

Advances in Surgical Management of Ocular Surface Disease.

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Abstract: Surgery has a definitive role in the management of severe ocular surface disease. This review considers the various options in surgical management and recent advances in the field. The surgical interventions available for ocular surface disorders include surface stabilization procedures, ocular surface transplantation, penetrating or lamellar keratoplasty and prosthokeratoplasty. The procedures which can be taken up for ocular surface stabilization include punctal occlusion, tarsorrhaphy, conjunctival flaps, anterior stromal puncture and phototherapeutic keratectomy. Ocular surface transplantation involves transplantation of conjunctival or limbal tissue, either autologous, from a living related donor or cadaveric, or amniotic membrane. Recent times have witnessed great strides in transplantation of tissue that has been expanded ex vivo, and the basic principles of ex vivo culture of limbal stem cells are considered, along with clinical results. Finally, keratoplasty options in these patients are evaluated.

Key words : Keratoplasty, ocular surface disorder, limbal transplantation, cultivated stem cells, keratoprosthesis, phototherapeutic keratoplasty

INTRODUCTION

Medical management is the mainstay in the management of ocular surface disease, with surgical therapy being reserved only for the most severe forms of the condition. Ocular surface disease encompasses a wide range of conditions such as dry eye, limbal stem cell deficiency disorders, ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Surgical management of these disorders has witnessed great advances in recent years, particularly with regard to limbal stem cell transplantation procedures. The ability to expand limbal stem cells ex vivo as well as use oral mucosal stem cells as a source of limbal stem cells have, among others, made it possible to visually rehabilitate patients in whom no surgery would have been possible even a few years ago.

SURGICAL INTERVENTIONS

Surgical interventions for ocular surface disorders may be best described under the following heads: A) Surface stabilization procedures; B) Ocular surface transplantation; C) Penetrating/lamellar keratoplasty in ocular surface disease; D) Prosthokeratoplasty in ocular surface disease.

A) Surface stabilization procedures¹

These techniques mostly form adjuncts to primary medical management of ocular surface disorders. The following procedures are included:

1) Punctal occlusion (temporary, permanent, excisional)²

Temporary occlusion is advisable as a trial before permanent occlusion. Options for achieving temporary punctal occlusion include dissolvable collagen plugs, sections of cat gut suture material, cyanoacrylate glue, or silicone plugs. Inferior and/or superior punctal occlusion may be performed.

Permanent punctal occlusion may be subsequently undertaken in patients who improve following the temporary procedure. This may be achieved by cauterization of the punctal orifice using argon laser, thermocauterization using either monopolar/bipolar cautery or, rarely, canalicularlectomy.

2) Tarsorrhaphy (temporary, partial, total, permanent)

Tarsorrhaphy reduces evaporative tear loss as well as trauma to the ocular surface during blinking movement of the eyelids. The advantages of a partial tarsorrhaphy are preservation of central vision

as well as the ability to examine the ocular surface.

Botulinum toxin injections may be used to create an iatrogenic ptosis that lasts approximately three months in most patients. Naik et al³ evaluated the effectiveness of anterior chemodeneration of levator palpebrae superioris with Botulinum toxin type A (Botox) to induce temporary ptosis for corneal protection, and assess the incidence of superior rectus underaction. Patients with ocular surface pathology requiring temporary tarsorrhaphy underwent transcutaneous anterior chemodeneration of levator palpebrae superioris with Botox. The onset and duration of ptosis, corneal healing, and superior rectus underaction was evaluated. Median dose of Botulinum toxin injection was 12.5 U (range 10-15 U). The mean palpebral fissure height of 9 mm (SD±2.1 mm) before injection, reduced to 2.8 mm (SD±1.9 mm) at 1-week post-injection. More than 50% reduction in palpebral fissure height was seen in nine out of 10 eyes (90%, 95% CI 71.4-100%) at 1 week, seven of nine eyes (77.8%, 95% CI 50.6-100%) at 2 weeks, and two of nine eyes (22.2%, 95% CI 0-49.4%) at 4 weeks, and returned to pretreatment level after mean duration of 9.2 weeks (range 5-16 weeks). Superior rectus underaction was not noted in any of the patients. Corneal pathology improved in all cases. Anterior placement of the toxin injection may avoid superior rectus underaction.

Cyanoacrylate glue may be used to create an adhesion between the upper and lower eyelids⁴.

These more conservative techniques may be used to demonstrate therapeutic benefit prior to more invasive surgery.

Subcutaneous implantation of gold weights in the upper lid may also serve the same purpose, with the advantage of complete reversibility^{5,6}. Silver et al⁷ evaluated commercially available, thin platinum weights and compare complication rates and visibility rates with literature-reported data for gold weights. The platinum implant significantly reduced both capsule formation phenomena and extrusion compared with gold weights and could be considered as an alternative to the more conventional gold implants.

Klein et al⁸ have described reversible marginal tarsorrhaphy as a salvage procedure for periocular burns.

3) Conjunctival flap (partial, total)

These may be partial (advancement or bucket-handle) as often used for a peripheral corneal lesion, or total (Gundersen). The recruitment of the conjunctival vasculature onto the avascular cornea enables

healing of a persistent epithelial defect or severe inflammatory focus. Lim et al⁹ evaluated the indications, complications, and long-term outcomes of a Gundersen flap for the treatment of various ocular surface conditions in an Asian population. Surgical success defined as attainment of a stable ocular surface with resolution of symptoms and no flap retraction or dehiscence resulting in reexposure of the corneal surface was achieved in all eyes. Complications including infection, progression of the inflammatory or infectious disease process beneath the flap, flap loss from epithelial ingrowth, and epithelial cyst formation were encountered in 6 patients (24%). The Gundersen flap should be considered as a means of stabilizing globe integrity in the management of cases of severe ocular surface disease, particularly when visual potential is poor.

4) Anterior stromal puncture

First described by McLean et al in 1986, anterior stromal puncture is useful in the management of recurrent corneal erosions (dystrophic, post-infectious or post-traumatic).¹⁰ Though most commonly achieved using a 25-, 27- or 30-gauge needle, the YAG laser has also been used.

Tsai et al¹¹ evaluated the clinical outcomes in patients with recurrent corneal erosions who received anterior stromal puncture by use of neodymium:yttrium-aluminum-garnet (Nd:YAG) laser. Of 33 eyes, sixteen eyes were completely symptom free and twelve eyes had mild pain but no evidence of recurrent corneal erosion after operation. Five eyes had repeated episodes of recurrent corneal erosion. Eyes with a traumatic cause responded better to Nd:YAG laser therapy than those without. The frequency of corneal erosions and the severity of pain significantly improved in eyes with macroform and symptom-only recurrence. There was no significant change in refraction, but corneal surface regularity slightly improved after surgery. No adverse reaction was observed during follow-up.

The traumatic disruption of Bowman's layer incites fibroblastic transformation of the underlying stromal keratocytes and fibrin deposition, thereby enhancing epithelial adhesion to the Bowman's layer.

