EPITHELIAL TRANSPLANTATION FOR THE MANAGEMENT OF SEVERE OCULAR SURFACE DISEASE

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ABSTRACT

Purpose: First, to present a new classification of epithelial transplantation procedures for ocular surface disease; second, to present our experience with a keratolimbal allograft procedure for limbal stem cell deficiency; and third, to make recommendations for the indications and postoperative management of epithelial transplantation procedures.

Methods: A review of all epithelial transplantation procedures was performed. A classification of these procedures based on the source of donor tissue and the tissue transplanted was proposed.

In addition, a review of 25 eyes of 21 patients who underwent a keratolimbal allograft was completed. Ocular surface stability, improvement of visual acuity, success of subsequent keratoplasties, and preoperative risk factors were evaluated. Results were compared with those of other epithelial transplantation procedures for ocular surface disease. On the basis of the results of published studies, as well as ours, a recommendation for the indication of the various procedures was made.

Results: Epithelial transplantation for ocular surface disease can be classified as one of the following procedures: conjunctival autograft (CAU), conjunctival allograft (CAL), conjunctival limbal autograft (CLAU), cadaveric conjunctival limbal allograft (c-CLAL), living related conjunctival limbal allograft (lr-CLAL), or keratolimbal allograft (KLAL).

Evaluation of our keratolimbal allograft patients revealed that 18 of 25 eyes (72%) developed a stable ocular surface. Fifteen eyes (60%) demonstrated a significant improvement in visual acuity. Persistent epithelial defects and symblephara were successfully managed with this procedure. Six of 13 (46%) subsequent keratoplasties were successful. Patients with limbal deficiency due to Stevens-Johnson syndrome had a significantly worse outcome. Patients with preoperative conjunctival keratinization also had a significantly worse outcome.

Indications for epithelial transplantation are as follows: For patients with unilateral cicatrizing conjunctival disease, the first option should be CAU. For patients with unilateral limbal deficiency, CLAU is the proce-

dure of choice. For patients with bilateral disease lr-CLAL should be considered first. If this procedure is not available, then consideration of KLAL is warranted.

Conclusions: Classification of the various epithelial transplantation procedures based on anatomy is useful for an accurate comparison and discussion of the procedures.

KLAL is a useful technique in the management of severe ocular surface disease due to limbal deficiency. However, patients with preoperative conjunctival keratinization have a poor prognosis. Consideration of a CLAU or a lr-CLAL should be made for ocular surface disease on the basis of whether the disease is unilateral or bilateral.

The importance of HLA and ABO typing, as well as the protocol for immunosuppression in the allograft procedures for limbal deficiency, needs further study.

INTRODUCTION

OCULAR SURFACE ANATOMY

The ocular surface is composed of the tear film and the epithelium of the cornea and conjunctiva. Stratified, nonkeratinized epithelium covers the entire cornea as well as the bulbar and palpebral conjunctiva. The corneal epithelium is constantly being sloughed and renewed. The renewal process involves centripetal and circumferential cellular migration from the limbus in addition to vertical movement from the basal layers.

Recent studies have shown that the cells responsible for the renewal of the corneal epithelium are located at the limbus. Davanger and Evensen[1] observed that pigmented limbal cells moved centrally in the cornea, suggesting centripetal migration. From this observation, they speculated that cells in the limbal area likely are involved in normal corneal epithelial renewal.

Schermer and coworkers[2] postulated that corneal epithelial stem cells are located at the limbus. They based their theory on the patterns of expression of a cornea-specific 64K keratin that is present in all corneal epithelial cells except the limbal basal cells. This finding indicates that the limbal basal cells are less differentiated than those found in other areas of the corneal epithelium.

Cell kinetic studies of the hematopoietic system, intestine, and epidermis indicate that stem cells and transient amplifying cells (TAC) make up the proliferating cells of epithelium.[3,4] Stem cells are present in all self-renewing tissues.[5] They compose a small subpopulation of the total tissue

and make up 0.5% to 10% of the total cell population.[6,7] Stem cells are long-lived, have a long cell cycle time, have an increased potential for error-free proliferation with poor differentiation, and demonstrate a capability to divide in an asymmetric manner.[8-10] This asymmetric cell division allows one of the daughter cells to remain a stem cell while the other differentiates to become a TAC. The TAC then differentiates into postmitotic cells (PMC) and finally to terminally differentiated cells (TDC). Both the PMC and TDC are incapable of cell division.[8] Schermer and coworkers[2] proposed the cell proliferation scheme for the cornea as follows: limbal basal cells (stem cells) → basal corneal epithelium (TAC) → suprabasal corneal epithelium (TDC) (Fig 1).

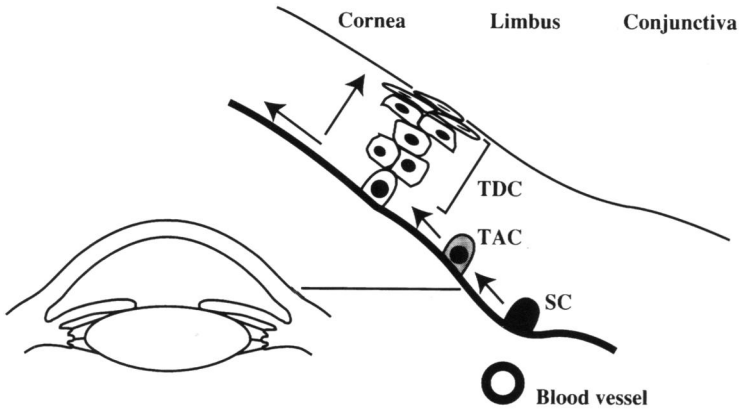


FIGURE 1
Schematic representation of limbal stem cells (SC). These are located at basal epithelium of limbus and give rise to transient amplifying cells (TAC) of basal corneal epithelium, and finally to terminally differentiated cells (TDC), which compose the suprabasal corneal epithelium.

Cotsarelis and coworkers[11] 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 labelling indicated that these cells exhibited a long cell cycle. Ebato and associates[12] reported that human ocular limbal epithelial cells grew better in culture and had a higher rate of mitotic activity than peripheral corneal epithelial cells.

A variety of factors appear to play a role in maintaining the limbal stem cells and thus a viable corneal surface. A significant difference between the limbus and central cornea is the presence of blood vessels at the limbus. These vessels help form the Palisades of Vogt, allowing close approximation of vessels to the limbal epithelium.[13] These vessels also provide the

limbal epithelium with both increased nutrition and greater interaction with blood-borne cytokines.[14]

The basement membrane of the limbus is also different from that of the central cornea in that there are anchoring fibrils and a rough undulating surface that enhance epithelial cell adhesion.[14] The limbus possesses an abundance of type IV collagen,[15] which can be detected in the conjunctival and limbal basement membranes but not in that of the central cornea.

The bulbar conjunctiva consists of 6 to 9 layers of stratified squamous epithelial cells organized in an irregular fashion. This irregular orientation stands in direct contrast to the more orderly epithelium of the cornea. The forniceal conjunctival epithelium is 2 to 3 cell layers thick over the superior tarsus and 4 to 5 layers thick over the inferior one. The forniceal conjunctival epithelium tends to be more columnar in nature, while the palpebral conjunctival epithelium is more cuboidal.

The normal conjunctival epithelium contains goblet cells. These cells are unicellular, mucin-secreting glands that account for about 5% to 10% of the total number of basal cells. The highest density of goblet cells lies in the medial forniceal and palpebral regions, near the tear-drainage apparati of the lids. The function of the mucin secreted by the goblet cells is to coat the surface of the epithelium. Mucin, which is extremely hydrophilic, allows the otherwise hydrophobic epithelium to be wettable. Mucin also maintains the stability of the tear film. The existence of a location of stem cells for conjunctival goblet and nongoblet epithelial cells is not known. It has been speculated that if conjunctival stem cells do exist, they would be equally distributed throughout the conjunctiva.[16]

STEM CELL DEFICIENCY

Conditions that severely compromise the ocular surface can result in profound morbidity for patients. Conjunctival sequelae include inflammation, scarring, and aqueous tear as well as mucin deficiency. Corneal disease from a compromised ocular surface includes neovascularization, scarring, ulceration, and perforation. Pain and loss of vision are common complications in the severe cases.

