THE ROYAL COLLEGE OF OPHTHALMOLOGISTS

Focus

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Management of Limbal Stem Cell Deficiency

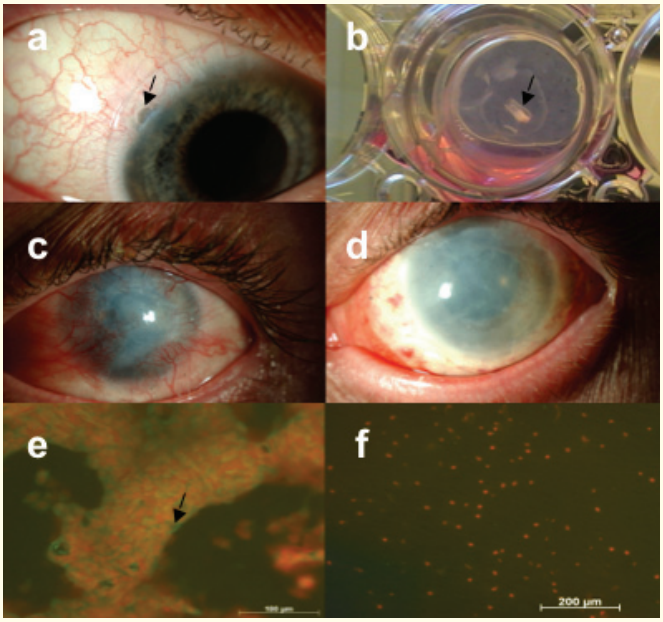

Figure 1. Donor site of limbal biopsy (a); limbal SCs cultured in vitro as a limbal explant (b); clinical photograph pre- (c) and post-LSC transplantation (d); pre-op Ck 19 staining of corneal impression indicating LSCD (e) and post-op restored corneal epithelial phenotype (f).

Normal visual function requires an intact ocular surface. The integrity of this surface is maintained in humans by two highly specialised epithelia, the conjunctival and the corneal epithelia. The concept of the limbal location of corneal epithelial stem cells (SCs) has revolutionised our understanding and therapeutic approaches for treating patients with complicated ocular surface diseases.

Cumulative evidence indicates that a small proportion of limbal epithelial cells in the basal layer are the SCs for the corneal epithelium, the so-called limbal stem cells (LSCs). The limbal epithelium is therefore crucial in maintaining the cell mass of corneal epithelium under normal conditions and plays an important role in corneal epithelial wound healing.

Clinical Presentation and Diagnosis

A deficiency or absence of LSCs explains the pathogenesis of severe ocular surface disorders often characterised by conjunctivalisation of the cornea. Ocular surface diseases associated with severe limbal damage often present with photophobia, chronic inflammation, persistent epithelial defects, scarring and neovascularisation of the cornea. This is known as limbal stem cell deficiency (LSCD) and definitive treatment of severe symptomatic LSCD requires the transplantation of healthy LSC containing limbal epithelium to restore corneal epithelial function.

A large number of both acquired and congenital ocular surface diseases can cause LSCD. Congenital causes are rarer and include epidermal dysplasia and aniridia. Acquired diseases are more common and include chemical or thermal burns, radiation keratitis, Stevens-Johnson syndrome and contact lens induced keratopathy[1].

Several studies have concluded that the demonstration of conjunctival epithelium containing goblet cells on the corneal surface by impression cytology is diagnostic of LSCD[1]. In humans, cytokeratin staining of impression cytology specimens can also demonstrate LSCD (Fig. 1e). CK3 and CK19 have been demonstrated to discriminate between corneal and conjunctival epithelium: CK3 stains all layers of normal human corneal epithelium but does not stain the conjunctiva, whereas CK19 stains the conjunctival epithelium but not the superficial corneal epithelium.

Classification and Management

Damage to the LSCs may be partial or total, and unilateral or bilateral. In partial LSCD, there is still the presence of some functioning LSCs. In cases with good vision, where the patient is relatively asymptomatic, conservative management is often indicated[2]. However, where there is decreased vision, significant irritation and persistent epithelial defects, surgical management, including the mechanical debridement of the conjunctival epithelium from the surface of the cornea and/or amniotic membrane transplantation, may be indicated[3].