5) Phototherapeutic keratectomy (PTK)

PTK is useful in patients with recurrent corneal erosions and is less likely to produce visually significant scarring as compared to anterior stromal puncture. The reported rate of success, regarding alleviation of symptoms and prevention of recurrence of epithelial erosion, ranges between 74% and 100%.¹⁰ The mechanism by which excimer laser ablation prevents recurrent erosion may lie in the strong bonds formed between the epithelial basement membrane and Bowman's membrane post-operatively. The technique of treatment varies widely. Excimer laser can be used transepithelially or after debridement of the epithelium. No serious complications have been noted.

(B) Ocular surface transplantation

The integrity of the self-renewing corneal epithelium relies on the existence of stem cells, which are located in the limbal basal layer. These limbal stem cells are supported by a unique stromal microenvironment: the stem cell niche. Destructive loss of limbal stem cells or dysfunction of their stromal niche causes what is clinically referred to as limbal stem cell deficiency, characterized by conjunctivalization of the cornea, vascularization, chronic inflammation, and persistent epithelial defects. Severe ocular surface disease resulting from LSCD as in chemical injury, Stevens-Johnson syndrome (SJS) and ocular cicatricial pemphigoid are devastating conditions that represent a major clinical challenge. Conventional corneal transplants alone in these conditions are destined to fail. Ocular surface reconstruction by means of amniotic membrane

transplantation and limbal transplantation has been effective to some extent. More recently cultivated limbal stem cell transplantation has been shown to be a promising treatment modality in the management of severe limbal stem cell deficiency.¹²

1) Conjunctival transplantation

Thoft in 1977 described the first transplantation procedure for severe OSD when he proposed conjunctival transplantation for monocular chemical burns.

a) Conjunctival autograft (CAU): An autologous free conjunctival graft is obtained from the superior bulbar conjunctiva and sutured to the scleral bed. As a component of pterygium surgery (Figure 1a,1b), the autograft is most frequently fashioned from the ipsilateral eye, but in patients with previous surgery or scarring, the fellow eye is used as the source of the graft. A thin, Tenon-free conjunctival graft of adequate size is harvested. A 1-mm oversize in each diameter with regard to the donor site is generally adequate. Significant post-operative inflammation is usual, necessitating use of steroid\antibiotic eyedrops for about a month after surgery. The harvest site most often rapidly re-epithelialises. Conjunctival rotational autografting and annular conjunctival autografting are modified techniques that may prove useful under special circumstances. Table 1 summarises some of the most recent advances and concepts in pterygium surgery.

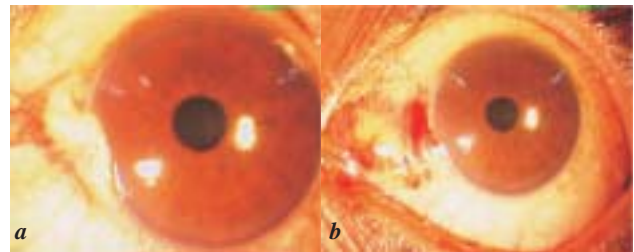


Figure 1a,1b: Pterygium excision with ipsilateral superior bulbar conjunctival autograft: (a) pre-operative and (b) post-operative appearance

b) Living-related conjunctival allograft: This is mostly performed as a conjunctival-limbal allograft for limbal stem cell transplantation procedures, as described below.

2) Limbal transplantation: Limbal stem cell transplantation enables restoration of phenotypic corneal epithelium to the corneal surface, promotes the barrier function of the limbus and also enhances surface lubrication, thereby providing an improved milieu for maintenance of corneal clarity.

A) Conjunctival limbal autograft (CLAU) : This procedure is indicated for patients requiring epithelial stem cell transplantation for management of corneal surface disease due to unilateral limbal stem cell deficiency, most often following chemical or thermal injuries.²⁰ (Figure 2a,2b)It is essential that the donor eye be free from any condition that may predispose it to subsequent development of limbal stem cell deficiency. The risk of donor epithelial problems can be minimised by harvesting less than six clock hours of limbal tissue and a moderate amount of conjunctiva. Gentian violet markings within the graft help delineate the epithelial surface of the graft. At the time of harvesting the graft, dissection is performed into the peripheral cornea approximately 1 mm beyond the peripheral corneal vascular arcades. Correct orientation of the graft is vital. The major benefit is that the need for systemic immunosuppression is eliminated. The donor eye is treated with antibiotic\steroid drops 3 to 4 times daily until epithelialization is complete (usually 1 to 2 weeks).

Table 1: Pterygium surgery:Recent advances and concepts

	Surgery performed	Comments	Complications/results	Recurrence
1) Hall et al ¹³ (August 2009)	Pterygium excision with conjunctival autograft	Tisseel fibrin glue vs vicryl sutures (25 eyes in each group)	Fibrin glue advantages:Shorter surgical time(12 min vs 26 min); Significantly less pain on post-operative days 1,2;	6 and 12 month recurrence rates not significantly different between the two groups (0/24 in the glue group and 2/23 in the suture group)
2)Srinivasan et al ¹⁴ (Feb 2009)	Primary pterygium surgery with conjunctival autograft	Comparison of Tisseel fibrin glue with sutures (20 eyes in each group)	Fibrin glue advantages:Significantly less post-operative inflammation at 1 month and 3 months;Graft remained equally stable in both groups;	-
3) Farid et al ¹⁵ (Jan 2009)	Pterygium excision with conjunctival autograft	Comparison of recurrence rates between fibrin glue(n=27) and absorbable sutures(n=20) used for graft fixation	-	Recurrence rates significantly less with fibrin glue(3.7%) as compared to absorbable sutures(20%)
4) Hirst LW ¹⁶ (July 2009)	P.E.R.F.E.C.T. (Pterygium Extended Removal Followed by Extended Conjunctival Transplant) for recurrent pterygium	111 patients with recurrent pterygium	Complications: 1 patient had an absent graft at week 1 One patient developed an exotropia; one patient lost four lines of vision due to a corneal ulcer	No recurrences at 1 year (all but two patients followed up for at least one year);
5) Haider et al ¹⁷ (April 2009)	Primary pterygium excision with conjunctival autograft	In 38 eyes, the conjunctival autograft was rotated by 90 degrees , in 15 eyes by 0 degrees and in 21 eyes by 180 degrees .	-	The recurrence rates (regrowth of the pterygium more than 1 mm over the cornea) were 3% in 90 degrees rotation, 28.6% in 0 degree rotation, and 15.8% in 180 degrees rotation (p=0.033). The overall recurrence rate for all rotation autograft surgeries was 10.8%.
6) Ozer et al ¹⁸ (April 2009)	Primary pterygium excision surgery	Compared the long-term follow-up results of the bare sclera technique (BST), limbal-conjunctival autograft technique (LCAT) and amniotic membrane graft technique (AMGT) in primary pterygium excisions.	Corneal epithelialization time was earlier in group 2 than in groups 1 (p < 0.01) and 3 (p < 0.05). Postoperative complications were not seen in any of the groups.	The recurrence rate was significantly lower in group 2 than in groups 1 and 3 (p < 0.001).
7) Bahar et al ¹⁹ (May 2008)	Pterygium excision with conjunctival autograft	Compared the long-term outcomes and recurrence rate of extensive versus limited subconjunctival pterygium excision	-	The recurrence rate in the limited excision group was 12.1% compared with only 4.3% in the extensive pterygium excision group (p = 0.14). The mean time to recurrence was shorter with limited excision compared to extensive excision . Younger age (p = 0.0003), larger area of corneal involvement (p = 0.004), worse preoperative visual acuity (p = 0.01), and limited pterygium excision (p = 0.04) significantly increased the risk for recurrence.

