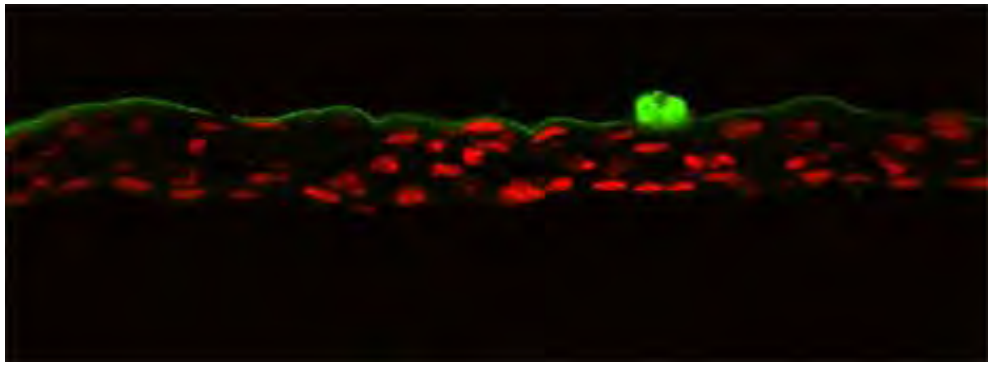
Oral mucosal epithelium (in vivo)



Corneal epithelial cell sheet



Oral mucosal cell sheet



(Hori Y, et al Exp Eye Res 2008)

Clinical Application

- **Subjects**

  Severe total limbal stem cell deficiency:

  Stevens-Johnson syndrome, Ocular cicatricial pemphigoid, Chemical burn, Thermal burn, Aniridia, etc

