

# Corneal Limbal Stem Cells

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## Corneal limbal stem cells: histological and physiological summary

A normal ocular surface is covered by corneal and conjunctival epithelium. The cornea is covered by stratified squamous epithelium with tight intercellular junctions making the optical surface clear and smooth. In contrast, conjunctival epithelium contains squamous epithelium, goblet cells and numerous vascularization in the stroma. The corneal epithelium is a rapid self-renewing tissue that loses its surface cells to the tear film. When the corneal surface is destroyed, which may come from various factors, the epithelial defect can be rapidly healed. This possible because corneal epithelial cells are repopulated by corneal limbal stem cells. Since 1986, Schermer et al.<sup>1</sup> proposed that corneal stem cells located in the basal layer of the limbus, generate and maintain the corneal epithelium. These cells are the ultimate source of corneal cellular proliferation and differentiation and centripetal migration from periphery to central of the cornea<sup>2,3</sup>. The stem cells (SC) which are in some fractions of the limbal basal epithelium generate corneal transient amplifying cells (TAC), which are located in the corneal basal epithelium. Both cells are classified as progenitor cells in the proliferative compartment and give rise to non-proliferative, differentiative compartment which are post-mitotic cells (PMC) and terminally differentiated cells (TDC) at the superficial layers. This suprabasal movement at the corneoscleral limbus creates a barrier that prevents conjunctival epithelium to migrate to the cornea and hence separates the conjunctiva from the cornea<sup>3</sup>. (Fig 1)

## Clinical manifestations of corneal limbal stem cell deficiency

When the full thickness of the limbal epithelium is completely destroyed, it will produce abnormal corneal surface and termed "limbal stem cell deficiency". They are characterized by conjunctival epithelial ingrowth invading the cornea (conjunctivalization), corneal vascularization, chronic inflammation, poor epithelial integrity and manifest as irregular surface, recurrent epithelial erosion and corneal persistent epithelial ulcer, destruction of the basement membrane and fibrous ingrowth<sup>3</sup>. These cause photophobia, corneal opacity, corneal ulcer, corneal perforation and decrease or loss of vision. Limbal stem cell deficiency is classified to two categories. (Fig 2) Category 1 is caused by destruction of limbal stem cell from the external injuries such as chemical burn, Steven Johnson's syndrome, multiple surgeries, contact lens induced, and severe microbial infection. Category 2 includes hypofunction of stem cells due to insufficient

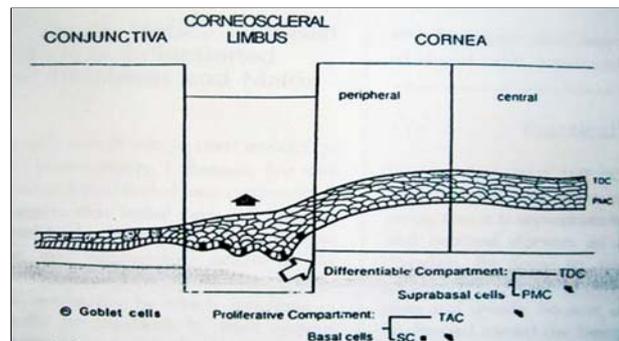


Fig 1. A Schematic drawing of the corneal limbal stem cell<sup>3</sup> (with permission)

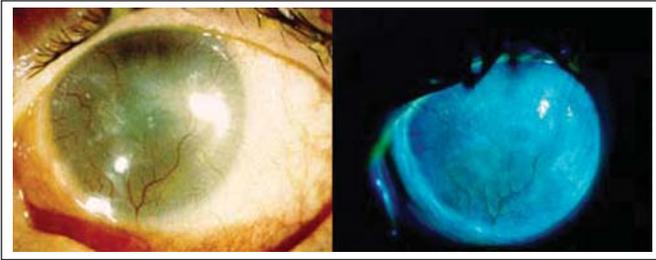
stromal support such as aniridia, multiple endocrine deficiency, ischemic neurotrophic keratopathy, or idiopathic. The definite diagnosis of limbal stem cell deficiency can be made by existence of conjunctival goblet cells in the corneal surface area. It is detected by impression cytology.

## Treatment of corneal limbal stem cell deficiency

Patients with limbal stem cell deficiency are poor candidate for penetrating keratoplasty because a corneal donor has only corneal transient amplifying cells. Moreover, corneal neovascularization increases the risk of graft rejection. Treatment of limbal stem cell deficiency begins with: avoidance of further injury to the residual corneal TAC or the remain SC cells by subsidence of any unnecessary drug treatment, promotion of wound healing by adding some growth factor and reduction of the inflammation by using topical non preservative steroid, autologous serum and non preservative tear<sup>3-5</sup>. The initial treatment is very important to retain limbal stem cells as much as possible.

Further treatment depends on the severity of the limbal stem cell deficiency. Partially mild corneal limbal stem cell deficiency may be treated by mechanical debridement of the conjunctival epithelium from the corneal surface<sup>1</sup>. In severe or total limbal stem cell deficiency, specific surgical treatments are necessary; they include; amniotic membrane transplantation<sup>4,6</sup>, limbal stem cell transplantation<sup>3-6</sup> and mucosal cell transplantation<sup>7</sup>.