The recipient eye is prescribed antibiotic eyedrops in addition to frequent application of preservative free artificial tears with topical steroids, three to four times daily to minimize post-operative inflammation.

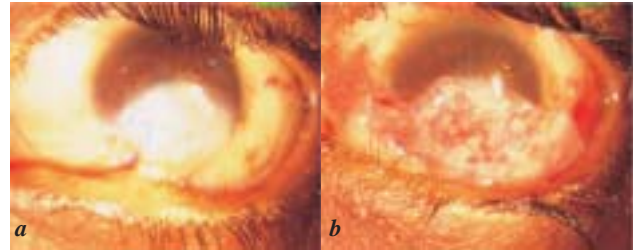


Figure 2a, 2b: Conjunctival-limbal autograft for post-chemical injury partial limbal stem cell deficiency (a) pre-operative and (b) post-operative day 1 appearance)

Soliman et al evaluated the usefulness of limbal stem cells and conjunctival autograft transplantation for the treatment of primary pterygium. 42 eyes of 42 patients with grade I-III primary pterygium underwent pterygium excision followed by superotemporal limbal stem cells and conjunctival autograft transplantation. Pterygium recurrences and complications within a mean follow-up period of 18.26 months (range, 10-28 months) were assessed.

RESULTS

There were no recurrences of pterygium growth except in 2 cases (4.75%). In addition, no significant complications were noted. No further surgical interventions were needed in any case except for reoperation in the 2 recurrent cases²¹.

a) Cadaveric conjunctival limbal allograft: This procedure is rarely used nowadays, largely due to success of ex vivo culture techniques for limbal stem cells.

b) Living-related conjunctival limbal allograft (lrCLAL): The best HLA-matched relative is the ideal donor. ABO matching is also advisable since expression of blood-group antigens has been demonstrated on epithelial cells. Both eyes of the donor must be evaluated to exclude any condition that may contraindicate donation such as glaucoma (and the potential need for filtering surgery in the future), chronic use of topical medications and history of contact lens wear with Thiomersal-containing preservatives (that may cause stem cell depletion) or a prior surgical history (and the possibility of iatrogenic stem cell deficiency). Donor screening for HIV 1 & 2, Hepatitis B and C is mandatory. Most commonly, two pieces of tissue, each between two- and three-clock hours at the limbus are resected from the 12 o'clock and six o'clock areas, 5 mm posterior to the limbus. In the post-operative period, the aim is to minimize inflammation and promote epithelialisation in both the donor and the recipient. In the recipient, vascularisation of the graft is essential for survival of limbal stem cells. Minimising graft movement due to lid action at the graft site can be achieved by keeping the eye patched between eyedrops for atleast the first week. A bandage contact lens may also help. The recipient is prescribed preservative-free antibiotic/steroid eyedrops 4 times daily. Autologous plasma or serum may be used to provide growth factors to the grafted epithelium. Preservative free tear supplements and/or early tarsorrhaphy may be necessary where indicated.

Systemic immunosuppression is advisable unless contraindicated. It is commenced on the day of surgery, most often with a combination

of systemic steroid and cyclosporine-A. While some discontinue immunosuppression after 12 to 18 months, others continue indefinitely. The addition of oral Azathioprine may permit lower doses of Cyclosporin-A. Yalcindag et al²² evaluated the efficacy and safety of tacrolimus in prevention of allograft rejection in high-risk allo-limbal grafts. Six eyes of six patients with severe limbal stem cell deficiency were included. All patients were started 0.1 mg x kg 1 x day (-1) tacrolimus orally 3 days before the surgery. The study concluded that limbal allo-graft transplantation for ocular surface reconstruction had a successful outcome with using systemic tacrolimus for immunosuppression.

Scocco evaluated the long-term outcome of HLA-matched Ir-CLAL for bilateral ocular surface disorders. 39 eyes of 32 patients with bilateral surface disorders and clinical diagnosis of limbal stem cell deficiency who underwent HLA-matched Ir-CLAL were included. Visual acuity (VA), ambulatory vision (> or = 20/200) and ocular surface stability were evaluated as main outcomes. One year after surgery, VA improved in 46.2%, ambulatory vision was achieved in 48.7% and a stable corneal surface was achieved in 84.6% of the eyes. At the final follow-up (mean, 48.7 ±30.6 months), 66.6% of the eyes that had gained VA one year after surgery maintained an improved VA (p=0.28), 94.7% of eyes that had achieved ambulatory vision one year after surgery maintained 20/200 or better (p<0.001) and 93.9% still had a stable corneal surface (p=0.043) at the final follow-up²³.

- c) **Keratolimbal allograft (KLAL):** In this technique, allogenic cadaveric limbal stem cells are transplanted to a recipient eye with severe ocular surface disease using peripheral cornea as a carrier. It is indicated in patients with severe bilateral OSD secondary to limbal stem cell deficiency. It is best suited for disease entities that primarily affect the limbus with minimal or no involvement of the conjunctiva, most typically aniridia. A healthy and stable tear film is essential for success of the procedure. (Figure 3)

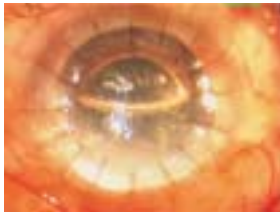


Figure 3: Keratolimbal allograft

The surgical techniques described include the corneoscleral crescent technique of Holland/Schwartz^{24,25} (wherein the donor corneo-scleral rim is sectioned into 2 halves), corneoscleral ring technique of Tsubota²⁶ (wherein ring-shaped donor corneoscleral rim bearing limbal stem cells is transplanted) and the homologous penetrating central limbo-keratoplasty (HPCLK) technique of Sundmacher²⁷ (here the donor corneo-scleral button is eccentrically trephined, resulting in one-third of the circumference of the button containing limbal tissue, thereby combining a penetrating keratoplasty and a limbal stem cell transplantation procedure). Lim et al²⁸ described an alternative and novel technique using cyanoacrylate glue to achieve successful limbal tissue dissection, from an organ culture media stored corneoscleral button, without an artificial anterior chamber.

Due to the fact that the limbal stem cells are antigenically more active than central corneal stromal and endothelial cells, systemic immunosuppression consisting of corticosteroid, Azathioprine and cyclosporine is recommended post-operatively.