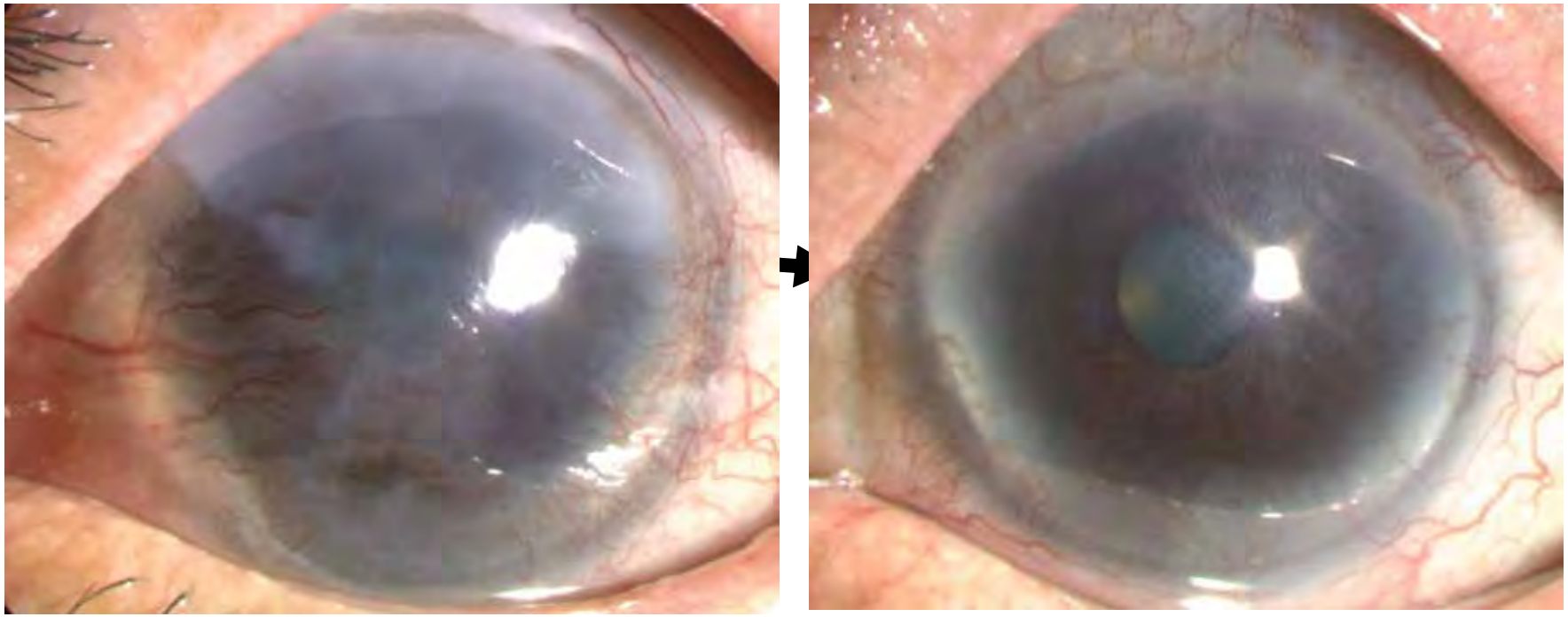
- **Surgery**

  Cell sheet transplantation using autologous cell soruce

- **Post-operative management**
  - Topical administration of 0.3 % levofuloxacin and 0.1 % betamethasone four times daily
  - Systemic administration of betamethasone (1-2 mg/day) for the first two weeks after surgery

CASE Autologous Oral Epithelial Cell Sheet Transplantation to Treat Ocular Pemphigoid

Pre-op **14 months post-op**



VA=20/2000 **VA=20/25**

Results of Initial 4 Cases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ORIGINAL ARTICLE

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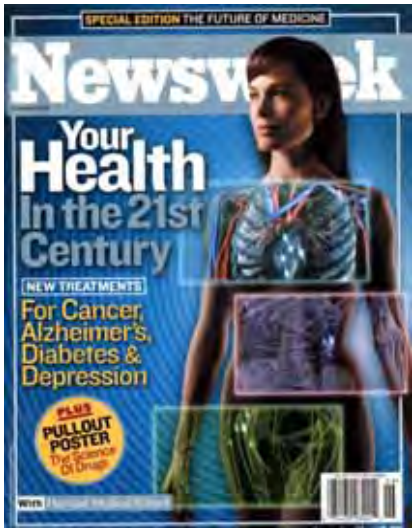
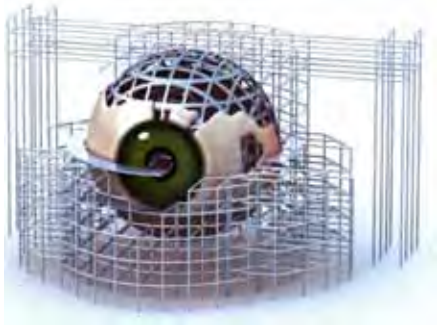
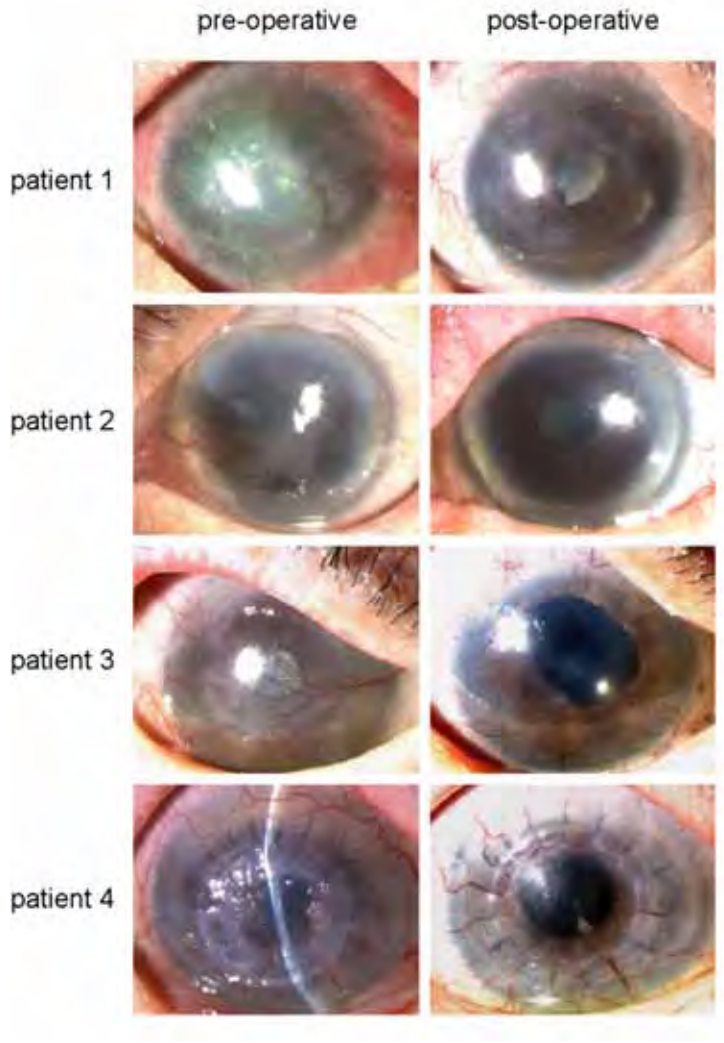
Corneal Reconstruction with Tissue-Engineered Cell Sheets Composed of Autologous Oral Mucosal Epithelium

