Review Article

Ocular Surface Stem Cells and Disease: Current Concepts and Clinical Applications

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Abstract

Corneal and conjunctival epithelial stem cells are responsible for the homeostasis and regeneration of the ocular surface epithelium. Corneal epithelial stem cells reside in the basal region of the limbus, while the conjunctival forniceal region appears to be the site that is enriched in conjunctival stem cells. Ocular surface disease arising from limbal stem cell deficiency is characterised by persistent epithelial defects, corneal vascularisation, chronic inflammation, scarring and conjunctivalisation, resulting in visual loss. Limbal stem cell transplantation replaces the corneal stem cell population in these eyes with the hope of restoring vision. More recently, the use of bioengineered ocular surface tissue-equivalents has had promising results, and may represent the future for replacement and regeneration of ocular tissues in various ocular disorders.

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Introduction

The ocular surface is a complex biological continuum responsible for the maintenance of corneal clarity, elaboration of a stable tear film for clear vision, as well as protection of the eye against microbial and mechanical insults. The ocular surface epithelium comprises corneal, limbal and conjunctival epithelia, of which the conjunctiva extends from the corneal limbus up to the mucocutaneous junction at the lid margin, and is divided anatomically into the bulbar, forniceal and palpebral regions (Fig. 1). The precorneal tear film, neural innervation and the protective blink reflex help sustain an environment favourable for the ocular surface epithelium.

Ocular Surface Stem Cells

Adult corneal and conjunctival stem cells represent a small, quiescent subpopulation of epithelial cells of the ocular surface. The limbus is a 1.5 mm- to 2 mm-wide area that straddles the cornea and bulbar conjunctiva. Corneal epithelial stem cells reside in the basal region of the limbus, and are involved in the renewal and regeneration of the corneal epithelium.[1-5] Following injury, these limbal basal stem cells are stimulated to divide and undergo differentiation to form transient amplifying cells (TACs) (Fig. 2). Subsequent cell divisions result in non-dividing post-mitotic cells (PMCs), which then terminally differentiate and migrate towards the central cornea and superficially, taking on the final corneal phenotype as terminal differentiated cells (TDCs). Their presence allows continued replacement and regeneration of tissues following injury, thereby maintaining a steady-state population of healthy cells.

The ocular surface is an ideal region to study epithelial stem cell biology, because of the unique compartmentalisation of the corneal epithelial stem cells within the limbal region, which provides us with a valuable opportunity to study the behaviour of stem cells and TACs, how they respond to various growth stimuli, and the mechanisms that modulate their growth and differentiation.[1-5]

By comparison, conjunctival stem cell biology has been much less investigated than that of corneal stem cells. Conjunctival and corneal epithelial cells have been shown to belong to 2 separate, distinct lineages.[6] Unlike corneal

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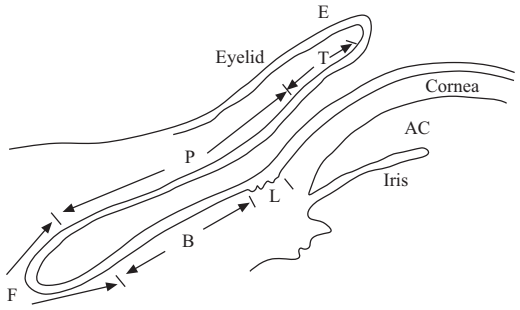

Fig. 1. Schematic diagram showing the ocular surface comprising the cornea, limbus and conjunctiva. The corneal stem cells are located in the limbus, while the conjunctival stem cells appear to be enriched in the conjunctival fornix.
AC: anterior chamber; B: bulbar conjunctiva; E: epidermis; F: fornix conjunctival; L: limbus; P: palpebral conjunctiva; T: transitional zone between the palpebral conjunctiva and epidermis (mucocutaneous junction)

epithelium, conjunctival epithelium consists of both non-goblet epithelial cells as well as mucin-secreting goblet cells. Wei et al[7,8] showed that both these populations of cells arise from a common bipotent progenitor cell. The conjunctival forniceal region appears to be the site that is enriched in conjunctival stem cells, although stem cells are also likely to be present in other regions of the conjunctiva.[7,8]

Identification of Stem Cells

To date, several putative stem cell markers have been proposed, although no single molecular marker that is specific for stem cells has been identified. This has significantly limited our capacity to study the characteristics and behaviour of these cells. Taking advantage of the slow-cycling characteristic of stem cells, an indirect method of labelling stem cells was developed.[9-11] Continuous administration of tritiated thymidine (^{3}H-TdR) for a prolonged period labels all dividing cells. Slow-cycling cells that remain labelled for a prolonged period are termed "label-retaining cells" and are believed to represent stem cells.[9-11] Cotsarelis et al[11] confirmed the presence of a small subpopulation of slow-cycling label-retaining limbal basal stem cells that had a significant reserve capacity and proliferative response to wounding.