Conditions that result in ocular surface disease from a loss of limbal stem cells have a variety of etiologies (Table I). Aniridia is a primary stem cell disorder that may result from a deficiency in the development or maintenance of limbal stem cells.[17-19] Secondary disorders of limbal stem cells are more common. In both chemical and thermal burns, there is direct injury to the limbal stem cells as well as the corneal and conjunctival epithelium, including the goblet cells. Stevens-Johnson syndrome (SJS) and ocular cicatricial pemphigoid may result in severe inflammation

TABLE I: OCULAR SURFACE DISEASE WITH LIMBAL STEM CELL DEFICIENCY

DISEASE	PATHOGENESIS OF LIMBAL DEFICIENCY
Aniridia	Abnormal development or maintenance of limbal stem cells
Chemical burn	Direct injury to limbal stem cells
Thermal burn	Direct injury to limbal stem cells
Stevens-Johnson syndrome	Inflammatory destruction of limbal stem cells
Multiple surgeries	Excision and/or mechanical destruction of limbal stem cells
Neoplasia	Replacement of normal limbal stem cells with neoplastic cells
Contact lens–induced keratopathy	Hypoxia and chemical toxicity to limbal stem cells

with conjunctival scarring, goblet cell depletion, aqueous tear deficiency, and loss of limbal stem cells.[20] In addition, severe contact lens–induced keratopathy, as well as multiple surgical procedures or chronic use of toxic topical medications, can also result in limbal stem cell deficiency.[21]

When a significant number of limbal stem cells are lost, conjunctival epithelial cells invade and populate the corneal surface. This process of conjunctivalization results in a thickened, irregular, unstable epithelium, often with secondary neovascularization, inflammatory cell infiltration, and disruption of the basement membrane.[22] Impression cytology typically demonstrates the presence of goblet cells and conjunctival epithelial cells on the diseased corneal surface.[23,24] Punctate corneal epithelial defects and larger confluent defects are common and may ultimately lead to corneal scarring and loss of vision.

Conjunctival transdifferentiation is based on the concept that conjunctival epithelium can transform into a cornealike epithelium.[25,26] This theory suggests that the corneal epithelium can be regenerated from a conjunctival source. More recently, studies have presented evidence that the conjunctival epithelium cannot transdifferentiate into epithelium that is truly phenotypically corneal. Studies have shown that the protein and keratin composition,[27,28] glycolytic enzyme and glycogen content,[29] normal tensile strength,[30] and paracellular permeability[31] of transdifferentiated conjunctival epithelium differ from that of true corneal epithelium. In

addition, long-term studies of human corneas where partial conjunctivilization has occurred failed to show transdifferentiation of conjunctiva to corneal phenotype after several months of follow-up.[32] Small buds of corneal epithelium that were seen along the line of contact of conjunctival and corneal phenotypes suggest replacement, rather than transdifferentiation, of one phenotype by the other.[33] These studies indicate that conjunctival transdifferentiation does not result in the biochemical or physiologic transformation of conjunctival epithelium into corneal epithelium.

Debridement of a conjunctivalized corneal epithelium results in a reinvasion of the abnormal epithelium. A penetrating or lamellar keratoplasty with a normal epithelial surface results in a stable surface as long as the donor epithelium is present. However, after the eventual donor epithelial sloughing, the surface most often fails owing to conjunctivalization from the lack of limbal stem cells.[34,35,21,20,36] Because of the historically poor outcomes with conventional therapy in patients with limbal stem cell deficiencies, a more recent approach to managing these patients is based on the concept of epithelial transplantation.

CLINICAL STUDIES

In reviewing studies of epithelial transplantation for ocular surface disease, it is important to bear in mind the duration of follow-up when evaluating the efficacy of these procedures. Almost all of the procedures result in excellent short-term outcomes, because most donor epithelium does well in the short-term regardless of the procedure. Donor epithelium in routine penetrating keratoplasty may survive for several months without the need for re-epithelialization from the limbus. This survival time is known because epithelial rejection may be seen as late as 13 months postoperatively.[37] Thus it is conceivable that epithelium from the donor, whether it is derived from conjunctiva, limbus, or cornea, may survive many months before the source of regenerated epithelium is needed. Brown and associates[35] reported on 13 patients who underwent penetrating keratoplasty for severe alkali burn. In this report they stated that 9 patients had a clear graft with a follow-up range of 6 to 31 months. In a subsequent study published 2 years later, they reported that 4 of the 9 successful grafts had failed and 3 more had developed scarring of the anterior stroma due to epithelial problems.[21] Thus, included in the literature review that follows are only those cases with at least 6 months of follow-up.

Epithelial transplantation procedures can be divided into autografts and allografts. In the autograft procedure the donor tissue is obtained

from the fellow eye, thereby avoiding a major problem facing the allograft procedure, namely, immunologic rejection.

EPITHELIAL AUTOGRAFTS

Epithelial transplantation for severe ocular surface disease was first described by Thoft in 1977,[38] when he described conjunctival transplantation for monocular chemical burns (Table II). This conjunctival autograft procedure used several pieces of bulbar conjunctiva from a normal fellow eye as donor tissue. In 3 of 5 eyes, successful re-epithelialization of the cornea was achieved with accompanied improved vision and decreased neovascularization. The median follow-up time was 9 months, with a range of 8 to 10 months. The conjunctival autograft procedure was based on the concept of conjunctival transdifferentiation.[39-42]

TABLE II: STUDIES OF EPITHELIAL AUTOGRAFT PROCEDURES FOR SEVERE LIMBAL DEFICIENCY

AUTHORS	YEAR	PROCEDURE	CLASSIFICATION OF PROCEDURE	N*	MEDIAN F/U(M)	RANGE F/U(M)	IMPROVED VA(%)†	IMPROVED SURFACE(%)	SIMULTANEOUS/ SUBSEQUENT SUCCESSFUL PK/LK
Thoft[38]	1977	Conjunctival transplant	CAU	5	9	8-10	3/5 (60)	3/5 (60)	0/0
Thoft[43]	1982	Conjunctival transplant	CAU	17	36	12-60	10/17 (59)	16/17 (94)	0/2
Vastine et al[44]	1982	Autologous conjunctival transplant	CAU	7	16.5	10-18	1/7 (14)	7/7 (100)	0/1
Herman et al[45]	1983	Conjunctival autograft transplant	CAU	3	N/A	N/A-24	3/3 (100)	3/3 (100)	3/3
Kenyon, Tseng[56]	1989	Limbal autograft	CLAU	21	24	6-45	17/21 (81)	20/21 (95)	7/7
Jenkins et al[21]	1993	Limbal transplant	CLAU	5	24	12-36	3/5 (60)	3/5 (60)	0/0

CAU, conjunctival autograft; CLAU, conjunctival limbal autograft; F/U, follow-up; LK, lamellar keratoplasty; M, months; N/A, not available; PK, penetrating keratoplasty; VA, visual acuity.

*N, number of eyes in patients with severe limbal deficiency.
†Improvement of at least two lines of visual acuity.

In 1982 Thoft[43] reported on an additional 17 cases of ocular surface disease that were managed with a conjunctival autograft. Sixteen of these eyes achieved a stable ocular surface with a median follow-up of 36

months and a range of 12 to 60 months. Ten of these eyes had a significant improvement in vision. However, two subsequent penetrating keratoplasties failed.

Vastine and associates[44] also in 1982 reported on a procedure of "autologous conjunctival transplantation." This procedure was also a conjunctival autograft from the fellow eye. Of the 14 patients described, 7 had diagnoses consistent with limbal deficiency. The investigators reported that in all 7 cases, a stable ocular surface developed postoperatively with a median follow-up of 16.5 months and a range of 10 to 18 months.

In 1983 Herman and colleagues[45] described a variation of the previous reports of conjunctival autografts, in which a 360° ring of conjunctiva was obtained from the limbus extending 2 to 3 mm posteriorly from the fellow eye. Three eyes with severe surface disease were managed with this procedure. All eyes developed a stable surface and all had a subsequent successful penetrating keratoplasty. Specific data on time of follow-up were not given.

Although conjunctival autograft is useful to re-establish an intact ocular surface in patients with conjunctival scarring, there are concerns whether this procedure truly results in normal corneal epithelium.[46] Tsai and coworkers[47] compared the results of limbal transplantation to conjunctival transplantation in a rabbit model of ocular surface disease. They reported a significant decrease in corneal neovascularization with limbal transplantation and the resultant corneal epithelia displayed the corneal phenotype. Conjunctival transplantation resulted in a corneal epithelia with the phenotype of conjunctiva .

The technique of conjunctival autograft remains a valuable procedure for the management of fornix reconstruction and primary and recurrent pterygium. The success of conjunctival transplantation for the establishment of the corneal surface when a source of limbal stem cells is not available remains to be determined.

Procedures to transplant the limbus have been devised on the basis of the concept of limbal stem cells. Kenyon and Tseng[56] in 1989 described a modification of the conjunctival autograft, which he named limbal autograft transplantation. In this procedure, conjunctiva and limbus from a normal fellow were was used to manage diffuse limbal deficiency in unilateral ocular surface disease, or focal limbal deficiency in unilateral or bilateral disease. The technique used grafts of bulbar conjunctiva that extended approximately 0.5 mm onto the clear cornea centrally, thus containing limbal cells. Data on 21 cases with 6 months or more of follow-up were included. Preoperative diagnoses included acute and chronic chemical injuries, thermal injury, contact lens–induced keratopathy, and surface

disease secondary to multiple surgeries. 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 or regression of corneal neovascularization in 15 cases. No complications developed in the donor eyes. Seven of 7 patients underwent simultaneous or subsequent successful penetrating or lamellar keratoplasty.