Kohji Nishida, M.D., Ph.D., Masayuki Yamato, Ph.D., Yasutaka Hayashida, M.D., Katsuhiko Watanabe, M.Sc., Kazuaki Yamamoto, M.Sc., Eijiro Adachi, M.D., Ph.D., Shigeru Nagai, M.Sc., Akihiko Kikuchi, Ph.D., Naoyuki Maeda, M.D., Ph.D., Hitoshi Watanabe, M.D., Ph.D., Teruo Okano, Ph.D., and Yasuo Tano, M.D., Ph.D.



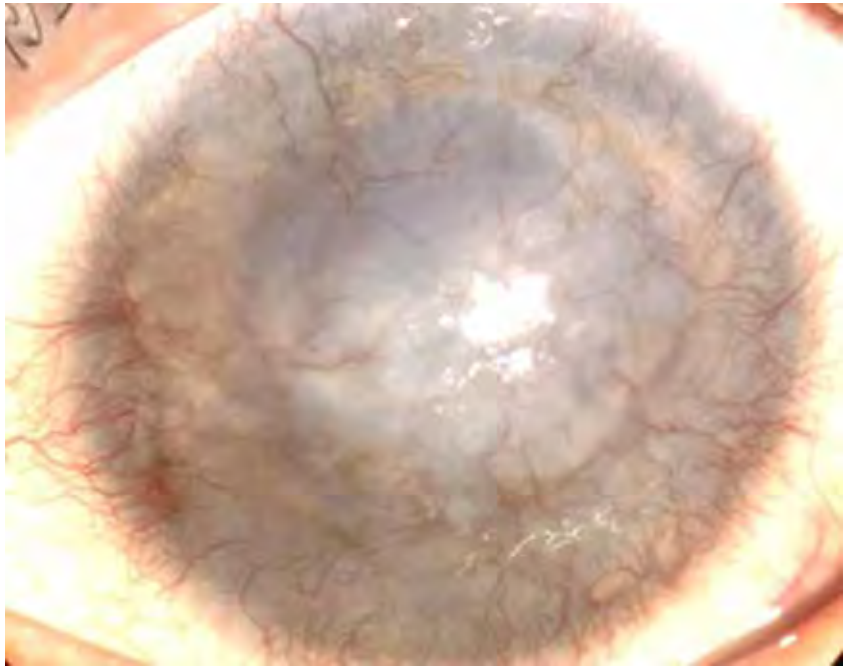




（Nishida K, et al. N Engl J Med 2004）

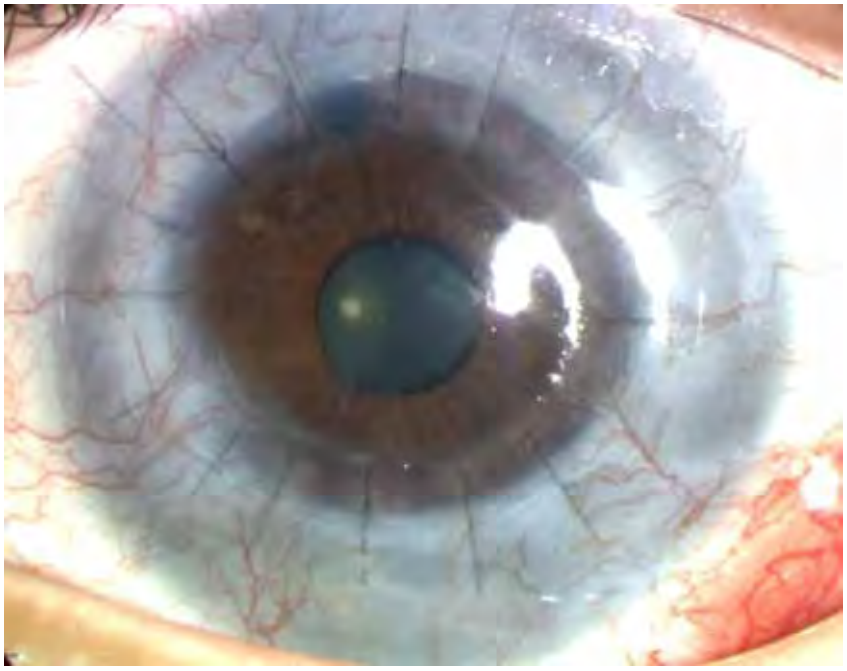
CASE Autologous Oral Epithelial Cell Sheet Transplantation with PKP (epithelium scraped off) to Treat Stevens-Johnson syndrome (post PKP)

