The management of severe ocular surface disease (OSD) has benefited from major breakthroughs in recent years. Previously, patients with OSD had a poor prognosis. Modern treatment of severe ocular surface disorder is quite different. Advancements in microsurgical techniques and understanding of the role of limbal stem cells have led to great improvement in both visual acuity and patient’s quality of life. It is very interesting to study the sequence of events that led to the birth of modern day regenerative medicine in ocular surface reconstruction.

**Conjunctival transplantation**

In 1977, Thoft described the conjunctival transplantation procedure, which is recognized as the forerunner of modern ocular surface transplantation. Thoft reported transplanting several pieces of bulbar conjunctival tissue from a normal fellow eye to four quadrants of the eye with damaged ocular surface epithelium and superficial vascularization. An epithelial front spread onto the corneal surface from the edges of each graft during the reepithelialization process. (Figure 1) His technique was based on the principle of conjunctival transdifferentiation, which postulated that the conjunctival epithelium could transform into cornea-like epithelium. In his report of 22 eyes, 19 obtained a good ocular surface, out of which two subsequent keratoplasties went on to fail. Although a conjunctival autograft is useful in reestablishing an intact ocular surface in patients with conjunctival scarring, concerns exist as to whether this procedure truly results in normal corneal epithelium. The technique of conjunctival autograft remains a valuable procedure for the management of fornix reconstruction as well as primary and recurrent pterygium.

**Keratoepithelioplasty**

In 1984, Thoft described the first allograft procedure for the management of severe OSD. (Figure 2) He called the procedure keratoepithelioplasty (KEP). His procedure involved the use of lenticules of peripheral cornea from a cadaveric donor cornea as a source of epithelium. A whole globe was used to obtain four pieces of partial thickness.

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**Figure 1:** Thoft’s conjunctival autograft. (a): Grafts are taken from uninjured fellow eye in four quadrants from areas normally covered by eyelid. (b): Placement of conjunctival grafts.

**Figure 2:** Thoft’s keratoepithelioplasty. (a): Preparation of lenticules from donor globe. (b): Placement of lenticules around the corneoscleral limbus.
Evolution

cornea. Lenticules were carved from the midperipheral cornea and consisted of an epithelium and a thin layer (0.2mm) of stroma that served as a carrier for delicate epithelium. The four lenticules were placed evenly around the corneoscleral limbus and sutured to the sclera. No limbal cells were used in this technique. The epithelium from the lenticules spread and covered the recipient cornea. Because cadaveric eyes, rather than the fellow eye, were used for the donor tissue, this technique was useful in treating patients with bilateral OSD. Although Thoft did not describe obtaining limbus with his KEP procedure, since there was not a good understanding of the stem cell theory at that time, it is possible that some stem cells were harvested with the peripheral corneal lenticules. In 1990, Turgeon and co-workers, including Thoft, reported on 13 additional patients managed with KEP. The technique described was modified from Thoft's original procedure to include limbal tissue with the peripheral corneal tissue in an attempt to transplant limbal stem cells. Of the 11 patients with at least 6 months' follow-up, 7 had a stable ocular surface, and 7 had improved visual acuity.

**Limbal Stem Cell Theory**

The single most important breakthrough in managing severe OSD was the understanding of the location and function of the limbal stem cells. In 1971, Davenger and Evenson speculated that the source for replacing the corneal epithelium lay at the limbus when they observed that pigmented limbal cells moved centrally. Schermer and co-workers studied patterns of corneal-specific 64K keratin expression and discovered that the corneal limbus basal cells are less differentiated than those found in other areas of the corneal epithelium. Cotsarelis and co-workers provided additional evidence that stem cells were located at the limbus when they found that tritiated thymidine was incorporated for long time intervals into limbal basal cells. This labeling indicated that these cells exhibited a long cell cycle. Ebato and associates reported that human ocular limbal epithelial cells grew better in culture and had higher rates of mitotic activity than peripheral corneal epithelial cells.