Liang et al²⁹ determined the long-term outcomes of keratolimbal allograft (KLAL). Prolonged oral mycophenolate mofetil and tacrolimus and short-term prednisone and acyclovir were administered in 12 eyes (10

consecutive patients) with total limbal stem cell deficiency after KLAL. Ten eyes underwent subsequent penetrating keratoplasty. During a follow-up of 61.2 months after KLAL, postoperative epithelial breakdown due to exposure occurred late in the period after PKP and remained a primary risk. Mean daily doses of 1.4 g of mycophenolate mofetil and 1.6 mg of tacrolimus were administered for a mean period of 52.7 months (SD, 22.5; range, 23-91 months) with few adverse effects. Keratolimbal allograft and PKP rejection was noted in 2 and 3 eyes, respectively, though there was a reversal in 1 eye in each group, yielding final KLAL and PKP survivals in 10 and 8 eyes, respectively, and ambulatory visual acuity of up to 20/20 in 10 eyes for 67.2% of the entire follow-up period.

Wylegala et al³⁰ analyzed the graft survival rate and stability of the corneal surface in patients who underwent limbal stem cell transplantation. Three surgical techniques were evaluated: conjunctival limbal autograft (CLAU), living-related conjunctival limbal autograft (Ir-CLAL), and keratolimbal allograft (KLAL) transplantations. Graft survival rate and the regularity of the corneal surface differed significantly between the allo- and autografts. The 3-year and 6-year graft survival rates were 76.1% and 61.9%, respectively, for the autologous transplantation group, and 59.4% and 46.3%, respectively, for the allogeneic transplantation group, thereby concluding that significantly better long-term outcomes were achieved with autotransplantation of the limbus compared with allogeneic limbal grafts from living-related and cadaveric donors.

- a) **Ex vivo expanded limbal autograft \ Living-related ex vivo expanded limbal allograft (Figure 4a,4b):** The various protocols for cultivation of limbal epithelium differ in the use of intact versus epithelially denuded amniotic membrane, suspension of epithelial cells rather than explants, co-cultivation of 3T3 fibroblast feeder layers, and air-lifting prior to transplantation¹². Pellegrini and colleagues³¹ first reported successful reconstruction of the ocular surface in LSCD using a suspension culture technique.

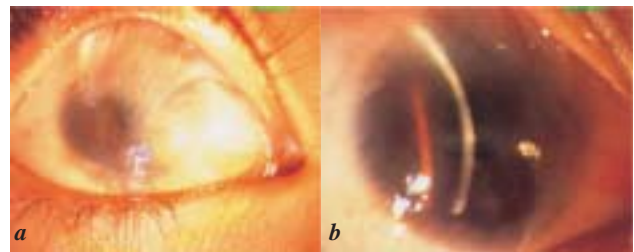


Figure 4: a) Pre-operative and b) 1st post-operative day appearance of ex vivo expanded limbal autograft for post chemical injury total limbal stem cell deficiency

The next advancement in this technique was the use of human amniotic membrane as a substrate for in vitro epithelial cell culture. Koizumi and colleagues³² first cultivated rabbit limbal epithelium on human amniotic membrane (HAM) in vitro and then, after transplantation onto rabbit ocular surface, confirmed the viability of the transplanted cultivated epithelium in vivo.

Koizumi and colleagues³³ demonstrated that both cell suspension and explant culture methods produced a healthy epithelial cell layer, with cells from the former being morphologically more superior.

Currently most investigators prefer the explant culture technique¹². The benefits of using explants are that they are easy to prepare and there is no danger of damaging the corneal epithelium through enzyme treatment.

The general principles of culturing the cells by explant culture technique involve the following steps: harvesting the limbal tissue (from the contralateral healthy eye in case of a unilateral LSCD, or from donor corneas for bilateral LSCD); selecting the appropriate carrier: a sheet of multi-layered epithelium, human amniotic membrane, collagen shields, or contact lens; preparation of human corneal epithelial medium; and

explant cultures.

Confirmation of growth can be done by various methods including-direct observation, whole mount stained preparation, histopathology, immunohistochemistry, thymidine incorporation and by flow cytometry using markers for cell cycle.

Although a number of investigators have included various reconstruction techniques using autogenous conjunctiva, mucous membrane grafts, collagen lattices, synthetic implants, and cell-suspension cultures, the most widely accepted universal substratum for explant cultures is the HAM³⁴.

The donor eye should be healthy without a history or physical signs of injury. (Figure 4) A 2mm² biopsy is usually taken from the superior temporal limbus and placed in a cellular transport medium for transportation to the laboratory. If an allogenic transplantation is planned, the recipient should be immunosuppressed pre-operatively. Systemic immunosuppression may be begun a week or two prior to surgery. Cyclosporin A is the prototype immunosuppressive agent. A therapeutic contact lens is placed after surgery and may need to be retained for 8 to 12 weeks to allow proper epithelial cell adherence. If tolerated, systemic immunosuppression should be continued for a year or even longer.

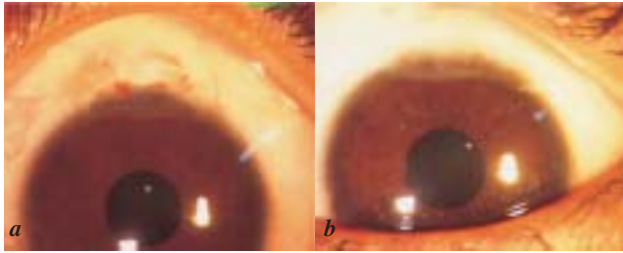


Figure 5a, 5b: 1 day and 1 month post-operative appearance of donor site for ex vivo expanded limbal autograft (fellow eye of same patient as in figure 4)

Omoto et al reported the efficacy of human bone marrow-derived mesenchymal stem cells as a source of feeder cells for the cultivation of transplantable corneal epithelial cell sheets³⁵.

Monteiro et al³⁶ demonstrated, using immunohistochemistry and reverse transcription-polymerase chain reaction, that human immature dental pulp stem cells express markers in common with limbal stem cells, such as ABCG2, integrin beta1, vimentin, p63, connexin 43 and cytokeratins 3/12. These cells were also shown to be capable of reconstructing the ocular surface after induction of unilateral total limbal stem cell deficiency in rabbits, as shown by morphological and immunohistochemical analysis using human-specific antibodies against limbal and corneal epithelium, thereby suggesting that human immature dental pulp stem cells share similar characteristics with limbal stem cells and might be used as a potential alternative source of cells for corneal reconstruction.

Kim et al³⁷ reported their experience with corneal epithelium, grown in vivo, transplantation in three patients with persistent epithelial defect (PED). The three patients had ocular surface disease unresponsive to standard treatments and were therefore chosen for transplantation. They underwent transplantation of epithelial sheets, grown in vivo, to the most affected eye. In vivo cultivation was carried out in the cornea of a living related donor. After epithelialization was completed, the epithelium grown on an amniotic membrane was harvested gently; it was then transplanted into the patient's eye after debridement of fibrovascular tissue. The cultivated epithelium was completely epithelialized by 2 weeks; it was well-differentiated with well-formed hemidesmosome. On immunohistochemical staining, p63, connexin 43, and Integrin beta4 were expressed in the cells on the epithelial sheet. The PED was covered completely and maintained for 4 weeks in all cases. However, corneal erosion recurred after 5 weeks in two cases. This novel technique

demonstrated that corneal epithelial cells can be expanded in vivo successfully on denuded amniotic membrane on a healthy cornea and harvested safely. A corneal epithelial sheet, grown in vivo, can then be transplanted to treat an eye with a severe ocular surface disease, such as total limbal deficiency.