Jenkins and associates[21] in 1993 reported on conjunctival limbal autografts in 5 patients with severe epitheliopathy secondary to chronic contact lens wear. Two of the 5 procedures failed, and 1 of the donor eyes developed epitheliopathy. These results are most likely due to the fact that the donor eyes were not normal in that they were also exposed to chronic contact lens wear. Therefore, it is important to adhere to the recommendation that conjunctival limbal autografts should be obtained only from eyes with normal epithelial surfaces.

Conjunctival autograft and conjunctival limbal autograft have been major advances in the management of severe unilateral ocular surface disease. These procedures are now widely used by many corneal surgeons, and numerous patients have benefited. However, these procedures are, for the most part, limited to patients with unilateral disease; they are not available for ocular surface disease patients with the greatest need, namely, those affected severely bilaterally.

EPITHELIAL ALLOGRAFTS

Because of the need to manage bilateral disease, allograft procedures for epithelial transplantation have been investigated. Relatively few studies of allograft procedures have been performed, and the number of patients reported in each study is small. These limits occur because of the relatively recent understanding of the location and function of limbal stem cells. The vast majority of patients has been reported since 1990 (Table III).

In 1984 Thoft[49] described the first allograft procedure for the management of severe ocular surface disease. He called this procedure keratoepithelioplasty. His procedure involved the use of lenticules of peripheral cornea from a cadaveric donor globe as a source of epithelium. A whole globe was used to obtain 4 pieces of partial-thickness cornea. Lenticules were carved from the midperipheral cornea and consisted of epithelium and a thin layer of stroma (0.2 mm thick) to serve as a carrier of epithelium. The 4 lenticules were placed evenly around the corneoscleral limbus and sutured to the sclera. Limbal cells were not 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

TABLE III: STUDIES OF EPITHELIAL ALLOGRAFT PROCEDURES FOR SEVERE LIMBAL DEFICIENCY

AUTHORS	YEAR	PROCEDURE	CLASSIFICATION OF PROCEDURE	N*	MEDIAN F/U(M)	RANGE F/U(M)	IMPROVED VA(%)†	IMPROVED SURFACE(%)	SIMULTANEOUS/ SUBSEQUENT SUCCESSFUL PK/LK
Thoft[49]	1984	Keratoepi-thelioplasty‡	KAL	4	N/A	9-30	3/4 (75)	3/4 (75)	0/1
Turgeon, Thoft[50]	1990	Keratoepi-thelioplasty	KLAL	11	8.5	6-48	7/11 (64)	7/11 (64)	0/0
Pfister[7]	1994	Homotrans-plant of limbal stem cells	c-CLAL	2	16.5	15-18	2/2 (100)	2/2 (100)	2/2
Tsai, Tseng[51]	1994	Allograft limbal transplant	KLAL	6	17.7	6-24	5/6 (83)	N/A	0/0
Tsubota, et al[52]	1995	Limbal allograft transplant	KLAL	4	9.0	7-15	4/4 (100)	4/6§ (67)	3/3
Kwitko, et al[54]	1995	Allograft conjunctival transplant	lr-CAL	11	15	7-29	5/11 (45)	10/11 (91)	0/0
Kenyon, Rapoza[55]	1995	Limbal allograft transplant	lr-CLAL	8	19.5	10-40	6/8 (75)	6/8 (75)	4/5
Holland	1996	Kerato-limbal allograft	KLAL	25	20	6-63	15/25 (60)	18/25 (72)	6/13

c-CLAL, cadaveric conjunctival limbal allograft; F/U, follow-up; KAL, keratoallograft; KLAL, keratolimbal allograft; LK, lamellar keratoplasty; lr-CAL, living related conjunctival allograft; lr-CLAL, living related conjunctival limbal allograft; M, months; N/A, not available; PK, penetrating keratoplasty; VA, visual acuity.

*N, number of eyes in patients with severe limbal deficiency.
†Improvement of at least two lines of visual acuity.
‡Original keratoepithelioplasty procedure included peripheral cornea, but not limbus, and therefore it is classified as a KAL.
§Two patients had repeat KLAL.

donor tissue, this technique was felt to be useful to treat patients with bilateral ocular surface disease. Four patients were reported in Thoft's study. Three patients had bilateral chemical burns, and 1 patient had severe atopic keratoconjunctivitis with symblepharon formation and persistent epithelial defects of both corneas. Following keratoepithelioplasty, 3 of the 4 patients developed a stable ocular surface and improved vision. The atopic keratoconjunctivitis patient, however, underwent a penetrating

keratoplasty 2 months after keratoepithelioplasty, and the graft failed secondary to a bacterial infection. The range of follow-up was 9 to 30 months, and the median could not be determined from the data reported.

In 1990 Turgeon and coworkers, including Thoft,[50] reported on 13 additional patients managed with keratoepithelioplasty. 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 of follow-up, 7 patients had a stable ocular surface and 7 patients had improved visual acuity. The median follow-up of these 11 patients was 8.5 months with a range of 6 to 48 months.

Pfister[7] in 1994 reported on "homotransplantation of limbal stem cells," in which cadaveric conjunctiva and limbus were removed from a corneoscleral rim in the management of alkali burns in 2 patients. Superficial keratectomy and conjunctival recession were performed on the recipient eyes. The donor conjunctival graft was thinned by reflecting the conjunctiva over the cornea and excising Tenon's capsule. The conjunctiva was excised as close to the limbus as possible. The ring of conjunctiva was then transferred to the recipient eye and sutured to the limbus with a running 9-0 nylon suture. Both patients achieved a stable ocular surface and underwent subsequent penetrating keratoplasty. At 15 and 18 months postoperatively, both patients had improved visual acuity and stable ocular surfaces.

A modification of Thoft's keratoepithelioplasty procedure was reported by Tsai and Tseng in 1994.[51] They described an "allograft limbal transplantation" procedure that utilized a whole globe to provide a keratolimbal graft. A suction trephine was used to make a 50% to 66% depth incision in the midperipheral cornea. A rounded steel blade made a 360° scleral incision approximately 1 mm from the limbus at the same depth as the corneal incision. Lamellar dissection of the keratolimbal tissue was completed with an angled rounded steel blade. The resultant keratolimbal ring was divided into three equal pieces and transferred to the recipient eye. Postoperatively, all patients were treated with oral cyclosporine A (CsA) in addition to topical corticosteroids. The starting dose of CsA was 8 to 12 mg/kg per day for the first week, then tapered to 4 to 5 mg/kg per day for 2 to 6 months. CsA was discontinued according to the disappearance of tortuous and engorged perilimbal vessels adjacent to the allograft.

Tsai and Tseng[51] reported on a total of 16 cases; however, 10 of these cases did not have diagnoses consistent with severe limbal deficiency. Two of these 10 patients had Terrien's marginal degeneration, while 1 had peripheral sclerocornea. Seven patients had chronic keratoconjunctivitis

secondary to a variety of causes including pterygium, Salzmann's nodular degeneration, elastoic degeneration, and inflammatory granulation. It is not clear whether these 10 patients had significant limbal deficiency. These disease entities mentioned are not included in any other reports of patients treated for severe limbal deficiency. The remaining 6 patients had diagnoses consistent with limbal deficiency. Five patients had chemical or thermal burns, and 1 had SJS.

The results of the 6 patients with diagnoses consistent with limbal deficiency and follow-up of 6 months or more revealed that 5 of 6 had improved vision. The sixth patient, who had SJS, developed an occurrence of fibrovascular tissue into the cornea. The median follow-up for these patients was 17.7 months with a range of 6 to 24 months. The stability of the ocular surface was not reported in this study.

Tsubota and colleagues[52] reported on a technique they termed "limbal allograft transplantation," another variation of a keratolimbal allograft. The donor tissue in this technique was obtained from a corneoscleral rim stored in media. A central corneal button was excised with a trephine and, in most cases, was used for a penetrating keratoplasty in the same patient at the time of keratolimbal allograft. The remaining corneoscleral rim was cut into two pieces. A lamellar dissection was used to remove the posterior portion, resulting in a thin piece of tissue made up of epithelium, Bowman's layer, and a small amount of stroma. Excess scleral tissue was removed with a scissors. The resultant crescent of tissue was then placed so that the limbus of the donor was positioned over the limbus of the recipient. Prior to transplantation, the corneoscleral rims were stored in corneal storage media for an average of 5 days. Tsubota's report is important because it is the first to employ stored tissue in media as a source for limbal tissue.