Pre-op



VA=HM

3 year post-op



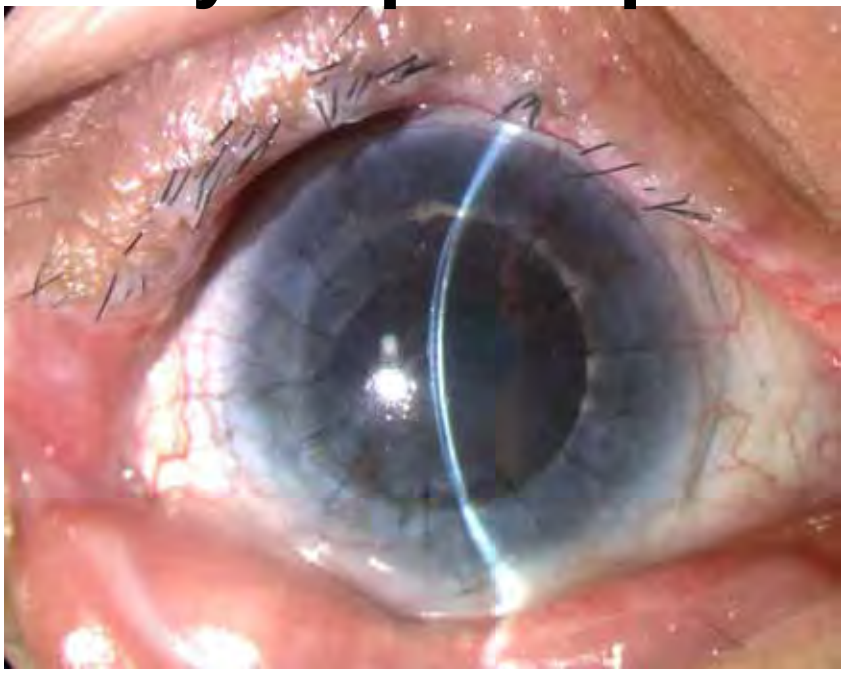
VA=20/20

CASE Autologous Epithelial Cell Sheet Transplantation with LKP (epithelium scraped off) and AMT to Treat Severe Thermal burn