Hashemi et al³⁸ undertook a study to establish the characterization of cultured oral mucosal epithelium thereby introducing them as an alternative source for reconstruction of ocular surface disease. The cultured oral epithelium shared the characteristics with corneal epithelium and hence could be an alternative source for ocular surface transplantation. Table 2 summarises some of the clinical studies of ex vivo cultivated limbal stem cell transplantation.

Table 2 : Summary of clinical studies of ex vivo cultivated limbal stem cell transplantation

Author/ year	Type of LSCT	Intervention	No of eyes	Mean follow up (Months)	Overall success rate	Improved BCVA from baseline No./total (%)	Comments
1) Pelligrini et al/1997 ³¹	Total-2	Autologous LSCT	2	24	Stable ocular surface	Improvement in BCVA	
2) Schwab et al/ 2000 ³⁹	Total-7 Partial-7	Autologous LSCT-10 Allograft LSCT-4	14	13	Stable ocular surface in 6/10 of autografts and all 4 of allografts	All patients improved in BCVA. >20/200 in 12/14 patients And > 20/30 in 7/14 patients	
3) Tsai et al ⁴⁰ / 2000	Total	Autografts	6	15	All eyes had stable ocular surface	83 % BCVA improved from 20/112 to 20/45	
4) Rama et al/ 2001 ⁴¹	Unilateral severe	Autologous LSCT	18	18.6		7/18 (38.9 %)	
5) Koizumi et al/ 2001 ⁴²	Total	Allografts-13	13	11.2	All eyes had stable ocular surface	BCVA improved by 2 lines or more in 10/13 eyes.	Subconjunctival tissue treatment with 0.04% mitomycin C
6) Shimazaki et al/2002 ⁴³	Total-13	Allograft LSCT-13	13	NA	46.2 % 3/8 eyes developed partial conjunctival invasion 2 eyes later developed epithelial defects		Corneal perforation-4 eyes Infectious keratitis-2 eyes
7) Sangwan et al/ 2005 ⁴⁴	Total- 14, partial-1	Autologous LSCT -11 Living related allograft LSCT-3 nonrelated allograft LSCT-1	15	15.3		10/15 (67 %)	In 15 cases PKP was done later
8) Sangwan et al/ 2005 ⁴⁵	Bilateral -4 Unilateral-84	Autologous LSCT	86	18.3			
9) Daya et al/ 2005 ⁴⁶	Total-10	Allografts-10	10	28	Improvement in ocular surface - 70 %	BCVA improvement - 40 %	
9) Nakamura et al/2006 ⁴⁷	Total-9	Allograft LSCT-7 Autologous LSCT-2	9	14.6	Stable ocular surface at last follow up	Improvement in more than 2 lines of preoperative BCVA	
10) Kawashima et al/ 2007 ⁴⁸	Total-6	Autologous-2 Allografts non related -3 Allograft related -1	6	25	Stable ocular surface at last follow up	Improvement in BCVA in 4 out of 6 eyes	All eyes subsequently underwent keratoplasty for visual rehabilitation
11) Shortt et al/ 2008 ⁴⁹		Autologous-3 Allografts-7	10	13	60% autografts 33%, allografts 71%	Improvement in more than 2 lines of preoperative BCVA	

3) Amniotic membrane transplantation: Amniotic membrane may be used as a graft to replace damaged ocular surface stromal matrix, as a dressing to prevent unwanted inflammatory insults from gaining access to the damaged ocular surface, a combination of both as well as a carrier for expanding epithelial stem cells ex vivo.

The proposed mechanisms for the benefits of amniotic membrane transplantation include prolonging life span and maintaining clonogenicity of epithelial progenitor cells, promoting non-goblet cell epithelial differentiation, promoting goblet cell differentiation when combined with conjunctival fibroblasts, excluding inflammatory cells because of anti-protease activities and suppressing the TGF- β signaling system and myofibroblast differentiation of normal fibroblasts.

The observed clinical effects include epithelialization with maintenance of normal epithelial phenotype and reduction of inflammation, vascularisation and scarring.(Figures 6a,6b)

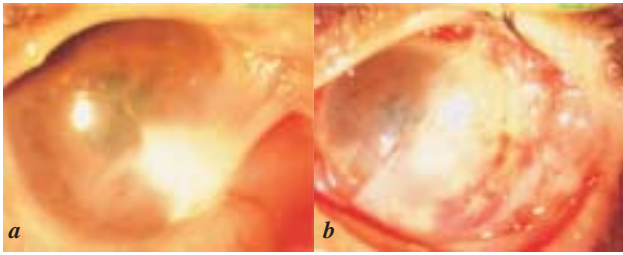


Figure 6a,6b: Amniotic membrane transplantation following chemical injury: pre-operative and 1st post-operative day appearance.

Shay et al, in a recent review⁵⁰, summarized recent literature data, showing how sight-threatening corneal complications of Stevens Johnson syndrome and its more severe variant, toxic epidermal necrolysis, can progressively develop from cicatricial pathologies of lid margin, tarsus, and fornix at the chronic stage. They concluded that such pathologies can be prevented with the early intervention of cryopreserved amniotic membrane transplantation to suppress inflammation and promote epithelial healing at the acute stage. Significant dry eye problems and photophobia can also be avoided with this intervention.

The role of amniotic membrane transplantation in surgery for pterygium deserves mention. Kucukerdonmez et al⁵¹ compared the results of amniotic membrane transplantation (AMT) using fibrin glue vs vicryl sutures in pterygium surgery. After the removal of pterygium, patients were randomised to undergo AMT using fibrin glue (Tisseel, 32 eyes) or 8-0 vicryl sutures (38 eyes). The average operation time in the fibrin glue and suture groups were 11.2±2.4 min (mean±SD) and 18.7±2.2 min, respectively (P=0.018). Significantly fewer post-operative symptoms were observed in the fibrin glue group at post-operative days 1, 7, and 14 (P<0.05 for all). At the end of the follow-up, true recurrence was developed in three eyes (9.4%) in the fibrin glue group, and in four eyes (10.5%) in the suture group (P=0.33). The rates of conjunctival recurrence were 21.9% (seven eyes) and 23.7% (nine eyes) in the fibrin glue and suture groups, respectively (P=0.38).

Kucukerdonmez et al⁵² in a separate study, compared the clinical results of conjunctival autograft and amniotic membrane transplantation (AMT) for primary and recurrent pterygium excision in an effort to determine whether AMT yields acceptable cosmetic results as conjunctival autografting in pterygium surgery. 78 eyes of 78 patients were divided into 2 groups: forty eyes (28 with primary and 12 with recurrent pterygia) received conjunctival limbal autograft (group 1), and 38 eyes (27 with primary and 11 with recurrent pterygia) received AMT (group 2) after extensive removal of pterygium. The results were evaluated by clinical examination and photographing and final appearances were graded from 1 to 4. Grade 4 represented true recurrence, whereas grade 3 indicated unacceptable cosmetic appearance. Recurrence rates of primary, recurrent, and all pterygia in group 1 and group 2 were 3.6%, 16.7%, and 7.5% and 3.7%, 18.2%, and 7.9%, respectively. There was no significant difference in recurrence rates between the 2 groups (P = 1.00 for all). The rates of final appearance as grade 3 were 10.0% in group 1 and 21.1% in group 2, and the difference was statistically significant (P = 0.048). No major complications occurred during the follow-up period. The study thereby concluded that although better results in the final appearance were achieved using conjunctival autografting than with AMT, AMT can be a useful alternative in pterygium surgery, especially in those patients who may have a limited amount of conjunctiva for future surgeries.