All patients were treated with intravenous corticosteroids for 8 days postoperatively, oral CsA for 2 days preoperatively and 1 month postoperatively, and maintenance topical CsA and corticosteroids. A total of 9 patients was reported. Three of these 9 patients had the diagnosis of aphakic bullous keratopathy, and 1 had corneal trauma with secondary scarring and neovascularization. Although patients with corneal edema may develop superficial neovascularization and abnormal epithelium, they do not represent severe limbal deficiency. This fact is supported by the excellent results of penetrating keratoplasty, including stability of the corneal surface, seen in other patients with aphakic and pseudophakic corneal edema.[53] One of the remaining patients in Tsubota's study had follow-up time of only 2 months and therefore will not be included in the data analysis. The remaining 4 patients had follow-up of 6 months or greater and

diagnoses consistent with severe limbal deficiency. Two had alkali burn, and 2 had ocular cicatricial pemphigoid. Of interest, preoperative testing of tear function in these patients revealed a Schirmer test with topical anesthesia to be 5 mm or greater in all patients. The Schirmer test results suggested that aqueous tear production was not severely compromised in these patients. Of these 4 patients, 2 underwent a simultaneous penetrating keratoplasty, and 1 underwent a penetrating keratoplasty 1 year after keratolimbal allograft.

Results of this study revealed that in 2 patients an irregular surface developed and in 2 patients the surface failed. In the latter 2 patients, the keratolimbal allograft was repeated and the surface stabilized. Visual acuity improved in all patients. However, the best vision reported in patients with at least 6 months follow-up was 20/100. The 3 penetrating keratoplasties performed on these patients were successful. One patient developed an endothelial rejection reaction that was successfully treated.

This study introduced the idea of using stored corneoscleral rims as a source of limbal tissue for allograft transplantation. The results of the ocular surfaces, as well as the penetrating keratoplasties, were promising. However, the study was limited by the very short follow-up. The median follow-up for those patients followed for at least 6 months was 9 months, with a range of 7 to 15 months.

Kwitko and colleagues[54] in 1995 described a technique they called "allograft conjunctival transplantation," whereby living related donors were used to provide conjunctival tissue. Limbal tissue was not transplanted in this study. One to 2 pieces of donor conjunctiva, approximately 5x8 mm in size, were transferred from the donor to the recipient eye, in which a 360° peritomy and superficial keratectomy had been performed. Each patient was treated with topical corticosteroids and antibiotics in the postoperative period.

Donor conjunctiva was obtained from donor siblings, and if tissue could not be obtained from a sibling, a parent was used for the donor tissue. One donor eye developed a granuloma at the site of the graft excision. After excisional biopsy, this lesion healed without recurrence. Human leukocyte antigen (HLA) typing and crossmatching were performed retrospectively in 8 patients and their donors.

The investigators reported on 12 eyes of 10 patients with severe limbal deficiency. Eight eyes had SJS or toxic epidermal necrolysis, 3 eyes had alkali burns, and 1 had a thermal burn. These eyes all appeared to have extreme pathologic condition in comparison to other studies as evidenced by the preoperative diagnosis, visual acuity, and status of the ocular surface. Three eyes had corneal keratinization, 3 had necrosis of the corneal

stroma, 1 had a trophic ulcer, and 1 had a persistent epithelial defect.

Examination of the data of patients with at least 6 months of follow-up revealed 5 of 11 eyes with improvement in visual acuity of at least two lines. Improved ocular surface was noted in 10 of 11 eyes as noted by increased corneal transparency, decreased neovascularization and photophobia, and lack of epithelial defects. The patient with a severe thermal burn had a persistent epithelial defect and stromal necrosis following conjunctival allograft. The epithelium eventually healed and the surface stabilized.

Three patients experienced epithelial rejection episodes, with no disturbance in the corneal surface in 2 of these patients. One case of rejection resulted in a severe disturbance of the corneal surface. Two of these cases had 100% incompatible HLA donor–recipient pairs, while the HLA typing of the third was unavailable. It appears that patients with identical or haplo-identical HLA matching were less likely to undergo epithelial rejection. The median follow-up time was 15 months with a range of 7 to 29 months.

This study was the first to describe the use of living related donor tissue in the treatment of ocular surface disease. It is interesting to note that these excellent results, obtained in patients with severe ocular surface disease, were obtained with the transplantation of conjunctival tissue alone, and not of limbal stem cells.

Kenyon and Rapoza[55] 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 the previously described 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 the conjunctiva.

The surgical technique involved harvesting of conjunctival limbal specimens 2 mm wide by 10 mm in circumferential length taken from the superior and inferior limbal zones. Each piece of tissue was maintained in a moist chamber for about 1 hour in the interval required for recipient preparation. The recipient eye underwent 360° conjunctival peritomy and superficial keratectomy. The donor tissue was then sutured to the limbus at the superior and inferior locations. Most patients underwent simultaneous lateral tarsorrhaphy, with simultaneous lamellar keratoplasty in 1 patient. Four patients had subsequent penetrating keratoplasty for visual rehabilitation.

Postoperative management included topical corticosteroids in all

cases, with topical and/or systemic CsA predominantly for HLA haploidentical or incompatible cases. Systemic CsA was initiated at the time of surgery at a dose of 10 mg/kg per day to attain a therapeutic blood level of approximately 500 ng/mL. The CsA dosage was tapered to 2 to 3 mg/kg per day to achieve a maintenance level of 100 ng/mL. The use of oral CsA ranged from 3 to 34 months with a mean of 11.25 months for those treated.

Evaluation of the 8 cases with follow-up of 6 months or more revealed a median follow-up of 19.5 months with a range of 10 to 40 months. Preoperative diagnoses included 4 cases of chemical burns, 2 cases of erythema multiforme, 1 case of limbal deficiency secondary to multiple surgeries, and 1 case of limbal deficiency secondary to atopy. Two patients with follow-up of 3 months were not included in this analysis.

Visual acuity improved substantially in 6 of 8 cases and was unchanged in the other 2 cases. Final visual acuity was 20/80 or better in 5 patients. Simultaneous or subsequent lamellar or penetrating keratoplasty was successful in 4 of 5 patients. The ocular surface remained stable in 6 of 8 patients. Two patients suffered recurrent erosions and epithelial defects. No episodes of epithelial rejection of either the limbal or corneal grafts were observed, and the etiologies of the 2 failures did not appear to be immunologic. One of the failures occurred in an HLA identical while the other occurred in an HLA haplo-identical match. Both patients with SJS did well with improvement in vision from hand motions to 20/40 and 20/60, respectively, with a follow-up of 21 and 34 months. This study was the first to describe the use of limbal tissue from a living related donor, with a conjunctival carrier used in combination with systemic immunosuppression.

PURPOSE OF THE PRESENT STUDY

Numerous procedures have been described for the surgical management of severe ocular surface disease secondary to limbal stem cell deficiency. The purpose of this thesis is threefold. First, we propose a new classification for the variety of surgical procedures available for the management of ocular surface disease. This classification will allow for the accurate comparison and discussion of the available procedures for epithelial transplantation. In addition, this classification should eliminate the confusion involved in conjunction with the multiple terms presently used in reference to these procedures.

Second, we present our experience with a keratolimbal allograft procedure for the management of severe ocular surface disease secondary to

limbal stem cell deficiency. We also compare the efficiency of this procedure in relation to the other available procedures.

Third, we make recommendations concerning indications for the various epithelial transplantation procedures and the accompanying medical management.

PROPOSED CLASSIFICATION OF EPITHELIAL TRANSPLANTATION PROCEDURES FOR SEVERE OCULAR SURFACE DISEASE

A variety of techniques have been reported for epithelial transplantation procedures in the management of severe ocular surface disease. Multiple terms have been used in previous studies, including conjunctival transplantation,[38] autologous conjunctival transplantation,[44] allograft conjunctival transplantation,[54] limbal autograft transplantation,[56] limbal conjunctival autograft,[57] limbal transplantation,[21] limbal allograft transplantation,[52,55] homotransplantation of limbal stem cells,[7] and keratoepithelioplasty[49,50]

Confusion arises when reviewing reports of these procedures. Multiple terms have been used by different investigators to describe the same technique, while the same term has been used for more than one technique. It is not often clear from the present terminology which of the various procedures is being performed. Therefore, a standardization of the classification of epithelial transplantation procedures for ocular surface disease would be useful.

All of the procedures share the goal of transplantation of a new source of epithelium for a diseased ocular surface. Although the different techniques have similar goals, they vary depending on the source of the donor tissue and whether the procedure is primarily a conjunctival or a limbal transplantation. Limbal transplantation procedures also vary depending on the carrier tissue used for the transfer of the limbal stem cells. Carrier tissue is needed in limbal transplantation because it is not technically possible to transfer limbal stem cells alone.

The source of donor tissue for epithelial transplantation can be the fellow eye (autograft), cadaveric whole globe (allograft), cadaveric corneoscleral rim (allograft), or a living relative (allograft). Conjunctival-only transplants transfer conjunctiva. Limbal transplants, on the other hand, utilize either conjunctiva or cornea as a carrier tissue for the fragile limbal stem cells. On the basis of the source of donor tissue, the carrier tissue employed, and whether the procedure is a conjunctival transplant or a limbal transplant, the following classification of surgical procedures for epithelial transplantation for ocular surface disease is proposed (Table IV).