Pre-op

VA=HM

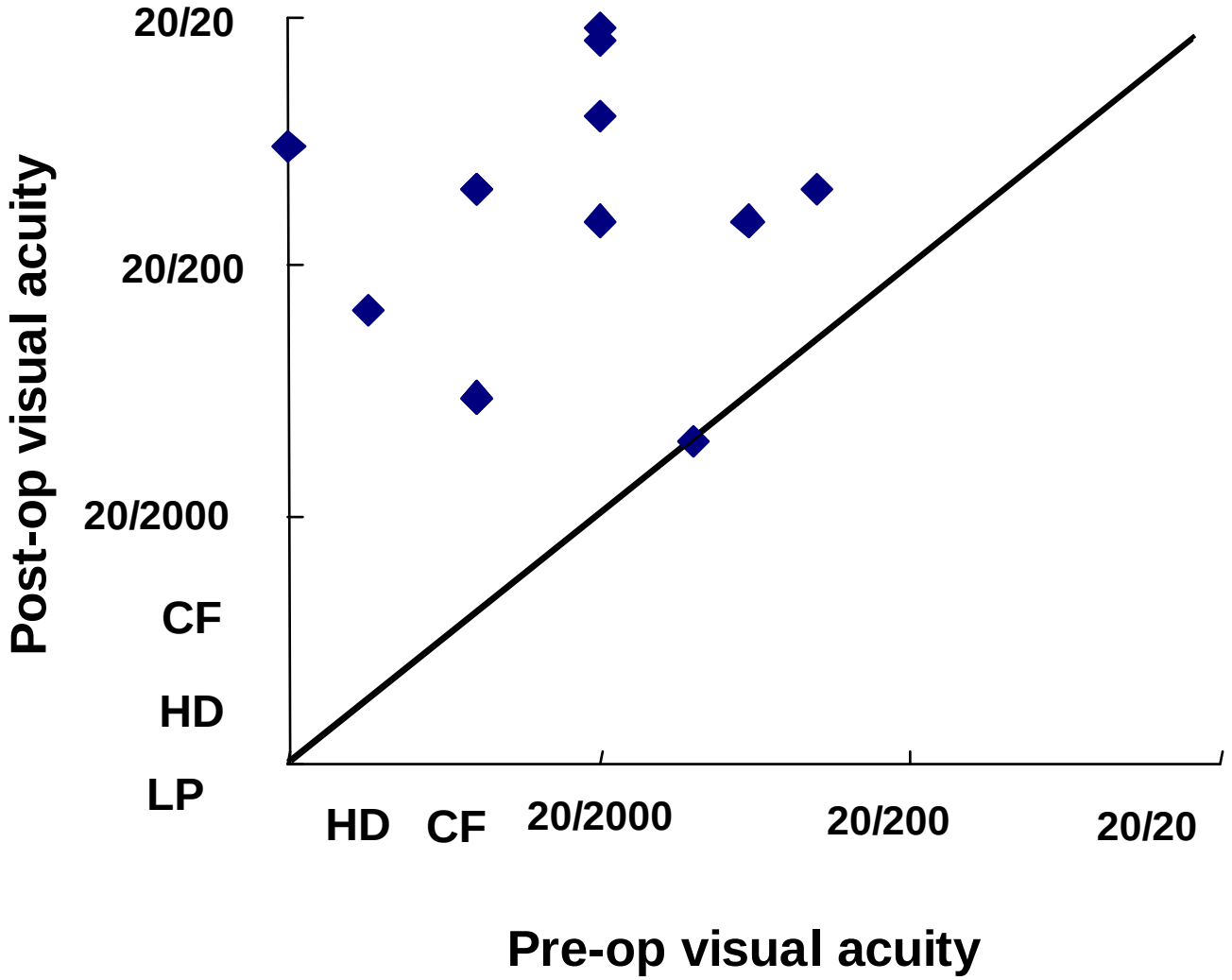
2 year post-op



VA=20/200

Surgical Results

Best-Corrected Visual Acuity

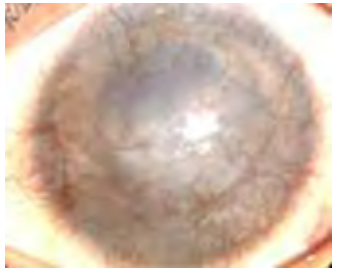


Issues to be solved

Corneal Neovascularization

Oral mucosal epithelial cell sheet induce peripheral neovascularization in some cases