**Limbal Autograft**

In 1989, Kenyon and Tseng were the first to take the limbal stem cell theory and apply it clinically. They built on the work of Thoft by modifying his conjunctival autograft surgery to include limbal stem cells. In this way, their procedure was the first to specifically transplant limbal epithelial stem cells for severe OSD. In this procedure, conjunctival and limbal tissue from a normal fellow eye was used to manage diffuse limbal deficiency in unilateral ocular surface disease, or focal limbal deficiency in unilateral or bilateral disease (Figure 3). Their technique used grafts of bulbar conjunctiva that extended approximately 0.5 mm onto the clear cornea centrally, thus containing limbal cells. The authors reported data on 21 cases with 6 months or more of follow-up. The results were impressive with rapid surface healing in 19 cases, stable ocular surface in 20 cases, improved visual acuity in 17 cases, and arrest of regression of corneal neovascularization in 15 cases.
No complications developed in the donor eyes. Seven of seven patients underwent simultaneous or subsequent successful penetrating or lamellar keratoplasty. A potential risk of limbal autograft transplantation is development of iatrogenic limbal stem cell deficiency in the donor eye. A recent study has shown that partial removal of full-thickness limbal zone will compromise the donor surface.

**Keratolimbal Allograft (KLAL)**

Tsai and Tseng reported a modification of Thoft’s keratoepitheliopasty procedure in 1994. They described an “allograft limbal transplantation” procedure that utilized a whole globe to provide a keratolimbal graft (Figure 4). A suction trephine was used to make mid-peripheral and scleral incisions, resulting in a continuous ring of keratolimbal tissue. The resultant keratolimbal ring was divided into three equal pieces and transferred to the recipient eye. Postoperatively, all patients were treated with oral cyclosporin A (CsA) in addition to topical corticosteroids. In 1995, Tsubota and colleagues reported a technique they termed “limbal allograft transplantation”, another variation of a keratolimbal allograft. Their technique was the first report of using stored corneoscleral rims for stem cell transplantation. By using stored tissue, they afforded patients several days with which to coordinate surgery after acquisition of suitable donor tissue. The major disadvantage of keratolimbal allograft is the high risk of rejection. Intensive immunosuppression by systemic corticosteroids and systemic and topical cyclosporine A was reported to improve the survival of limbal allografts and is recommended when performing KLAL and penetrating keratoplasty (PKP).

**Living-Related Conjunctival Allograft**

In 1995, Kwitko and co-workers described a technique called allograft conjunctival transplantation. In this report, they were the first to utilize a living relative as a source of donated ocular surface tissue. They harvested conjunctival tissue, and made a specific point of stating that they did not transplant limbal tissue. Donor conjunctiva was obtained from siblings, and if tissue could not be obtained from a sibling, a parent was used. Kenyon and Rapoza described a technique they called limbal allograft transplantation in which they transplanted limbal tissue with a conjunctival carrier from a living related donor. This technique was similar to Kenyon and Tseng’s technique of limbal autograft, except that the donor tissue was obtained from a living relative as opposed to the fellow eye. This technique differs from Kwitko’s living related conjunctival allograft technique in that Kenyon and Rapoza transplanted limbal tissue along with conjunctival.

The use of living-related conjunctival allografts may minimize the risks of rejection associated with the use of unrelated cadaveric donor tissue. However, the amount of tissue that can be transplanted from the living donor is limited to only 2 clock hours of limbal conjunctival tissue at the 12 and 6 o’clock positions from each eye, to minimize the risk of developing limbal deficiency in the donor.

**Human Amniotic Membrane Transplantation**

Further strategies to reconstruct the ocular surface have included the use of human amniotic-membrane transplantation (AMT). In 1940, de Röth reported the successful use of amniotic membrane transplant for conjunctival reconstruction in 1 of 6 patients following chemical burn injury. The reconstructed tissue in this patient was histologically similar to the normal bulbar conjunctiva. In 1995, Kim and Tseng used amniotic membrane for ocular surface reconstruction in a rabbit model of OSD. In their studies, they demonstrated that AMT facilitated epithelialization without allowing host fibrovascular ingrowth onto the membrane, and suggested that this procedure might be clinically useful for ocular surface reconstruction. Following on this work, in 1996 Tsubota and co-workers were the first to reconstruct human eyes with severely diseased ocular surfaces and limbal deficiency utilizing AMT. They combined AMT with a limbal stem cell allograft in 14 eyes of 11 patients with Stevens–Johnson syndrome and ocular cicatricial pemphigoid. Human amniotic-membrane transplantation is useful in conjunction with epithelial transplantation because it promotes epithelial growth without fibrovascular growth and reduces ocular surface inflammation. It supports differentiation of epithelial cells, is nonantigenic, and is resorbed in vivo.
Ex Vivo Expansion of Limbal Stem Cells