Conjunctival transplantation procedures can be either autografts or

allografts depending on the source of donor tissue. A conjunctival autograft (CAU) utilizes tissue from the fellow eye. A conjunctival allograft (CAL) can utilize donor tissue from a cadaver or living relative and be designated as a cadaveric conjunctival allograft (c-CAL) or living related conjunctival allograft (lr-CAL).

TABLE IV: PROPOSED CLASSIFICATION FOR EPITHELIAL TRANSPLANTATION PROCEDURES FOR OCULAR SURFACE DISEASE

PROCEDURE	ABBREVIATION	DONOR	TRANSPLANTED TISSUE
Conjunctival transplantation			
Conjunctival autograft	CAU	Fellow eye	Conjunctiva
Cadaveric conjunctival allograft	c-CAL	Cadaver	Conjunctiva
Living-related conjunctival allograft	lr-CAL	Living relative	Conjunctiva
Limbal transplantation			
Conjunctival limbal autograft	CLAU	Fellow eye	Limbus/ conjunctiva
Cadaveric conjunctival limbal allograft	c-CLAL	Cadaver	Limbus/ conjunctiva
Living-related conjunctival limbal allograft	lr-CLAL	Living relative	Limbus/ conjunctiva
Keratolimbal allograft	KLAL	Cadaver	Limbus/ cornea

Limbal transplantation procedures can be subdivided according to the donor and the carrier tissue. A conjunctival limbal autograft (CLAU) utilizes tissue from the fellow eye, and conjunctiva is the carrier. A cadaveric conjunctival limbal allograft (c-CLAL) utilizes a cadaveric donor for conjunctiva and limbus. A living related conjunctival limbal allograft (lr-CLAL) is a procedure in which a living relative donates conjunctiva and limbal tissue. Finally, a keratolimbal allograft (KLAL) utilizes a cadaveric donor,

and peripheral cornea is used to transfer the limbal stem cells.

In this study we evaluate the efficacy of KLAL for the management of severe ocular surface disease from a variety of causes.

PATIENTS AND METHODS

All patients eligible for KLAL were considered to have serious ocular surface disease manifested by chronic epitheliopathy and conjunctivilization of the corneal surface. KLAL was recommended for each of these patients on the basis of three specific characteristics for limbal deficiency. The first indication was a persistent epithelial defect unresponsive to conventional treatment such as lubrication, punctal occlusion, and tarsorrhaphy. The second indication was decreased visual acuity secondary to an irregular ocular surface. The third was an unstable ocular surface in patients requiring eventual penetrating or lamellar keratoplasty for visual rehabilitation.

All patients underwent complete ophthalmic examination, including Snellen visual acuity testing, slit-lamp biomicroscopic examination, intraocular pressure determination, dilated funduscopic evaluation, Schirmer testing, fluorescein and rose bengal testing, and slit-lamp photography. Evaluation of symblepharon was made and scored on a scale of 0 to 6 per eye in the following fashion. A score of 1 was given in the presence of symblepharon from palpebral conjunctiva to bulbar conjunctiva. A score of 2 was given if symblephara extended from palpebral conjunctiva to the limbus, and a score of 3 was given if symblephara extended from the palpebral conjunctiva to the cornea (ankyloblephara). Because both lids were evaluated for each eye, a maximum score of 6 could be achieved for anyone.

PREOPERATIVE CONSIDERATIONS

Ocular adnexa and anterior segment structures of patients with severe ocular surface disease were carefully examined. The eyelids were inspected for the presence of entropion or ectropion. Abnormal lid position was corrected prior to limbal transplantation. In addition, trichiasis and distichiasis were treated with cryotherapy or argon laser.

An assessment of the aqueous tear production was completed on all patients. Nonpreserved artificial tear lubricants were used by patients with aqueous tear deficiency. Patients with a low Schirmer test, a scant tear lake, and rose bengal and fluorescein staining consistent with aqueous tear deficiency also had permanent punctal occlusion. In addition, lateral tarsorrhaphy was considered at the time of KLAL.

It is crucial to elicit a past history of glaucoma from the patient's past

ocular history and physical examination. Many patients with severe ocular surface disease may have glaucoma, from either concurrent disease, as in patients with aniridia or multiple past surgical procedures, or long-term use of topical corticosteroids, as in SJS and alkali injuries. Glaucoma was managed aggressively in patients undergoing limbal transplantation for ocular surface disease to ensure the best prognosis for visual rehabilitation. Chronic glaucoma is a major risk factor for long-term poor visual functioning in patients undergoing otherwise successful limbal allograft.

SURGICAL TECHNIQUES

Donor Tissue Selection

Paramount to the success of KLAL is the careful evaluation and selection of the donor tissue. In this study, we transplanted tissue only from donors who were under the age of 50. Donor tissue was used only if it had no epithelial abnormalities and was available for transplantation within 36 hours of the death of the donor.

Preparation of the Recipient Eye

Either retrobulbar anesthetic with 7th cranial nerve block or general anesthesia was administered. Exposure was typically difficult in these patients because of superior and inferior symblephara. The initial incision was a limbal peritomy for 360°. In most patients with severe ocular surface disease, significant bleeding was encountered following the conjunctival incision. One quadrant was resected at a time, and hemostasis was maintained with topical epinephrine, thrombin, and wet-field cautery. In areas of symblepharon, conjunctival tissue was first recessed at the limbus and then undermined to allow the conjunctival tissue to fall back, not only to create a new fornix, but to supply tissue for a new palpebral surface as well (Fig 2). If the initial dissection were made at the fornix, and the symblepharon were simply excised, there would be a broad area of epithelial defect on the palpebral conjunctival side, leading to further symblepharon formation. Therefore, the symblepharon was actually used to help reconstruct the fornix and provide the epithelium of the palpebral surface. Care was taken to avoid damaging the superior or inferior rectus muscles in areas of broad symblepharon.

The conjunctiva was resected 4 to 5 mm from the limbus to allow an adequate-sized bed of sclera on which to position the KLAL tissue. Abnormal fibrovascular pannus and epithelium, which were typically present, were then removed from the surface of the cornea. Blunt dissection with a cellulose sponge was used initially to remove this abnormal tissue. Often, semi-sharp dissection with a rounded steel scalpel or blunt blade

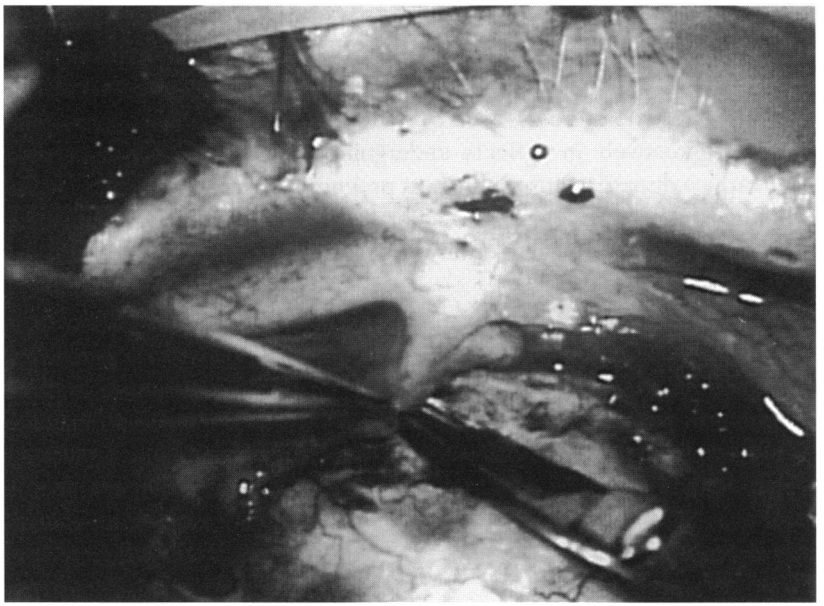


FIGURE 2
Recession of symblepharon. Initial incision is made at limbus and then undermined to allow symblepharon tissue to recess and create an epithelial surface for palpebral conjunctiva.

was used to create a smooth surface. Care was taken to ensure that the dissection continue in a lamellar fashion, remaining anterior, and that the deep layers of the corneal stroma were not disturbed. It must be borne in mind that the purpose of this dissection was removal of the abnormal fibrovascular conjunctivilized surface that had replaced normal corneal epithelium.

Harvesting of the Limbal Tissue

The purpose of performing KLAL is to provide healthy limbal stem cells to the recipient limbus. Because the limbal stem cells lie in a narrow, fragile portion of the limbus, they must be delivered attached to a more robust carrier tissue. This carrier tissue allows for safe transfer and secure attachment of the limbal stem cells to the recipient limbus. We have utilized 2 techniques to provide limbal stem cell and carrier tissue. Initially, we employed a lenticule technique in which the stem cells were delivered via multiple pieces of donor cornea. More recently, a technique utilizing a corneoscleral rim to provide crescents of tissue was employed.

