# Cultivated Oral Mucosal Epithelial Transplantation

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## INTRODUCTION :

The normal ocular surface is covered with highly specialized corneal, limbal, and conjunctival epithelial cells that, together with the tear film, maintain the surface integrity.1-3 Severe ocular surface damage (OSD) such as Stevens-Johnson syndrome (SJS), chemical injury and ocular cicatricial pemphigoid represents a serious clinical challenge. In such cases, the corneal epithelial stem cells in the corneal limbus are destroyed and the corneal surface is covered by invading neighbouring conjunctival epithelial cell which results in neovascularisation, chronic inflammation, ingrowth of fibrous tissue, and stromal scarring.4,5 Conventional management is generally unsatisfactory and the long term consequences tend to be devastating.

Attempts have been made to establish a surgical treatment for severe OSD. Although corneal epithelial transplantation (limbal transplantation or keratoepithelioplasty) and cultivated corneal epithelial stem cell transplantation have been developed to improve the outcome of ocular surface reconstruction, significant problems stiil remain. Transplantation from allogeneic donors carries the risk of rejection and the intensive, prolonged postoperative immunosuppressant therapy necessary to prevent inflammation and rejection markedly reduces the patients' quality of life, especially in younger patients. In cases with unilateral, but not bilateral damage, reconstruction of the affected ocular surface can be attempted by transplanting cultivated autologous corneal EC from the contralateral eye.

The possibility of reconstructing the human corneal surface using autologous oral mucosal epithelium is a current topic of research now a days. In rabbits, it has been an established surgical method using amniotic membrane (AM) as a carrier.6 But in human it is still debatable and research is still going on.

### Histological Correlation of oral mucosa and corneal epithelial cell.

The optical transparency of harvested cell sheet of oral mucosa almost resembles corneal epithelium. Ultrastructurally the expected microstructure of native cell including microvilli, tight junction, desmosome and basement membrane is similar to corneal epithelium7. Corneal epithelial cell and oral mucosal cell express keratin 3 as common marker. Basal cell in multi-layered cell sheet also express p63, a putative epithelial stem cell marker that is present in corneal epithelial cell8.

### Procedure of Tissue Engineering

#### *Preparation of human amniotic membrane*

With proper informed consent, human amniotic membrane (AM) is obtained at the time of elective caesarean section. Under sterile conditions, the membranes are cryopreserved at ?80°C.6 For the oral epithelial cultures, the AMs are made deprived of their amniotic EC by incubation with 0.02% ethylene diamine tetra-acetic acid (EDTA) at 37°C for 2 hours to loosen cell adhesion; this is followed by gentle scraping with a cell scraper.

### Preparation of oral mucosa

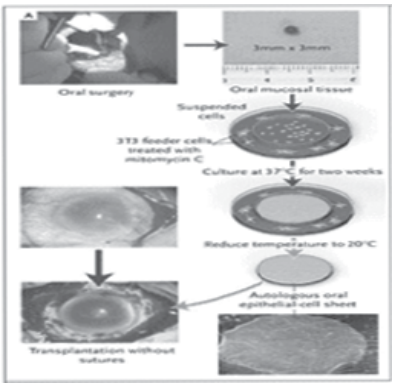
The presence of healthy oral mucosa is confirmed by a dentist before biopsy. All patients are followed up for tooth decay treatment if any, no alcohol or tobacco use, and regular brushing and iodine gargle.