In 1997, Pellegrini and co-workers described a procedure using autologous cultivated corneal epithelium to restore the ocular surfaces of two patients with unilateral alkali injury. They based their procedure on tissue culture work that had been done by Lindberg and co-workers in 1993. Pellegrini’s group used a 1–2 mm two full-thickness limbal specimen from the healthy fellow eye to create sheets of corneal epithelial cells in tissue culture. These epithelial sheets were then transplanted to the injured eye. Both patients were followed for more than two years, and both retained a stable ocular surface, implying that stem cells had been transplanted. In 2000, Tsai and co-workers separately published their results using ex vivo expanded limbal stem cells grown on human amniotic membrane (Figure 5). Tsai’s group expanded limbal epithelium on human amniotic membrane prepared as described by Lee and Tseng, and six eyes of six patients showed epithelialization within four days. Schwab’s group grew harvested limbal stem cells on human amniotic membrane that had been denuded of native epithelium in a technique described by Schwab the previous year. The ocular surface reconstruction was considered successful in all allograft patients, and in one of the three-autograft patients. As a much smaller amount of limbal tissue is obtained from the donor eye than is required for performing conjunctival limbal auto- or allografting, it minimizes potential future complications to the healthy donor eye.

Systemic Immunosuppression

Another important advancement in the evolution of ocular surface transplantation is the use of systemic immunosuppression. Rao and co-workers reported 9 eyes of 8 patients who underwent living- related conjunctival limbal allograft. All received the best HLA match available. Systemic immunosuppression was not used, and all ocular surfaces went on to fail. The authors felt that the cause of ocular surface failure was secondary to immune-mediated rejection. Daya et al presented a series of patients with living- related conjunctival limbal allograft. He described 10 eyes of 8 patients. All received the best HLA match available, and all received systemic immunosuppression. Eight eyes survived.

Cultivated oral mucosal epithelial transplantation (COMET)

Studies have shown that oral epithelial cells can be cultured and used as an alternative for allogogenous limbal transplants in case of bilateral LSCD. Cultivated mucosal epithelial transplantation using well-differentiated, stratified epithelial sheets on amniotic membrane allows a rapid re-epithelial cover over the entire corneal surface, resulting in early reduction of inflammation and cicatrization. This surgical approach dramatically improves the prognosis of severe ocular surface diseases, especially severely inflamed corneal stem cell deficiency. This new approach not only provides early epithelialization but also allows reconstruction of the corneal surface using autologous cultivated epithelium including the cornea and oral mucosa from a small number of cell sources after amplification.

The Use of Autologous Serum in the Development of Cultivated Epithelial Sheets

The currently preferred method of cultivating epithelial sheets requires the use of xenobiotic materials in the culture system, such as fetal bovine serum (FBS) and mouse-derived 3T3 feeder cells. However, the use of FBS in the culture system is a major concern, as bovine spongiform encephalopathy cannot be detected by any known in vitro assay. Various serum-free culture systems, developed to delete the FBS from the culture system, have mainly been used to study the roles of various growth factors. The clinical use of these serum-free culture systems has been limited because of their lower efficacy for cell proliferation, compared to FBS-supplemented medium.

Future goals

In view of the basic research and developments in the field of regenerative medicine for OSD, both past and present, great progress has been made in the fundamental understanding and development of a new therapeutic modality, such as the transplantation of cultivated oral mucosal epithelial sheets using tissue engineering techniques. Greater knowledge regarding epithelial stem cell behavior from non-ocular sources and the surrounding extracellular matrix will provide a foundation for the further development of treatments for severe OSD.

References

Evolution


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