Another characteristic of stem cells is their capacity to remain highly proliferative in vitro.[12-14] Cells that have the highest proliferative capacity (holoclones – with less than 5% of colonies being terminal) are considered stem cells.[12,13] Pelligrini et al[15] showed by clonal analysis that nuclear p63 was abundantly expressed by epidermal and limbal holocolones, but were undetectable in paraclones, suggesting that p63 might be a marker of keratinocyte stem cells. Other markers that have been suggested include alpha-enolase expression, as well as the presence of high levels of α_6-integrin and low to undetectable expression of the transferrin receptor (CD71), termed $\alpha_6^{bri}CD71^{dim}$ cells.[16,17] Connexin 43 was also found to be absent in the limbal basal cells, as was gap-junction mediated cell-to-cell communication, as detected by the lack of cell-to-cell tracer (Lucifer yellow) transfer.[18] This absence of intercellular communication may be an inherent feature of stem cells, reflecting the need for these cells to maintain the uniqueness of their own intracellular environment.

Ocular Surface Disease Arising from Stem Cell Deficiency

Limbal stem cell deficiency can be caused by a variety of hereditary or acquired disorders.[1-5,19-21] Inherited disorders include aniridia keratitis and keratitis associated with multiple endocrine deficiency, in which limbal stem cells may be congenitally absent or dysfunctional. Acquired conditions that may result in limbal stem cell deficiency include Stevens-Johnson syndrome, chemical injuries, ocular cicatricial pemphigoid, contact lens-induced keratopathy, multiple surgeries or cryotherapies to the limbal region, neurotrophic keratopathy and peripheral ulcerative keratitis. Acquired disorders form the majority of cases seen in the clinical setting.

Limbal stem cell deficiency is characterised by persistent or recurrent epithelial defects, ulceration, corneal vascularisation, chronic inflammation, scarring, and conjunctivalisation (conjunctival epithelial ingrowth), with resultant loss of the clear demarcation between corneal and conjunctival epithelium at the limbal region (Fig. 3).[1-5,19-21] Chronic instability of the corneal epithelium and chronic ulceration may lead to progressive melting of the cornea with the risk of perforation. The pathognomonic feature of limbal stem cell deficiency is the presence of conjunctival epithelial ingrowth of the corneal surface.[4,5] Impression cytology confirms the presence of conjunctival goblet cells on the surface.[22] Limbal deficiency may be localised (partial) or diffuse (complete).[19-21] In localised limbal stem cell deficiency, some sectors of the limbal and corneal epithelium are normal, and conjunctivalisation is restricted to the regions devoid of healthy epithelium.

The diagnosis of the presence of limbal deficiency is crucial as these patients are poor candidates for conventional corneal transplantation alone.[3,4] Conventional corneal transplantation does not address the problem of inadequate corneal epithelial replacement, and subsequent conjunctival ingrowth, vascularisation and inflammation ultimately result in graft rejection and failure.

Treatment of Limbal Stem Cell Deficiency

With the widespread acceptance of the limbus as the site of corneal stem cells, limbal stem cell transplantation was introduced as a definitive means of replacing the corneal stem cell population in the diseased eye.[4,5,23-27] Limbal autograft transplantation, first described in detail by Kenyon

and Tseng,[23] is essentially limited to unilateral cases or bilateral cases with localised limbal deficiency, where sufficient residual healthy limbal tissue is available for harvesting. In these cases, the procedure involves lamellar removal of 2 four-clock hour limbal segments (usually superior and inferior) from the healthy donor eye and transplantation of these segments to the limbal-deficient eye, after a complete superficial keratectomy and conjunctival peritomy to remove unhealthy diseased surface epithelium. For patients with bilateral diffuse disease, the use of allogeneic limbal grafts is required. In the case of cadaveric donors, the entire 360-degree annulus of limbus can be transplanted, either as an intact annular ring (Fig. 4), or in several contiguous segments. Limbal allografts may also be obtained from HLA-matched living-related donors, to reduce the risk of immunologic rejection.[27]

