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  Scaffold  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 follicle stem cells

Gastrointestinal stem cells

Neural stem cells

Hematopoietic stem cells
Pluripotent Stem Cells

Embryonic stem cells

induced pluripotent stem cells
Corneal Diseases

- Corneal epithelial disease
- Stromal disease
- Endothelial disease

- Epithelium
- Stromal tissue
- Endothelium

- Alkali, thermal burn
- Stevens-Johnson syndrome
- Corneal dystrophy
- Bullous keratopathy

Standard therapy: allogeneic corneal transplantation using eye bank cornea
Corneal Epithelial Stem Cell

Basal cells of Limbal epithelium
=stem cells
Corneal epithelial stem cells

Limbal stem cell deficiency

Congenital
- Aniridia

Idiopathic

Secondary
- Chemical burn
- Thermal burn

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
Cultured corneal epithelial sheet harvested by dispase treatment

Cell source: Corneal epithelial stem cell

Pellegrini et al. Lancet 1997
The problems of Previous Methods

Cell sheet harvest by dispase

- Confluent cells
- Enzymatic proteolysis

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 °C hydrophobic**
  - cell attachment
- **below 32 °C 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 transplantation

Limbal epithelium

2mmX2mm

Temperature-responsive culture dish

Tissue-engineered epithelial cell sheet

MMO-3T3 feeder

37ºC culture 2 weeks

20ºC harvest

(Nishida K, Transplantation 2004)
Cell sheet harvest

Cultivated corneal sheet

Cultivated oral mucosal sheet
Characteristics of cultivated human corneal epithelial sheet

(Nishida K, Transplantation 2004)
Autologous cell source

Unilateral diseases  Bilateral diseases
Transplantation of tissue-engineered epithelial cell sheet for corneal diseases

Histology

Normal cornea

Corneal epithelial cell sheet
(Nishida K et al. Transplantation, 2004)

Normal oral mucosa

Oral mucosal epithelial cell sheet
Immunostaining for MUC 16

Corneal epithelium (in vivo)

Corneal epithelial cell sheet

Oral mucosal epithelium (in vivo)

Oral mucosal cell sheet

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 source

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

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

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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 2 year post-op

VA=HM
VA=20/200
Surgical Results

Best-Corrected Visual Acuity

Post-op visual acuity

Pre-op visual acuity
Corneal Neovascularization
Oral mucosal epithelial cell sheet induce peripheral neovascularization in some cases
Quantification of Angiogenesis-Related Factors in Conditioned Medium from corneal sheet and oral sheet by ELISA

- 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.

(N=4 *, P<0.05 , Mann-Whitney test)
Development of cultured corneal endothelial cell sheet transplantation

1. Culture

2. Fabrication and harvest of cultured corneal endothelial cell sheet

3. Transplantation surgery

Cell density >3000/mm²

Corneal thickness

Corneal diseases

iPS cells production

Autologous cells

Corneal epithelial cells

Corneal endothelial cells

culture

Nanog, Sox2, Klf4

Autologous translation

遺伝子導入（Nanog, Sox2, Klf4等）
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 neovasacularization induced in peripheral cornea after oral mucosal cell sheet grafting should be controlled.
Acknowledgement

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