Lenticule. The lenticule technique involved lenticules of peripheral cornea and limbus obtained from a whole globe (Fig 3). This procedure

was a modification of Thoft's second description of keratoepithelioplasty.[50] To harvest the lenticules, a whole globe was placed on a prepped table and wrapped with a folded gauze to assist in stabilization. Under the operating microscope, a Graefe knife was used to slice lenticules of peripheral cornea and limbal tissue. The resultant lenticule was made up mostly of cornea with a small cuff of sclera and conjunctiva. The thickness of each lenticule was approximately one-third corneal depth, and each lenticule was approximately 5 mm long at the limbus by 3 mm in width. Thoft used one donor globe for each recipient eye, which typically provided 4 to 5 lenticules. In the present study, 2 globes were utilized for each recipient eye in order to provide additional stem cells and to cover as much area of bare sclera as possible.

After resection, each lenticule was laid on a firm surface, epithelial side up, and covered with corneal storage media. The lenticules were then placed around the limbus of the recipient eye. The orientation was reversed so that the donor corneal tissue overlay the recipient sclera, while the donor limbus overlay the recipient limbus. Each lenticule was secured with interrupted 10-0 nylon sutures placed at each wing as well as at the posterior surface. Six to 7 lenticules were usually required to surround the recipient limbus. The remaining lenticules were used to start a second row

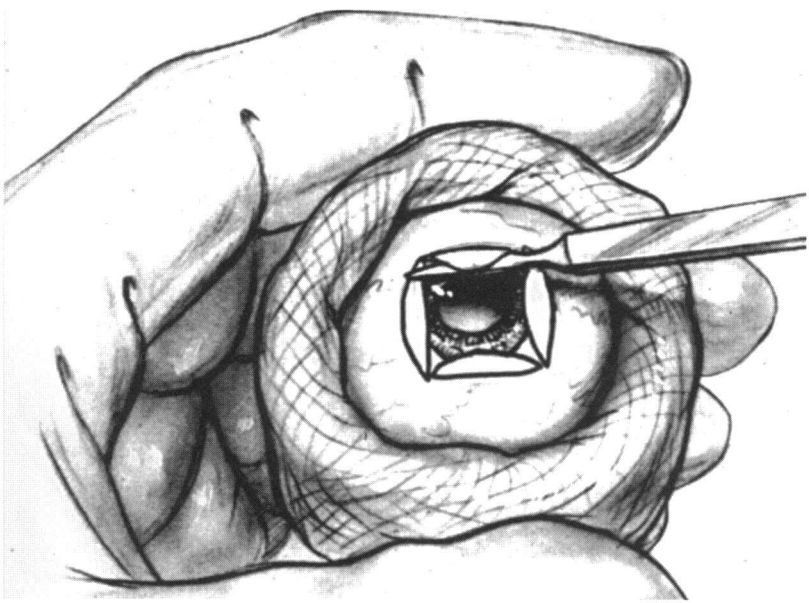


FIGURE 3A
Keratolimbal allograft lenticule technique. A whole globe is used to provide four or five lenticules.

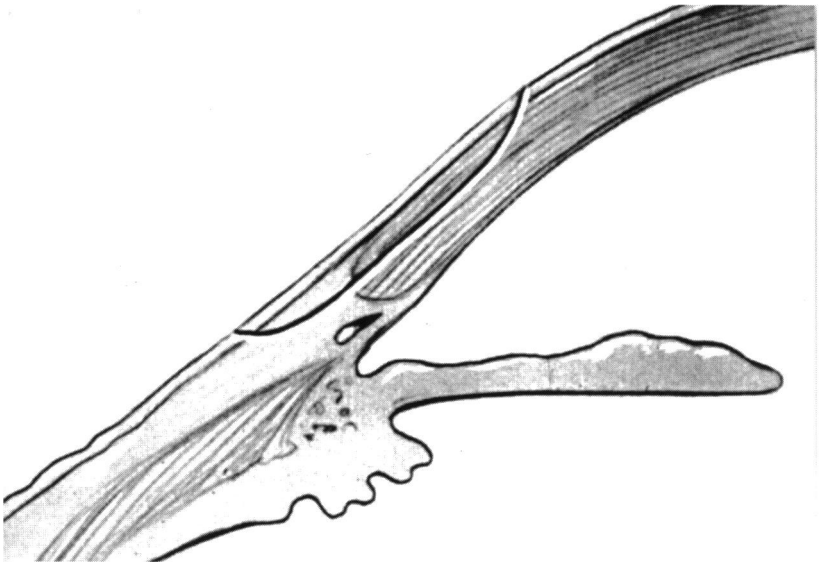


FIGURE 3B
Keratolimbal allograft lenticule technique. Cross section shows that lenticule is approximately a 30% depth incision to provide peripheral corneal and limbal tissue.

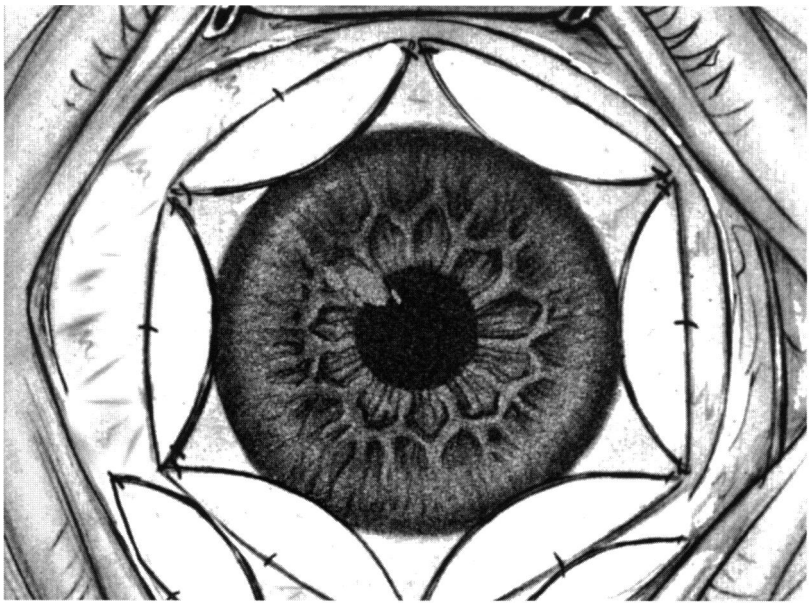


FIGURE 3C
Keratolimbal allograft lenticule technique. Seven to eight lenticules are aligned at the recipient limbus. Additional lenticules are used to start a second row to cover bare sclera and prevent symblepharon.

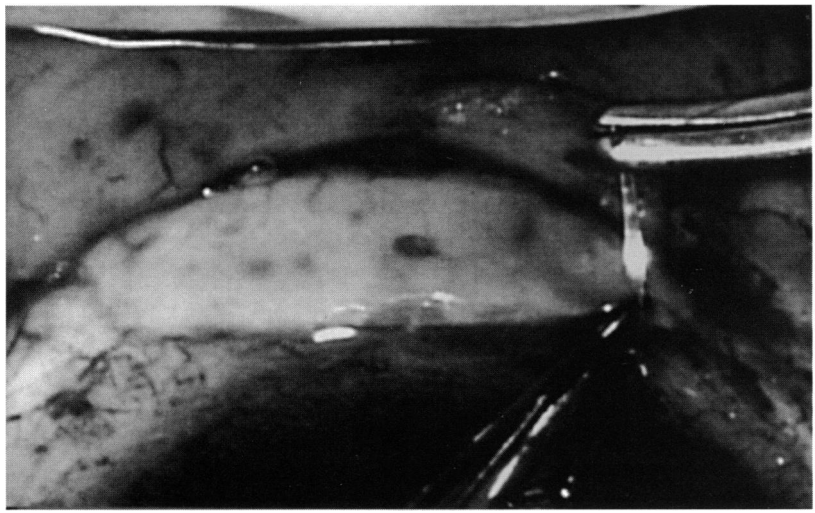


FIGURE 3D
Keratolimbal allograft lenticule technique. Each lenticule is sutured to anterior sclera with two or three 10-0 nylon sutures to align donor limbus to recipient limbus.

of tissue in areas of previous symblepharon. This tissue helped to cover any remaining nonepithelialized surface to prevent symblepharon recurrence. During the entire operation, the surface of the lenticules was moistened with balanced salt solution and a viscoelastic to prevent desiccation.

Corneoscleral Crescent. The corneoscleral crescent technique was a modification of the procedure first described by Tsubota and colleagues in 1995.[52] Unlike the lenticule technique, in which the whole globe was needed, a corneoscleral rim preserved in corneal storage media at 4° C was utilized for this technique (Fig 4). The central cornea of a corneoscleral rim was first excised with a 7.5-mm trephine. This corneal button was placed back into media for later use. The remaining corneoscleral rim was sectioned into equal halves. The posterior half of each crescent, including posterior stroma, Descemet's membrane, and endothelium, was removed by lamellar dissection using a super-sharp rounded steel blade. This posterior tissue was then discarded. At this stage, scissors were used to dissect the peripheral excess scleral tissue. Scleral tissue 1 mm peripheral to the limbus remained after this dissection.