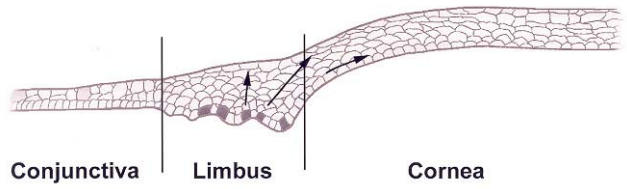

Fig. 2. The limbal basal epithelial cells (shaded) are believed to contain corneal stem cells. These cells divide to form transient amplifying cells which migrate centrally to occupy the basal layer of the cornea (indicated by arrows). Subsequent cellular divisions result in post-mitotic cells (PMCs) which occupy the suprabasal layers. Progressive differentiation of PMCs result in terminally differentiated cells in the superficial layers.

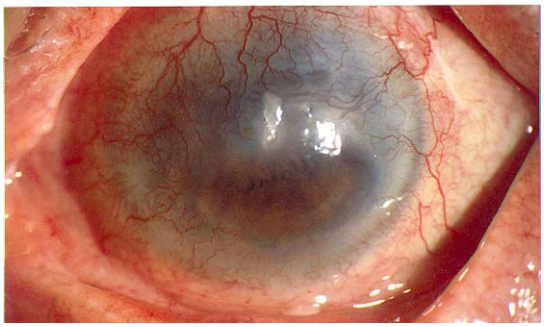

Fig. 3. Limbal stem cell deficiency in a patient with Stevens-Johnson syndrome. There is loss of transparency of the cornea, stromal scarring, corneal vascularisation and conjunctivalisation.

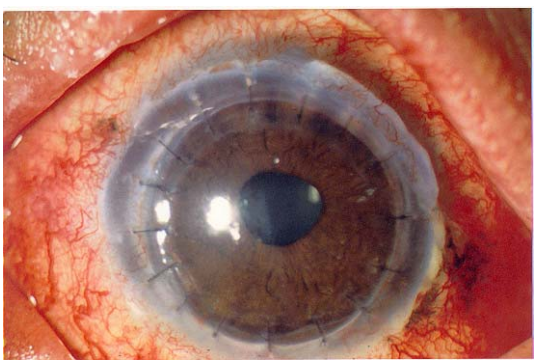

Fig. 4. 360-degree annular limbal allograft transplantation combined with corneal transplantation in a patient with Stevens-Johnson syndrome.

Limbal transplantation may be combined with penetrating keratoplasty, performed either at the same setting or as a staged procedure. In cases of severe ocular surface disease, there is often associated conjunctival and lid pathology, which may require adjunctive surgical procedures to augment the reconstruction of the ocular surface, such as lamellar keratoplasty, conjunctival transplantation, forniceal reconstruction with release of symblepheron, and correction of cicatrising lid disease.[24-28]

The use of allogeneic grafts is associated with the risk of graft rejection, and patients may require long-term systemic immunosuppression with cyclosporine, FK506 or mycophenolate mofetil. Despite several studies which reported good early success rates with limbal allograft transplantation, subsequent reports suggest that approximately 50% of these grafts fail within 3 to 5 years.[29,30]

More recently, human amniotic membranes have been shown to facilitate wound healing by promoting epithelial cell migration and adhesion, by possessing growth factors that encourage healing, and by their anti-inflammatory properties.[31,32] As such, amniotic membrane transplantation

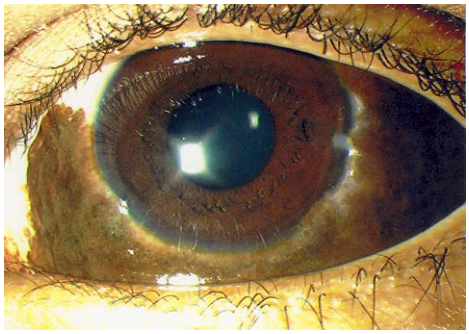

Fig. 5a. Extensive conjunctival naevus in a 9-year-old child.