Park et al⁵³ investigated the efficacy of permanent amniotic membrane transplantation (AMT) for the treatment of various ocular surface diseases. The amniotic patches were grafted for the treatment of neurotrophic ulcers (n=15), inflammatory corneal ulcers (n=15), scleral ulcers (n=11), painful bullous keratopathy (n=8) and pterygium as an adjuvant to a conjunctival autograft (n=13). Cryo-preserved or freeze-dried amniotic membrane (AM) were used. The overall success rate, the interval to epithelialization, pain-subsiding time, and complications were evaluated. The pain relief and the full epithelialization interval in the bullous keratopathy patients given the cryo-preserved AM were compared with those given the freeze-dried AM. The success rate in the patients with neurotrophic ulcer, inflammatory corneal ulcer, scleral ulcer and bullous keratopathy were 93.3%, 66.7%, 92.9% and 100%, respectively. A conjunctival autograft with AMT showed a 100% success rate without recurrence. The time to re-epithelialization was 24.4±24.2, 20.4±5.8 and 16.9±7.0 days in patients with neurotrophic, inflammatory and scleral ulcers, respectively (p=0.431). The pain relief interval in the cryo-preserved and freeze-dried AM group was 17.7 and 23.3 days, and the re-epithelialization interval was 29 and 22 days, respectively, which was insignificant.

(C) Penetrating/lamellar keratoplasty in ocular surface disease:

Ocular surface reconstruction is, most often, a sequence of procedures culminating in an optical penetrating/lamellar keratoplasty. These procedures aim to optimize conditions for optical function by ensuring a normal interface between the lids and the globe, normal tear function and, finally, cellular replacement or reconstruction. Adequacy of the stem cell reserve, aqueous- or mucin-deficiency dry eye and anatomic lid abnormalities are factors critical to the survival of a corneal graft. Adequate stem cell supply, adequate tear function and anatomically functional lids are essential to prevent persistent non-healing epithelial defects, secondary ulceration with stromal melting, vascularisation and conjunctivalisation and, ultimately, immune graft rejection.

There are three basic approaches: 1) simultaneous stem cell graft and penetrating keratoplasty using the same donor tissue, 2) the staged approach, which consists of the appropriate stem cell surgery followed by penetrating or lamellar keratoplasty at a later date, and 3) a large eccentric donor graft supplying both limbal stem cells and the optimal keratoplasty.

Shi et al⁵⁴ compared the therapeutic outcomes between penetrating keratoplasty (PK) combined with keratolimbal allograft (KLAL) transplantation and corneoscleral transplantation in patients with severe corneal burns. Thirty-eight patients (39 eyes) diagnosed with severe corneal burns in stable status with vascularization and corneal opacity were included. Combined PK with KLAL transplantation was performed in 23 eyes (group A) and corneoscleral transplantation in 16 eyes (group B). The main outcome measures were postoperative complications and long-term visual acuity and rejection. The incidence of postoperative complications (corneal epithelium defect, hyphaema and hypotony) in group A were obviously less than those in group B. Fifteen eyes (65%) in group A and four eyes (25%) in group B had best-corrected visual acuity of >0.05 at 24 months (P = 0.022). Limbal stem cell rejection occurred in eleven grafts (48%) in group A and eight grafts (50%) in group B (P = 1.000). Nine grafts (39%) in group A and 12 grafts (54%) in group B had endothelial rejection (P=0.049). Thus, PK combined with KLAL transplantation may reduce the risk of postoperative complications. Long-term prognosis appears better than corneoscleral transplantation in the treatment of severe eye burns.

Mendicutte et al⁵⁵ reported a new surgical procedure for the treatment of ocular surface diseases associated with severe limbal insufficiency.

Four patients with severe ocular surface disease who required stem cell transplantation and keratoplasty for the correction of limbal insufficiencies underwent large diameter lamellar keratoplasty with microkeratome. When limbal dysfunction was associated with limited alteration of the ocular surface and transparent deep corneal stroma, only the anterior corneal stroma was transplanted. When the entire corneal thickness was compromised, both anterior and deep donor buttons were transplanted. Patients remained stable and improved their visual acuity after surgery. Best-corrected visual acuity ranged from 20/200 to 20/30. No corneal graft rejections were found. The main complication found in one of patients was a central stromal opacity which required a secondary penetrating keratoplasty. This technique enables a single-stage surgical procedure and the use of a single donor which reduces the risk of rejection. In addition, better refractive results are achieved due to the quality of the interface and the absence of corneal sutures

(E) Prosthokeratoplasty in ocular surface disease: Complex issues of material bio-compatibility and technical difficulty limit the success of prosthokeratoplasty. The primary complications have included dislocation and extrusion of the prosthesis, retroprosthetic membrane formation, secondary glaucoma, endophthalmitis, uveitis, retinal detachment, vitreous haemorrhage, ad leakage around the keratoprosthesis with resulting hypotony, among others.

The osteo-odonto-keratoprosthesis (OOKP) and the Boston keratoprosthesis are the most frequently performed these days.

Patients with severe disturbances of the ocular surface, such as those with Stevens-Johnson syndrome, have an extremely poor prognosis. Noncicatrising conditions, on the other hand, may do quite well, while surface diseases such as ocular cicatricial pemphigoid and chemical burns occupy the prognostic mid-ground. The key factor appears to be the degree of inflammation.

Implantation of a keratoprosthesis device is generally undertaken when other surface reconstruction methods for visual rehabilitation have failed, or are deemed unfeasible. Candidates would include those with severe bilateral chemical or thermal injury and patients with ocular cicatricial pemphigoid or Stevens-Johnson syndrome.

Aldave et al⁵⁶ report the usefulness of the Boston type I keratoprosthesis in the management of corneal opacification, corneal limbal stem cell failure, or both in a large single-surgeon series. The Boston type I keratoprosthesis was demonstrated as an effective means of managing repeat corneal graft failure and corneal limbal stem cell failure with or without corneal opacification in patients with both unilateral and bilateral visual impairment.