The crescents were then placed on the recipient eye with the anterior corneal edge overlying the recipient limbus. The first crescent was positioned superiorly, centered at the 12-o'clock meridian, and the second crescent was positioned inferiorly, centered at the 6-o'clock meridian. Both were secured with several interrupted 10-nylon interrupted sutures.

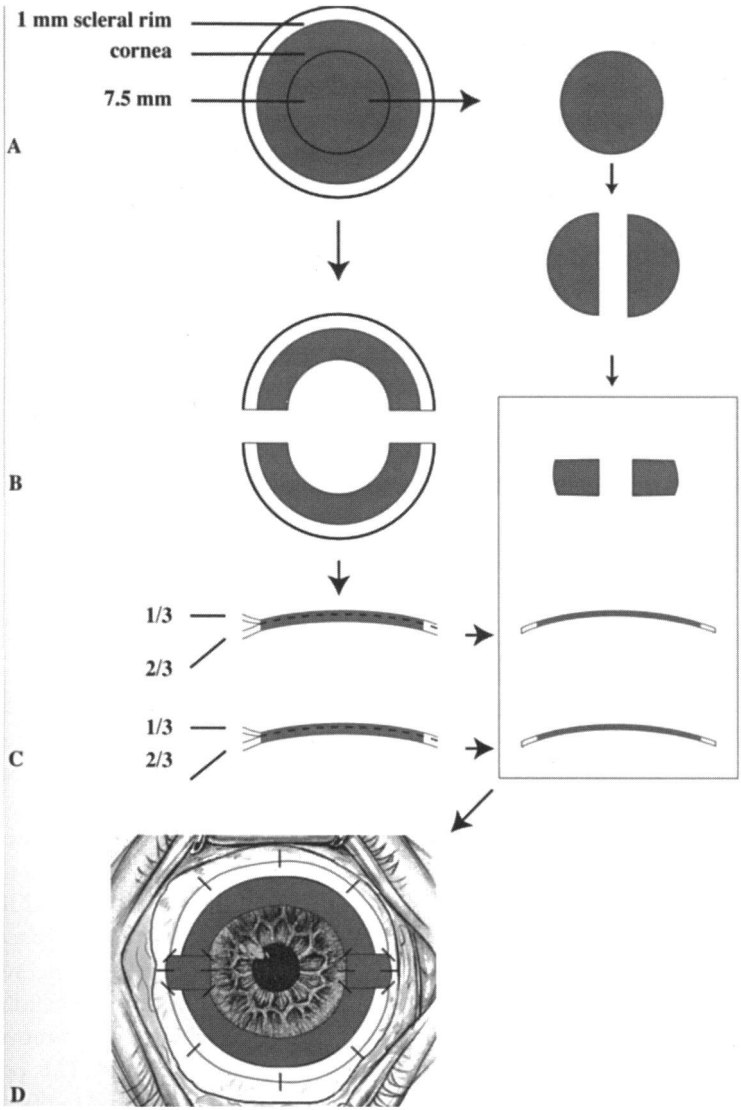


FIGURE 4

Keratolimbal allograft crescent technique. A, Donor corneoscleral rim with central 7.5 mm of cornea removed using trephine. B, Remaining corneoscleral rim is cut into equal halves, forming two crescents. C, Each corneoscleral crescent undergoes lamellar dissection to remove posterior two thirds of tissue. D, Remaining epithelium and anterior stromal crescent are placed at recipient limbus. Previously trephined central cornea is cut into equal halves, thinned to one third thickness, trimmed, and placed into gap areas between crescents.

Using this procedure, the entire recipient limbus, except for small areas at the 3-o'clock and 9-o'clock positions, was covered by healthy donor stem cell tissue.

Unlike Tsubota's technique, we utilized the previously trephined central cornea to cover the remaining gaps between the two crescents. The cornea was sectioned into equal halves. Each corneal piece button was dissected to 30% to 50% thickness using a rounded steel blade, and the posterior tissue was discarded. Each half was sewn into place over the previously described gaps at the 3-o'clock and 9-o'clock positions using interrupted 10-0 nylon suture. Although this tissue did not provide stem cells, it did provide healthy epithelium and acted as a barrier to conjunctiva-like tissue migrating through the gaps between the crescents.

There were several advantages to the corneoscleral crescent technique as compared with the lenticule one. First, the use of the corneoscleral crescent allowed the tissue preservation time to be extended, because the corneoscleral crescent procedure used a storage media. Second, there were areas of conjunctivilization that originated through gap areas in the lenticules. The corneoscleral crescent technique minimized these gaps. Third, the operative time for the corneoscleral procedure was less than that of the lenticule procedure. Fourth, the corneoscleral crescent technique required tissue from only one donor eye, while the lenticule technique was best accomplished with tissue from two. The ideal tissue for KLAL was difficult to obtain because most donor tissue did not meet the criteria for the quality of the epithelium. The corneoscleral crescent technique allowed more patients to be treated with KLAL.

Postoperative Management

All patients received topical antibiotics until the corneal and conjunctival epithelial defects were healed. Patients initially received topical corticosteroids every 2 to 4 hours while awake, based on level of inflammation. They were maintained on topical corticosteroids indefinitely, unless the KLAL grafts were determined to have failed, in which case the corticosteroids were discontinued. Patients received oral prednisone, 1 mg/kg per day, which was slowly tapered over 3 months. Twelve of 25 eyes were treated with topical CsA 2% following KLAL. The earliest patients undergoing KLAL in our series were not treated with topical CsA. Because of the concern of late allograft failure, possibly due to rejection, routine use of topical CsA was subsequently instituted. Four one-eyed patients were also placed on a regimen of oral CsA, 5 to 7 mg/kg per day, after penetrating keratoplasty to decrease the risk of graft failure due to rejection. Nonpreserved artificial tear lubricating drops were prescribed for all

patients. A therapeutic contact lens was placed if patients experienced significant discomfort or cessation of epithelial healing.

Post-KLAL Keratoplasty

Twelve eyes underwent penetrating keratoplasty and 1 eye underwent lamellar keratoplasty following KLAL to visually rehabilitate eyes with scarred corneas. Aside from one penetrating keratoplasty performed concomitant with KLAL, these procedures were performed about 3 months after the previous KLAL, at a point when the ocular surface appeared stable. The donor corneas were carefully selected so that no corneas with epithelial abnormalities were used.

In all keratoplasty patients, an 8.25-mm donor button was placed into an 8.0-mm wound, and the wound was closed with 16 interrupted 10-0 nylon sutures. Four patients underwent extracapsular cataract extraction with placement of a posterior chamber intraocular lens, and 2 had placement of a secondary sutured posterior chamber intraocular lens at the time of penetrating keratoplasty. Following keratoplasty, topical antibiotics and corticosteroids were used in the same regimen as previously described for the KLAL procedure.

CONJUNCTIVAL IMPRESSION CYTOLOGY FOLLOWING KLAL

Nine of the 25 eyes underwent conjunctival impression cytology following KLAL. Following topical proparacaine, cellulose acetate disks were placed on the superior, nasal, and temporal bulbar conjunctiva and KLAL lenticules. If possible, a disk was also placed on the inferior tarsal conjunctiva. An ophthalmodynamometer applied 40 to 45 gm of pressure for 2 seconds to the bulbar conjunctiva and 70 to 75 gm of pressure to the inferior palpebral conjunctiva. The disks were stained with hematoxylin and periodic acid–Schiff (PAS) reagents. Impression cytology was performed preoperatively or on the fellow eye, if present, for comparison. The disks were graded by a masked observer according to the system of Nelson and associates.[58,59]

STATISTICAL METHODS

Summary statistics were calculated for all eyes and subjects and also within diagnosis subgroups. These statistics included means and medians for continuous or ordered variables and percents for categorical variables. Associations between categorical data items were analyzed by chi-square or Fisher's exact tests. Logistic regression was used to evaluate several variables in combination as they related to success or failure of the ocular surface. Nonparametric methods were applied to the symblepharon score.

The Wilcoxon signed-ranks test assessed the change in score before and after KLAL, and the Kruskal-Wallis procedure determined if differences between diagnoses existed. All statistical tests were conducted as two-sided tests with a P value of 0.05 used to decide statistical significance.

This study has limitations. First, the number of patients was relatively small, and the patients were evaluated retrospectively. With a larger study, important issues such as crescent versus lenticule techniques, level of immunosuppression, and the value of tissue typing may have been addressed. Patients with limbal stem cell deficiency are not common, and a single, single-center, prospective study would be painstaking. However, our study, especially when considered in combination with previously reported ones, is useful in evaluating a number of important factors.

Second, a potential limitation was that 2 surgical techniques were employed in this study. The reason for the change from lenticule to the crescent technique of KLAL was that the former required 2 globes and the latter procedure utilized only 1 globe. The ideal tissue for KLAL was difficult to obtain, and a waiting list typically existed for patients awaiting KLAL. Therefore, we felt it was warranted to conserve tissue by converting from the lenticule to the crescent technique.