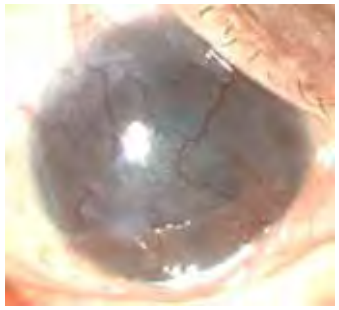
No neovascularization

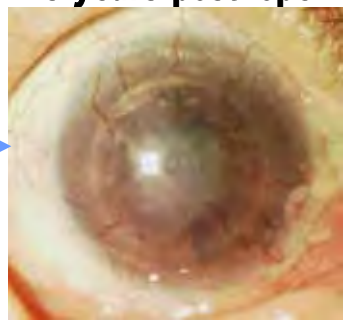


preope

3 years post-ope

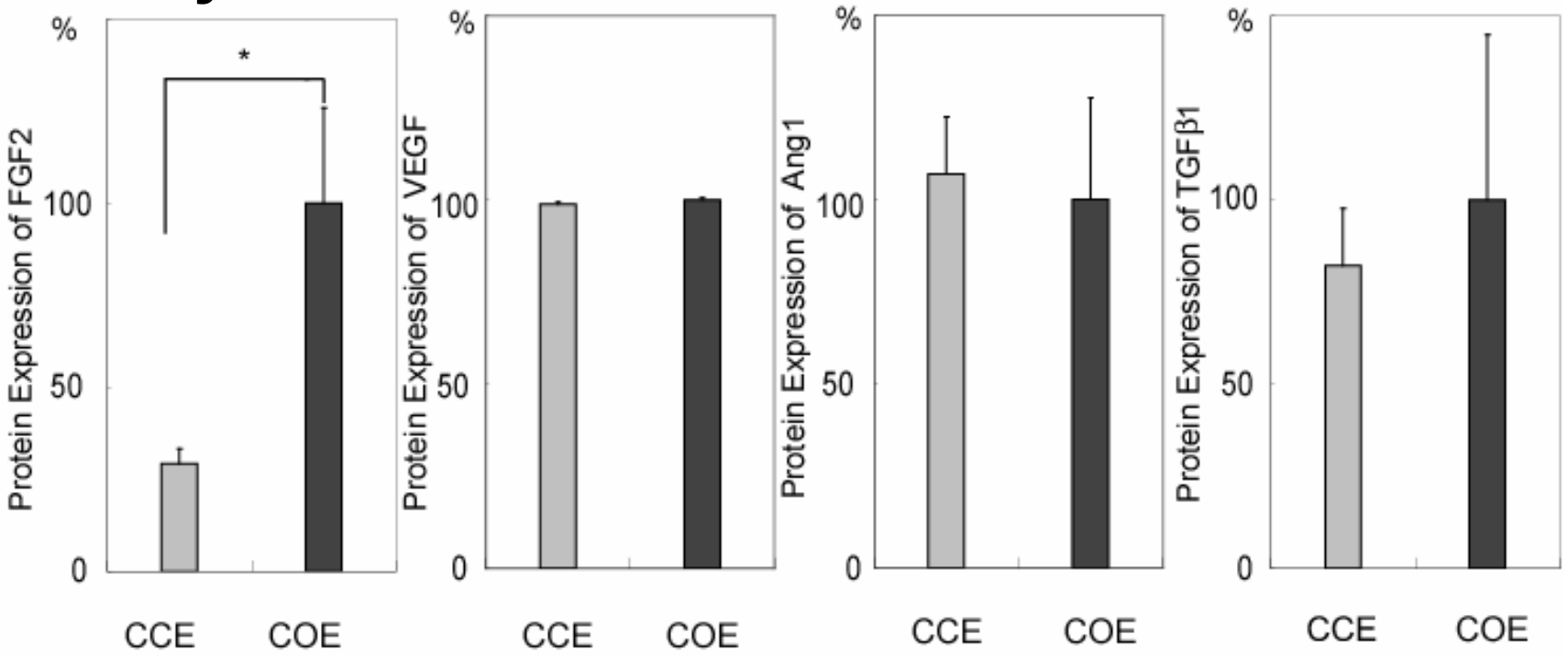
Neovascularization





2 years post-ope

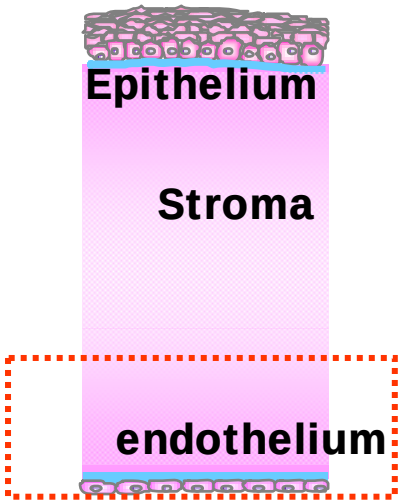
Quantification of Angiogenesis-Related Factors in Conditioned Medium from corneal sheet and oral sheet by ELISA