Tan et al⁵⁷ have evaluated the efficacy and preliminary safety of the osteo-odonto-keratoprosthesis in end-stage corneal and ocular surface disease. Sixteen adults of Asian ethnic origin, bilaterally blind with end-stage corneal blindness from Stevens-Johnson syndrome, or severe chemical or thermal burns were included. Osteo-odonto-keratoprosthesis surgery was performed in two stages-in stage 1, an autologous canine tooth is removed, modified to receive an optical polymethyl methacrylate cylinder, and implanted into the cheek. The ocular surface is denuded and replaced with full-thickness buccal mucosa. Stage 2 surgery, performed 2 to 4 months later, involves retrieval of the tooth-cylinder complex and implanting it into the cornea, after reflection of the buccal mucosal flap, corneal trephination, iris and lens removal, and anterior vitrectomy. Concurrent glaucoma and vitreoretinal procedures are also performed at either stage, as required. Intraoperative complications included expulsive hemorrhage (keratoprosthesis device not implanted), tooth fracture (n = 1), oronasal fistula (n = 1), and mild inferior optic tilt (n = 1). Anatomical stability and keratoprosthesis retention was

maintained in all eyes, with no dislocation, extrusion, retroprosthetic membrane formation, or keratoprosthesis-related infection. Other complications not directly related to device insertion included retinal detachment (RD) related to silicone oil removal (n = 1) and endophthalmitis related to endoscopic cyclophotocoagulation performed 1 year after OOKP surgery (n = 1). Eleven patients (73.3%) attained a stable best spectacle-corrected VA of at least 20/40 or better, whereas 9 (60%) attained stable 20/20 vision. Four patients achieved their best visual potential, ranging from 20/100 to counting fingers vision, related to preexisting glaucomatous optic neuropathy or previous RD.

REFERENCES

- Holland EJ, Mannis MJ: Ocular surface disease: Medical and surgical management 2002 Springer-Verlag.
- Tost FH, Geerling G: Plugs for occlusion of the lacrimal drainage system. *Dev Ophthalmol.* 2008;41:193-212.
- Naik MN, Gangopadhyay N, Fernandes M, Murthy R, Honavar SG: Anterior chemodeneration of levator palpebrae superioris with botulinum toxin type-A (Botox) to induce temporary ptosis for corneal protection. *Eye (Lond).* 2008 Sep;22(9):1132-6.
- Leahey AB, Gottsch JD, Stark WJ: Clinical experience with N-butyl cyanoacrylate (Nexacryl) tissue adhesive. *Ophthalmology.* 1993 Feb;100(2):173-80.
- Razfar A, Afifi AM, Manders EK, Myers EN, Johnson JT, Ferris RL, Deleyiannis FW: Ocular outcomes after gold weight placement and facial nerve resection. *Otolaryngol Head Neck Surg.* 2009 Jan;140(1):82-5.
- Nazi V, Marras C, Broggi G: Upper eyelid gold weight implants in patients with facial nerve palsy: Surgical technique. *J Neurosurg Sci.* 2006 Dec;50(4):107-10.
- Silver AL, Lindsay RW, Cheney ML, Hadlock TA: Thin-profile platinum eyelid weighting: a superior option in the paralyzed eye. *Plast Reconstr Surg.* 2009 Jun;123(6):1697-703.
- Klein MB, Ahmadi AJ, Sires BS, Engrav LH: Reversible marginal tarsorrhaphy: a salvage procedure for periocular burns. *Plast Reconstr Surg.* 2008 May;121(5):1627-30.
- Lim LS, How AC, Ang LP, Tan DT: Gundersen flaps in the management of ocular surface disease in an Asian population. *Cornea.* 2009 Aug;28(7):747-51.
- Das S, Seitz B: Recurrent corneal erosion syndrome. *Surv Ophthalmol.* 2008 Jan-Feb;53(1):3-15.
- Tsai TY, Tsai TH, Hu FR, Hou YC: Recurrent corneal erosions treated with anterior stromal puncture by neodymium: yttrium-aluminum-garnet laser. *Ophthalmology.* 2009 Jul;116(7):1296-300.
- Burman S, Sangwan V: Cultivated limbal stem cell transplantation for ocular surface reconstruction. *Clin Ophthalmol.* 2008 September; 2(3): 489-502.
- Hall RC, Logan AJ, Wells AP: Comparison of fibrin glue with sutures for pterygium excision surgery with conjunctival autografts. *Ophthalmology: Clin Experiment Ophthalmol.* 2009 Aug;37(6):584-9.
- Srinivasan S, Dollin M, McAllum P, Berger Y, Rootman DS, Slomovic AR: Fibrin glue versus sutures for attaching the conjunctival autograft in pterygium surgery: a prospective observer masked clinical trial. *Br J Ophthalmol.* 2009 Feb;93(2):215-8.
- Farid M, Pirnazar JR: Pterygium recurrence after excision with conjunctival autograft: a comparison of fibrin tissue adhesive to absorbable sutures. *Cornea.* 2009 Jan;28(1):43-5.
- Hirst LW: Recurrent pterygium surgery using pterygium extended removal followed by extended conjunctival transplant: recurrence rate and cosmesis. *Ophthalmology* 2009 Jul;116(7):1278-86.
- Haider TA, Al-Amin LM, Bamashmus MA, Ali SF: Ninety degree conjunctival rotation autograft versus zero and 180 degree rotation autograft for pterygium surgery. *Saudi Med J.* 2009 Apr;30(4):519-23.
- Ozer A, Yildirim N, Erol N, Yurdakul S: Long-term results of bare sclera, limbal-conjunctival autograft and amniotic membrane graft techniques in primary pterygium excisions. *Ophthalmologica.* 2009;223(4):269-73.
- Bahar I, Kaiserman I, Weisbrod M, McAllum P, Slomovic A: Extensive versus limited pterygium excision with conjunctival autograft: outcomes and recurrence rates. *Curr Eye Res.* 2008 May;33(5):435-40.
- Fernandes M, Sangwan VS, Rao SK, Basti S, Sridhar MS, Bansal AK, Dua HS: Limbal stem cell transplantation. *Indian J Ophthalmol.* 2004 Mar;52(1):5-22.
- Soliman Mahdy MA, Bhatia J: Treatment of primary pterygium: role of limbal stem cells and conjunctival autograft transplantation. *Eur J Ophthalmol.* 2009 Sep-Oct;19(5):729-32.
- Yalcindag FN, Incel O, Ozdemir O: Effectiveness of tacrolimus in high-risk limbal allo-graft transplantation. *Ann Ophthalmol (Stokio).* 2008 Fall-Winter;40(3-4):152-6.
- Scocco C, Kwitko S, Rymer S, Marinho D, Bocaccio F, Lindenmeyer R: HLA-matched living-related conjunctival limbal allograft for bilateral ocular surface disorders: long-term results. *Arq Bras Oftalmol.* 2008 Nov-Dec;71(6):781-7.
- Holland EJ, Djalilian AR, Schwartz GS: Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. *Ophthalmology.* 2003 Jan;110(1):125-30.
- Crossdale CR, Schwartz GS, Malling JV, Holland EJ: Keratolimbal allograft: recommendations for tissue procurement and preparation by eye banks, and standard surgical technique. *Cornea.* 1999 Jan;18(1):52-8.
- Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimamura S, Bissen-Miyajima H, Shimazaki J: Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med.* 1999 Jun 3;340(22):1697-703.
- Reinhardt T, Sundmacher R, Spelsberg H, Althaus C: Homologous penetrating central limbo-keratoplasty (HPCLK) in bilateral limbal stem cell insufficiency. *Acta Ophthalmol Scand.* 1999 Dec;77(6):663-7.