Amniotic membrane can restore abnormal basement membrane, damaged stromal matrix and microenvironment. It contains numerous growth factors, antiapoptotic effect on epithelial cells. Also, it prolongs the survival of the stem cells, and it contains protease inhibitors and anti-inflammatory cytokines. It can promote epithelial healing, and reduced inflammation, vascularization and scarring.



**Fig 2.** Clinical manifestations of Steven Johnson's syndrome patient in Siriraj Hospital. Corneal limbal stem cell deficiency manifested by conjunctivalization 360° invading cornea, and corneal opacity from fibrous scar (A). Positive fluorescein staining demonstrated that corneal surface was covered with conjunctival epithelial cells instead of corneal epithelial cells (B).

In partial limbal stem cell deficiency, amniotic membrane transplantation alone may be sufficient to improve the corneal surface.

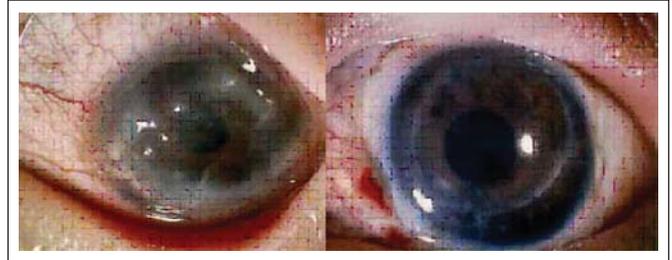
In case of total limbal stem cell deficiency, limbal cell transplantation is needed. The concept of limbal stem cell transplantation is to harvest the limbal stem cell by taking the superficial keratolimbal tissue from a donor source and transplant to the limbal deficiency patient. This concept was first proposed by Thoft in 1977 and the first limbal stem cell transplantation was introduced by Kenyon and Tseng in 1989. At Siriraj Hospital, we started to perform amniotic membrane transplantation and limbal transplantation in 1997<sup>6</sup>. The classification of limbal stem cell transplantation is defined by donor sources as follows:

1) Limbal autograft<sup>3,4</sup>: limbal tissue is harvested from the healthy eye of a patient and transplanted to the same patient. It can be used in unilateral disease such as unilateral chemical burn. There is no graft rejection but it has a problem of limited donor size. Fig. 3 demonstrates an autograft for unilateral chemical burn performed at Siriraj Hospital in 1998.

2) Limbal allograft<sup>3,4</sup>: limbal tissue is harvested from a healthy eye of a patient's living relative (living related limbal allograft) or from cadaveric tissue. The advantage of allograft especially from cadaveric tissue is large numbers of stem cells can be transplanted. The graft can perform 360 degree coverage of the whole limbus. It benefits bilateral diseases such as Steven Johnson's syndrome or bilateral chemical burn or unilateral disease's patient who is afraid of damage to the healthy fellow eye. However, the disadvantage is the high risk of graft rejection and the patient has to be on immunosuppressive agents for a long period of time or life long. Fig 4 demonstrates a allograft on Steven Johnson's patient from a cadaveric eye performed at Siriraj Hospital in 1999.



**Fig 3.** Autolimbic stem cell transplantation for unilateral chemical burn. (A) Preoperative. (B) After lysis symblepharon, amniotic membrane transplantation and 2 areas of autolimbic transplantation. The transplanted areas were located at superior and inferior limbus, 2 mm long (Arrow).



**Fig 4.** Steven Johnson's patient suffered from total corneal limbal stem cell deficiency (A). (B) After amniotic membrane transplantation and allolimbic stem cell transplantation 360° at the limbal area (Arrow).

3) Ex vivo limbal stem cell expansion<sup>7</sup>: limbal stem cells isolated from a healthy eye of a patient or from a cadaveric eye are grown in culture on amniotic membrane, after 2 weeks, the membrane and cells were transplanted to the patient's diseased eye. The limitation of allograft transplantation is the high incidence of graft rejection whereas the limitation of autograft is the limited donor tissue. These problems cause cultivated epithelial transplantation is the interesting direction for the future of stem cell deficiency. In ex vivo autolimbic stem cell expansion, the number of the stem cell can increase from 2 mm donor size to 2 cm epithelial cell size on amniotic membrane in 2 weeks and there is no need of immunosuppressive agent after transplantation. For ex vivo allolimbic stem cell culture, it is postulated that amniotic membrane may have anti-inflammation properties and hence reduce the graft rejection.

Cultivated autologous oral mucosal epithelial cell transplantation is a new modality for treatment of limbal stem cell deficiency<sup>7</sup>. It can eliminate the requirement of immunosuppression. Mucosal epithelial sheet from an oral mucosa is cultured on amniotic membrane and transplanted back to his/ her own corneal and limbal surfaces. The epithelial sheet possesses an intact epithelium and improves the ocular surface. It may benefit in bilateral diseases especially for Steven Johnson's patients who have high incidence of rejection of limbal transplantation. However, there is some peripheral corneal neovascularization which is a sign of limbal stem cell deficiency. The study reported improvement of the vision. The long-term results will depend on the future follow up.

In summary, the corneal limbal stem cells are a major key to maintain the health of the ocular surface. The understanding how the cells regulate and the appropriate management would help a patient to maintain a good vision and quality of life.

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