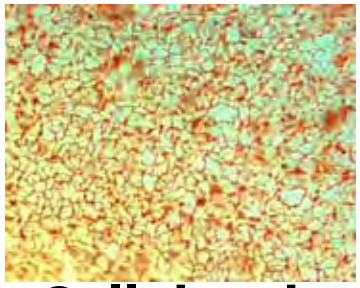
(N=4 *; P<0.05, Mann-Whitney test)

- **FGF2 was significantly higher in oral sheet than in corneal sheet., indicating that FGF2 is a candidate involved in the induction of corneal neovascularization after oral sheet transplantation.**
- **Anti-FGF2 therapy may control the neovascularization after oral sheet transplantation.**

Development of cultured corneal endothelial cell sheet transplantation

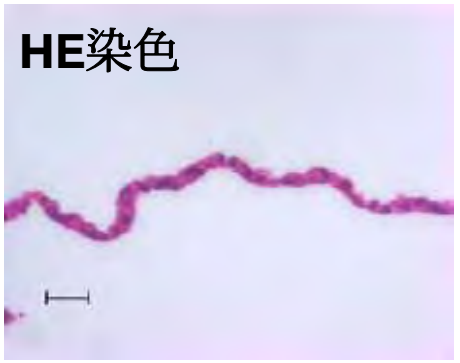


1.culture

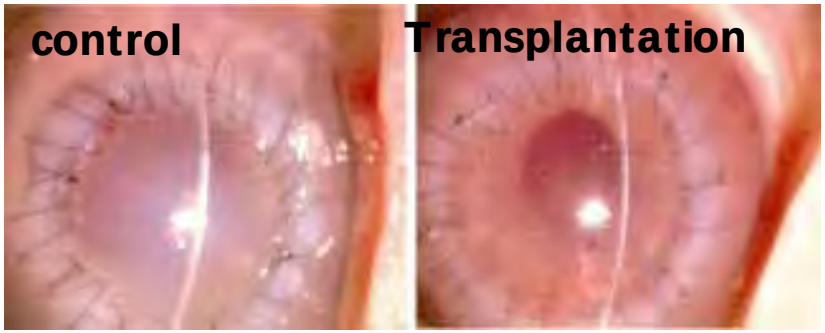


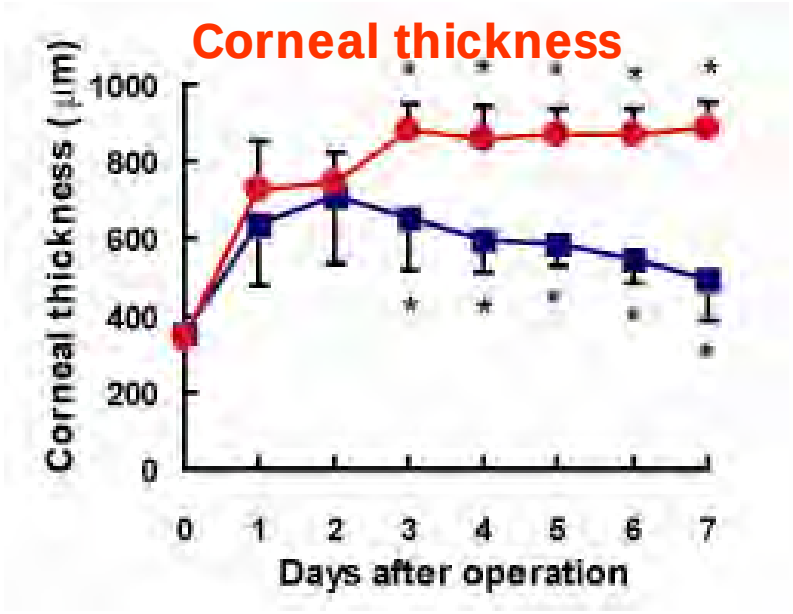
Cell density
>3000/mm²

2. Fabrication and harvest of cultured corneal endothelial cell sheet

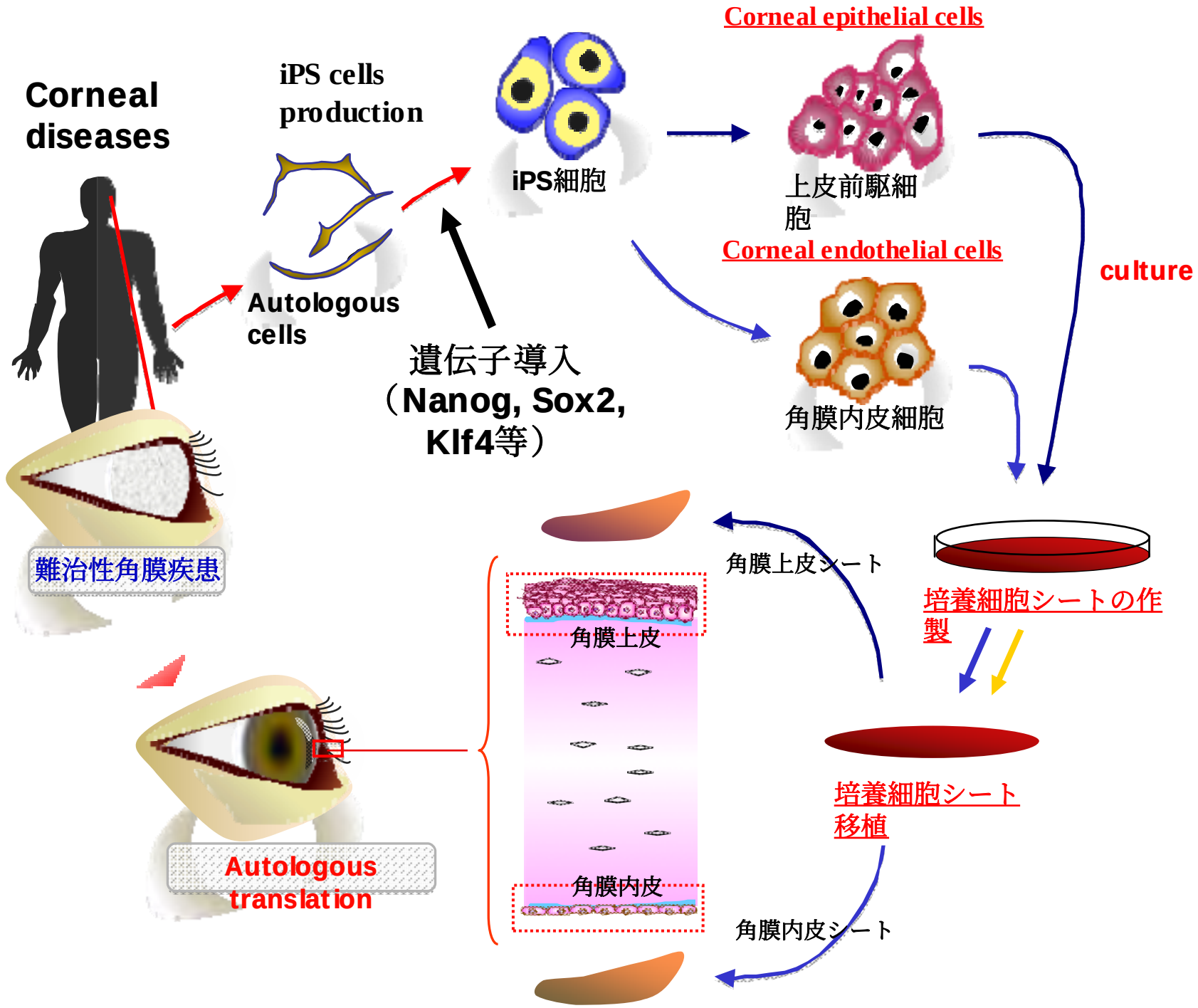


3. Transplantation surgery





(Sumide T, Nishida K, et al FASEB J 2006) *(Ide T, Nishida K, et al Biomaterials 2005)*


Corneal diseases
iPS cells production
Autologous cells
遺伝子導入
（Nanog, Sox2, Klf4等）
iPS細胞
Corneal epithelial cells
上皮前駆細胞
Corneal endothelial cells
角膜内皮細胞
culture
難治性角膜疾患
角膜上皮シート
培養細胞シートの作製
角膜上皮
角膜内皮
Autologous translation
培養細胞シート移植
角膜内皮シート

Conclusions

1. Clinical application of cell sheet transplantation using autologous limbal or oral mucosal epithelial stem cells are effective for reconstructing the corneal surface.

2. Corneal neovasucularization induced in peripheral cornea after oral mucosal cell sheet grafting should be controlled.

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