28. **Lim LT, Bhatt PR, Ramaesh K.** Harvesting keratolimbal allografts from corneoscleral buttons: a novel application of cyanoacrylate adhesive. *Br J Ophthalmol.* 2008 Nov;92(11):1550-1.
29. **Liang L, Sheha H, Tseng SC.** Long-term outcomes of keratolimbal allograft for total limbal stem cell deficiency using combined immunosuppressive agents and correction of ocular surface deficits. *Arch Ophthalmol.* 2009 Nov;127(11):1428-34.
30. **Wylegala E, Dobrowolski D, Tarnawska D, Janiszewska D, Gabryel B, Malecki A, Siekiera U.** Limbal stem cells transplantation in the reconstruction of the ocular surface: 6 years experience. *Eur J Ophthalmol.* 2008 Nov-Dec;18(6):886-90.
31. **Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M.** Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *Lancet.* 1997 Apr 5;349(9057):990-3.
32. **Koizumi N, Fullwood NJ, Bairaktaris G, Inatomi T, Kinoshita S, Quantock AJ.** Cultivation of corneal epithelial cells on intact and denuded human amniotic membrane. *Invest Ophthalmol Vis Sci.* 2000 Aug;41(9):2506-13.
33. **Koizumi N, Cooper LJ, Fullwood NJ, Nakamura T, Inoki K, Tsuzuki M, Kinoshita S.** An evaluation of cultivated corneal limbal epithelial cells, using cell-suspension culture. *Invest Ophthalmol Vis Sci.* 2002 Jul;43(7):2114-21.
34. **Grueterich M, Espana EM, Tseng SC.** Ex vivo expansion of limbal epithelial stem cells: amniotic membrane serving as a stem cell niche. *Surv Ophthalmol.* 2003;48:63-46.
35. **Omoto M, Miyashita H, Shimamura S, Higa K, Kawakita T, Yoshida S, McGrogan M, Shimazaki J, Tsubota K.** The use of human mesenchymal stem cell-derived feeder cells for the cultivation of transplantable epithelial sheets. *Invest Ophthalmol Vis Sci.* 2009 May;50(5):2109-15.
36. **Monteiro BG, Serafim RC, Melo GB, Silva MC, Lizio NF, Maranduba CM, Smith RL, Kerkis A, Cerruti H, Gomes JA, Kerkis I.** Human immature dental pulp stem cells share key characteristic features with limbal stem cells. *Cell Prolif.* 2009 Oct;42(5):587-94.
37. **Kim JT.** Epithelium transplantation on persistent epithelial defects with limbal stem cell deficiency. *J Korean Med Sci.* 2008 Jun;23(3):502-8.
38. **Hashemi H, Salehnia M, Kamali M, Beigi Boroujeni M.** The histological characteristics of cultured oral epithelium in different culture conditions. *Iran Biomed J.* 2009 Apr;13(2):109-15.
39. **Schwab IR, Reyes M, Isseroff RR.** Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea.* 2000 Jul;19(4):421-6.
40. **Tsai RJ, Li LM, Chen JK.** Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med.* 2000 Jul 13;343(2):86-93.
41. **Rama P, Bonini S, Lambiase A, Golisano O, Paterna P, De Luca M, Pellegrini G.** Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. *Transplantation.* 2001 Nov 15;72(9):1478-85.
42. **Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S.** Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. *Ophthalmology.* 2001 Sep;108(9):1569-74.
43. **Shimazaki J, Aiba M, Goto E, Kato N, Shimamura S, Tsubota K.** Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. *Ophthalmology.* 2002 Jul;109(7):1285-90.
44. **Sangwan VS, Matalia HP, Vemuganti GK, Ifthekar G, Fatima A, Singh S, Rao GN.** Early results of penetrating keratoplasty after cultivated limbal epithelium transplantation. *Arch Ophthalmol.* 2005 Mar;123(3):334-40.
45. **Sangwan VS, Matalia HP, Vemuganti GK, Fatima A, Ifthekar G, Singh S, Nutheti R, Rao GN.** Clinical outcome of autologous cultivated limbal epithelium transplantation. *Indian J Ophthalmol.* 2006 Mar;54(1):29-34.
46. **Daya SM, Watson A, Sharpe JR, Gileadi O, Rowe A, Martin R, James SE.** Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction. *Ophthalmology.* 2005 Mar;112(3):470-7.
47. **Nakamura T, Inatomi T, Sotozono C, Ang LP, Koizumi N, Yokoi N, Kinoshita S.** Transplantation of autologous serum-derived cultivated corneal epithelial equivalents for the treatment of severe ocular surface disease. *Ophthalmology.* 2006 Oct;113(10):1765-72.
48. **Kawashima M, Kawakita T, Satake Y, Higa K, Shimazaki J.** Phenotypic study after cultivated limbal epithelial transplantation for limbal stem cell deficiency. *Arch Ophthalmol.* 2007 Oct;125(10):1337-44.
49. **Shortt AJ, Secker GA, Rajan MS, Meligoni G, Dart JK, Tuft SJ, Daniels JT.** Ex vivo expansion and transplantation of limbal epithelial stem cells. *Ophthalmology.* 2008 Nov;115(11):1989-97.
50. **Shay E, Kheirkhah A, Liang L, Sheha H, Gregory DG, Tseng SC.** Amniotic membrane transplantation as a new therapy for the acute ocular manifestations of Stevens-Johnson syndrome and toxic epidermal necrolysis. *Surv Ophthalmol.* 2009 Nov-Dec;54(6):686-96.
51. **Kucukerdonmez C, Karalezli A, Akova YA, Borozan M.** Amniotic membrane transplantation using fibrin glue in pterygium surgery: a comparative randomised clinical trial. *Eye.* 2009 Jun 5. [Epub ahead of print]
52. **Kucukerdonmez C, Akova YA, Altinors DD.** Comparison of conjunctival autograft with amniotic membrane transplantation for pterygium surgery: surgical and cosmetic outcome. *Cornea.* 2007 May;26(4):407-13.
53. **Park JH, Jeoung JW, Wee WR, Lee JH, Kim MK, Lee JL.** Clinical efficacy of amniotic membrane transplantation in the treatment of various ocular surface diseases. *Cont Lens Anterior Eye.* 2008 Apr;31(2):73-80.
54. **Shi W, Gao H, Wang T, Xie L.** Combined penetrating keratoplasty and keratolimbal allograft transplantation in comparison with corneoscleral transplantation in the treatment of severe eye burns. *Clin Experiment Ophthalmol.* 2008 Aug;36(6):501-7.
55. **Mendicute J, Bidaguren A, Martinez-Soroa I, Gibelalde A.** Automated large diameter lamellar keratoplasty and stem cell transplantation for the treatment of ocular surface diseases with limbal insufficiency. *Eur J Ophthalmol.* 2008 Jul-Aug;18(4):641-4.
56. **Aldave AJ, Kamal KM, Vo RC, Yu F.** The Boston type I keratoprosthesis: improving outcomes and expanding indications. *Ophthalmology.* 2009 Apr;116(4):640-51.
57. **Tan DT, Tay AB, Theng JT, Lye KW, Parthasarathy A, Por YM, Chan LL, Liu C.** Keratoprosthesis surgery for end-stage corneal blindness in asian eyes. *Ophthalmology.* 2008 Mar;115(3):503-510.e3.

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