**Cultivation of oral mucosal epithelial cells**

Oral mucosal biopsy specimens, each measuring approximately 2-3 mm2, are taken from patients under local anaesthesia 2-3 weeks before transplantation. Submucosal connective tissues are removed with scissors to the extent possible; the resulting samples are cut into small explants that are immersed three times (10 minutes at room temperature) in phosphate buffered saline solution containing antibiotics, (50 IU/ml penicillin-streptomycin and 5 µg/ml amphotericin B). The explants are then incubated at 37°C for 1 hour with 1.2 IU dispase and treated with 0.25% trypsin-EDTA solution for 10 minutes at room temperature to separate the cells. Enzyme activity is stopped by washing with Dulbecco's modified Eagle's medium and Ham's F12 medium (1:1) containing 10% fetal bovine serum, insulin (5 ?g/ml), cholera toxin (0.1 nmol/l), human recombinant epidermal growth factor (10 ng/ml), and penicillin-streptomycin (50 IU/ml). The oral EC (1×105/ml) are then seeded onto denuded AM spread on the bottom of culture inserts, and co-cultured with MMC inactivated 3T3 fibroblasts. The culture is submersed in medium for 1-2 weeks and then exposed to air by lowering the level of the medium (air lifting) over the course of 1 week. Cultures were incubated at 37°C in a 5% CO2 95% air incubator; the medium is changed every day.

**Surgical procedures**

At first the conjunctivalised tissues on the corneal surface and the subconjunctival tissues are removed using surgical scissors. Then the subconjunctival fibroblasts are treated for 5 minutes with 0.04% mitomycin C, followed by vigorous repeated washing with saline9. The cultivated oral epithelium on AM is transplanted onto the corneal surface of the damaged eye and is secured with 10-0 nylon sutures at the limbus.To confirm its quality,fluorescein staining of the cultivated oral epithelial sheet is done and then covered with a therapeutic soft contact lens.




**Postoperative management**

Postoperatively, 0.3% ofloxacin and 0.1% dexamethasone is instilled four times a day; the doses are tapered to a maintenance dose at 2-3 months, depending on the severity of inflammation. Betamethasone (1 mg/day) and cyclophosphamide (50 mg/day), administered to prevent postoperative inflammation and conjunctival fibrosis and are stopped 1-2 months after surgery. Both renal and liver functions are monitored periodically.

**REVIEW OF LITERATURE**

We reviewed a total of seven authentic studies including three animal and four human studies.As per Gipson et al10,who took sample from rabbit oral mucosa and transplanted into rabbit cornea, all the autografts remained adherent until animal was killed and the corneolimbal allograft survived more than central cornea. NakamuraT et al6,11 found cultivated rabbit oral epithelial cells show similarity with corneal epithelial cells and xenotransplantation of human cultivated oral epithelial cells is feasible.They also confirmed that epithelial cells were very similar to corneal epithelium as revealed by electron microscopy and the presence of keratin 4,13 and 3 as per immunohistochemistry. NishidaK, et al12 in their observational case series of oral mucous membrane transplantation in four patients found that, complete reepithelization of corneal surface was possible in all the 4 eyes. Maximum visual acquity was achieved in all the four patients within 10 weeks.However stromal vascularization was seen by them in their patients. Leonard P et al13 in their observational series of 10 patients with a follow up period of 12.6 months found that complete corneal epithelization was possible within 2-5 days .VA improved by 2 lines in 9 of 10 eyes .The oral epithelial sheets cultivated in autologous serum and fetal bovine serum were similar in nature. IntomiT et al14 in their observation series of 15 cases with a follow up period of 20 months found that 14 of 15 sheets resulted in total reepithelization ,10 of 15 eyes survived for at least 34

months ,5 eyes i.e.33% showed long standing epithelial defect and 10 eyes improved to post op VA of more than 2 lines. Intomi T15 et al in their observational case series of cultivated oral mucosal epithelial transplantation and penetrating keratoplasty in severe limbal disorder concluded that ocular surface can be successfully reconstructed with no risk of complication.VA of 20/125 in one patient and 20/100 in others suggest this treatment to be beneficial for corneal graft. Nakamura T et al16 in their observation cases series of six cases with a follow up period of 13.8 months found that visual acuity was improved in all eyes and the corneal surface remained stable, although all eyes manifested mild peripheral neovascularisation.

**Merits**

It can be used for B/L severe limbal stem cell deficiency without any risk of graft rejection.The immunosuppressant drugs is not required as it is an autograft procedure.Buccal mucosa is well accessible.It can be used in conditions where limbal cell transplantation is contraindicated.

**Demerits**

Neovascularization of cornea is one of the important demerits and further the cultivated epithelial sheet is not exactly the same as corneal epithelium.

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