Total LSCD or severe partial LSCD can be treated in two ways:
(1) Direct limbal transplantation
(2) Culturing the limbal epithelium in a controlled environment followed by transplantation.

In addition, the above two procedures may need to be combined with penetrating keratoplasty often as a staged procedure.

Limbal Transplantation. Kenyon & Tseng were the first to propose the treatment of LSCD with healthy limbal tissue grafts using the procedure termed Conjunctival Limbal Autograft (CLAU) from the healthy fellow eye[4]. When

LSCD is diffuse and bilateral the fellow eye cannot be used as a source of LSCs. Consequently, transplantation of allogeneic limbal epithelial SCs from a cadaveric donor, i.e. Keratolimbal Allograft (KLAL) or from a living-related donor, i.e. living-related Conjunctival Limbal Allograft (lr-CLAL) is indicated. In both procedures, systemic immunosuppression for a prolonged, if not indefinite, period will have to be administered to prevent allograft rejection. However, the major reported problem encountered in both these techniques has been the failure of limbal allografts. This may be explained in part by the severity of the ocular surface damage, which significantly interferes with normal ocular surface homeostasis. Prior to any grafting procedure it is important to improve the ocular surface by using conservative measures such as preservative-free tear substitutes, autologous serum drops, punctal occlusion, bandage contact lens or surgical intervention such as correction of lid abnormalities. In addition, failure of the procedure may also be in part due to allograft rejection.

The main disadvantage of limbal epithelial autografts and living-related limbal epithelial allografts is the sizeable amount of healthy tissue required for the procedure with a consequent risk of creating LSCD to the healthy donor eye. In cases of all limbal allografts, there are considerable risks involved with lifelong potent immunosuppression, including infection and tumour formation, for a condition that is sight- rather than life-threatening. These problems often leave patients with no viable treatment option.

Ex vivo expanded limbal epithelial transplantation. An emerging approach uses a fraction of the amount of limbal epithelium and grows the epithelial cells in the laboratory for transplantation back into the patient at a later date (often 2 to 4 weeks). In 1997, Pellegrini and co-workers were the first to report this technique[5]. As is the case with previous techniques, the LSCs are taken from the healthy fellow eye of the patient, if possible, or in cases of allograft from a live donor, usually a sibling or a parent. Less often, LSCs are taken from a cadaver.

Ex vivo expansion of limbal epithelium in culture represents a substantial advance in ocular surface reconstruction[6]. The LSC containing limbal epithelium can be cultured in vitro as a limbal explant (Fig. 1b) or as single cell cultures on various substrates that can be easily handled during surgery. Laboratory experiments have demonstrated that amniotic membrane is an ideal substrate to preserve and expand LSCs in culture before transplantation. This technique has been used to reconstruct the corneal surface of patients with LSCD (Fig. 1c, d, e & f). Most importantly, since only a small biopsy is removed from the donor eye, it avoids the unnecessary risk of large limbal removal from a normal healthy eye. Typically, a 1-2 mm by 1-2 mm limbal biopsy (Fig. 1a) is sufficient to produce enough LSCs to cover the whole cornea as opposed to the 4-6 clock hours of limbal tissue needed for direct limbal transplantation. Several patients with LSCD have now been treated by this method, although the success rate varies according to the cause of the original deficiency[7].

It has been proposed that in patients with bilateral total LSCD, small pieces of the patient's own oral mucosal epithelium containing SCs could be cultured in the laboratory and then transplanted back to the patient's eye with LSCD, thus eliminating the requirement of potent immunosuppression. This approach has been applied to humans with encouraging early results[8], providing an exciting possibility of treating this difficult group of patients with bilateral blindness caused by total LSCD and definitely warrants further studies. In addition, the use of human embryonic SCs to generate corneal like epithelial lineages holds much promise and in the future we may be able to produce these cells for potential therapeutic purposes[9].

Summary

LSCD is a devastating, blinding and painful ocular surface disease that requires an understanding of the important principles and current treatment methods relevant to ocular surface disorders. *Ex vivo* expansion of LSC containing limbal epithelium is a novel technique that has been successfully used to treat a number of patients with severely LSC deficient eyes. By using a more refined technique to identify and grow SCs *in vitro* with animal-free products, this procedure will become more widely used. However, long-term prospective studies are needed.

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