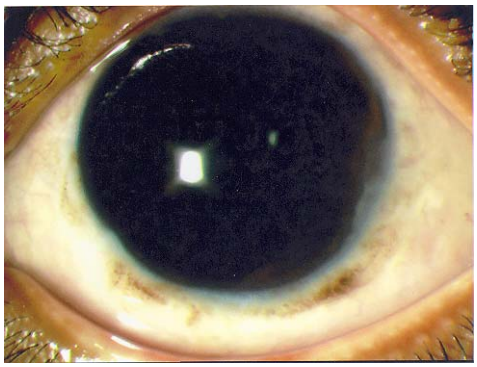

Fig. 5b. Transplantation of a cultivated conjunctival tissue-equivalent resulted in a good cosmetic result with minimal recurrence or scarring.

has been used in the treatment of persistent epithelial defects, limbal stem cell deficiency and reconstruction of the ocular surface.[31,32]

Bioengineered Ocular Surface Equivalents for Transplantation

Limbal autograft surgery overcomes the problem of immunologic rejection but may only be used for patients with unilateral limbal stem cell deficiency. Because fairly large segments are required, this places the donor eye at risk and may eventually result in surgically induced limbal stem cell deficiency in the donor eye. The use of autologous cultivated limbal stem cell transplantation has been employed to overcome this problem.[33-37]

The ex vivo expansion of limbal epithelial stem cells in vitro, followed by transplantation, provides a new modality for the treatment of limbal stem cell deficiency.[34-37] For this procedure, only a small limbal biopsy (approximately 2 mm^2) is required, which minimises potential damage to the healthy contralateral donor eye. This is then cultivated on various substrates, such as human amniotic membranes or fibrin-based substrates, which results in a composite graft tissue that is then transplanted onto the diseased eyes. Although the long-term results and safety of this procedure are yet to be determined, reasonable success of up to 1 year of follow-up has been achieved.[34-37]

Previous investigators have demonstrated that these amniotic membrane cultures preferentially preserved and expanded limbal epithelial stem cells that retained their in vivo properties of being slow-cycling, label-retaining, and undifferentiated.[38] This novel technique proves to be a promising therapeutic option for patients with unilateral or bilateral ocular surface disease, as only small amounts of tissue are required for the expansion of cells, which minimises iatrogenic injury to the donor eye. The use of these bioengineered corneal surface tissues with a complement of stem cells may thus provide a safer and more effective treatment option.

More recently, we demonstrated the development of serum-free derived conjunctival tissue-equivalents.[39,40] These cultivated cells were shown to remain proliferative in vitro and in vivo, and also retained their innate phenotypic characteristics.[39] These stratified conjunctival equivalents were used in the treatment of a variety of ocular surface disorders resulting in good cosmetic and functional outcomes (Fig. 5).[40] Because of the earlier epithelialisation of the ocular surface, these eyes appeared to have a more rapid recovery with a reduction in postoperative ocular surface inflammation. This is particularly useful for extensive or bilateral conjunctival disorders. The use of these conjunctival equivalents is advantageous as it removes the need for harvesting conjunctival autografts and causing iatrogenic injury to the remaining normal tissue, and encourages faster ocular surface rehabilitation.

The use of bioengineered tissue replacements represents the future for replacement and regeneration of various tissues and organs. Its use in the treatment of ocular surface disease has been promising. In addition, the use of autologous cultivated stem cells allows the patient's own cells to be cryopreserved, so that repeat grafts may be constructed from these cells in the case that the first graft fails.

Conclusion

Our knowledge and understanding of ocular surface stem cells has led to improvements in the management of ocular stem cell deficiency. Conjunctivalisation, recurrent corneal epithelial defects, vascularisation and inflammation are hallmarks of patients with severe limbal stem cell deficiency. Conventional penetrating keratoplasty has been shown to have uniformly poor results in view of the hostile milieu of these diseased eyes. Limbal stem cell transplantation and more recently, the use of bioengineered ocular surface equivalents have revolutionised the treatment of these difficult cases by replacing the depleted stem cell population.

Much remains to be learnt about the structural and biochemical components of the stem cell niche, and the regulatory mechanisms involved in the differentiation of stem cells. The development of specific stem cell markers will greatly improve our understanding of these cells and their behaviour in normal and diseased states, thereby allowing us to develop more successful treatment modalities for ocular surface disorders.

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