RESULTS

Twenty-five eyes of 21 patients with severe limbal deficiency underwent KLAL at our institution between 1989 and 1995 (Table V). Eight eyes of 7 patients had had previous alkali burns. Seven eyes of 5 patients carried the diagnosis of congenital aniridia. Four eyes of 3 patients had had prior episodes of SJS. The remaining group was designated as "other" and was composed of 1 eye of 1 patient with corneal and conjunctival intraepithelial neoplasia, one with acid burn, one with epidermolysis bullosa with recurrent ankyloblepharon, and 3 eyes of 3 patients who had undergone multiple previous ocular surgeries. One of the multiple previous surgery patients had been exposed to topical mitomycin C during 1 of the previous procedures.

Six patients were female for a total of 7 eyes, and 15 patients were male for a total for 18 eyes. The age at time of KLAL ranged from 4 to 72 years, with a mean of 40 and a median of 42 years. Nine patients had KLAL performed on only the left eye, 8 had KLAL on only the right eye, and 4 had KLAL performed on both eyes. The mean follow-up was 26.4 months, with a median of 20 months and a range of 6 to 63 months.

TABLE V: PATIENT DATA FOR KERTOLIMBAL ALLOGRAFT (KLAL)

CASE NO. AGE (YR)/SEX	DISEASE	EYE	PED	OTHER ABNORMAL FINDINGS	KLAL TECHNIQUE	EPITHELIAL HEALING (DAYS)	FOLLOW-UP (MO)	KERATINIZATION	SCHIRMER TEST <2 MM	SIMULTANEOUS OR SUBSEQUENT PK OR LK (OUTCOME)	INITIAL VA	FINAL VA	OCULAR SURFACE
1/35/M	Alkali burn, thermal burn	OD	yes	Glaucoma	Lenticules	25	20	yes	no	no	LP	HM	Failed
2/25/M	Alkali burn	OD	no	Amblyopia, ankyloblepharon	Lenticules	8	45	no	no	PK (failed)	HM	CF	Stable
3/57/M	Alkali burn	OD	no	Glaucoma	Lenticules	44	37	yes	yes	PK (failed)	CF	CF	Failed
4/27/M	Alkali burn	OD	yes	None	Lenticules	6	10	no	no	no	20/80	20/200	Failed
5/49/M	Alkali burn	OS	yes	Glaucoma	Lenticules	10	42	no	no	PK (clear)	HM	20/80	Stable
6/70/M	Alkali burn	OD	no	Failed PK	Lenticules	26	63	yes	no	PK (subepithelial scarring)	HM	20/200	Stable
7/27/M	Alkali burn	OS	no	Glaucoma	Lenticules	55	37	no	no	PK (clear)	20/300	20/25	Stable
8/25/M	Alkali burn	OS	yes	None	Crescent	15	10	no	no	LK (clear)	HM	20/80	Stable
9/42/M	Aniridia	OD	no	Amblyopia	Crescent	13	7	no	no	no	20/400	20/200	Stable
10/53/F	Aniridia	OD	yes	Glaucoma	Lenticules	16	53	no	no	no	20/400	20/50	Stable
11/57/F	Aniridia	OS	no	Glaucoma	Crescent	4	6	no	no	no	20/200	20/50	Stable
12/40/M	Aniridia Multiple surgeries	OS	no	Glaucoma	Lenticules	61	28	no	no	PK (failed)	LP	HM	Failed
13/58/M	Aniridia	OD	no	Amblyopia, glaucoma	Lenticules	14	45	no	no	PK (clear)	20/200	20/80	Stable
14/61/M	Aniridia	OS	no	Amblyopia, glaucoma	Crescent	10	8	no	no	no	LP	20/80	Stable
15/10/F	Aniridia	OS	yes	Amblyopia	Crescent	12	6	no	no	no	20/80	20/80	Stable

Table V continued on next page.

TABLE V: KLAL PATIENT DATA (CONTINUED)

CASE #1 AGE (YRS)/SEX	DISEASE	EYE	PED	OTHER ABNORMAL FINDINGS	KLAI TECHNIQUE	EPITHELIAL HEALING (DAYS)	FOLLOW-UP (MONTHS)	KERATINIZATION	SCHIRMER TEST <2 MM	SIMULTANEOUS OR SUBSEQUENT PK OR LK (OUTCOME)	INITIAL VA	FINAL VA	OCULAR SURFACE
16/42/F	SJS Multiple surgeries	OS	yes	none	Lenticules	5	31	yes	yes	no	20/400	20/80	Stable
17/22/M	SJS	OD	no	Glaucoma, failed PK	Lenticules	14	49	yes	yes	PK (failed)	HM	HM	Failed
18/24/M	SJS	OS	no	Glaucoma, failed PK	Lenticules	3	14	yes	yes	PK (failed)	HM	HM	Failed
19/26/F	SJS	OS	no	Glaucoma, failed PK	Lenticules	22	35	yes	yes	PK (failed)	HM	HM	Failed
20/44/M	Multiple surgeries	OD	yes	Atopy	Lenticules	3	13	no	no	PK (failed)	HM	HM	Stable
21/43/F	Multiple surgeries MMC	OS	no	none	Lenticules	4	16	no	no	no	20/80	20/30	Stable
22/08/M	Multiple surgeries Epidermolysis bullosa	OS	no	Ankyloblepharon	Lenticules	7	15	no	no	no	HM	20/150	Stable
23/04/F	Multiple surgeries	OS	no	Ankyloblepharon, amblyopia	Crescent	5	7	no	no	no	20/100	20/60	Stable
24/55/M	CIN	OD	yes	Glaucoma	Lenticules	8	45	no	no	PK (clear)	LP	20/80	Stable
25/43/M	Acid burn	OD	yes	none	Lenticules	4	19	no	no	no	20/80	20/60	Stable

CF, count fingers; CIN, corneal and conjunctival intraepithelial neoplasia; HM, hand motion; LK, lamellar keratoplasty; LP, light perception; OD, right eye; OS, left eye; MMC, mitomycin C toxicity; PED, persistent epitheliall defect; PK, penetrating keratoplasty; SJS, Stevens-Johnson syndrome; VA, visual acuity.

OCULAR SURFACE

In the immediate postoperative period, the corneal epithelium healed in all patients. In no case did a patient develop a significant persistent epithelial defect. The early epithelial movement off the KLAL tissue was both anterior and posterior. Injection of the recipient conjunctiva was common in the early postoperative course.

The healing corneal epithelium grew in a pattern similar to the shape of the donor tissue. The KLAL lenticules resulted in migration of epithelium in a curvilinear pattern, while tissue grew from the crescents in an even, circumferential line (Fig 5). The mean time to healing was 16 days, with a median of 10 days and a range of 3 to 61 days. Three eyes took longer than a month to heal, while 19 eyes healed in 3 weeks or less and 17 eyes healed within 2 weeks. In all eyes, the KLAL tissue had intact epithelium following surgery. With time there was neovascularization at the peripheral edge of the tissue. In addition, the KLAL gradually thinned and became more difficult to visualize.

The ocular surface of all patients was graded as either "stable" or "failed." A surface was called stable if there were minimal to no fluorescein staining, and an absence of epithelial defects, neovascularization, and epithelial opacities (Fig 6).

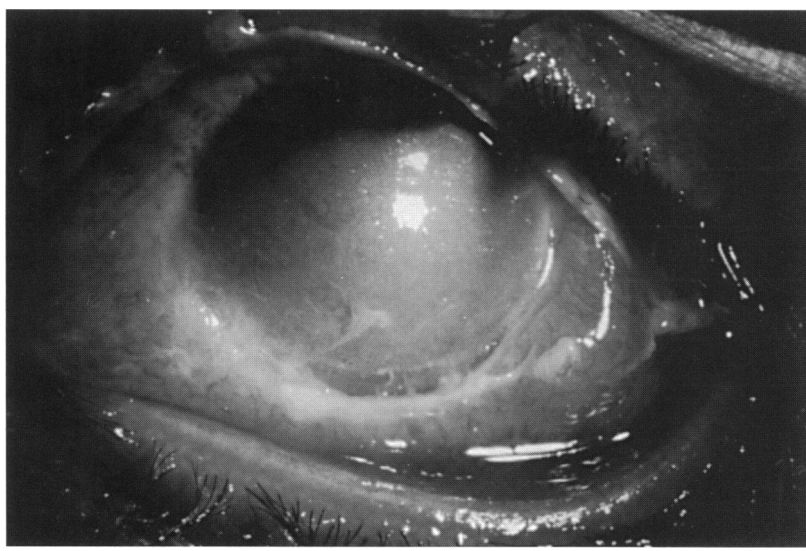


FIGURE 5A

Case 8. Preoperative appearance showing corneal and conjunctival scarring and inflammation and persistent epithelial defect from severe alkali burn.

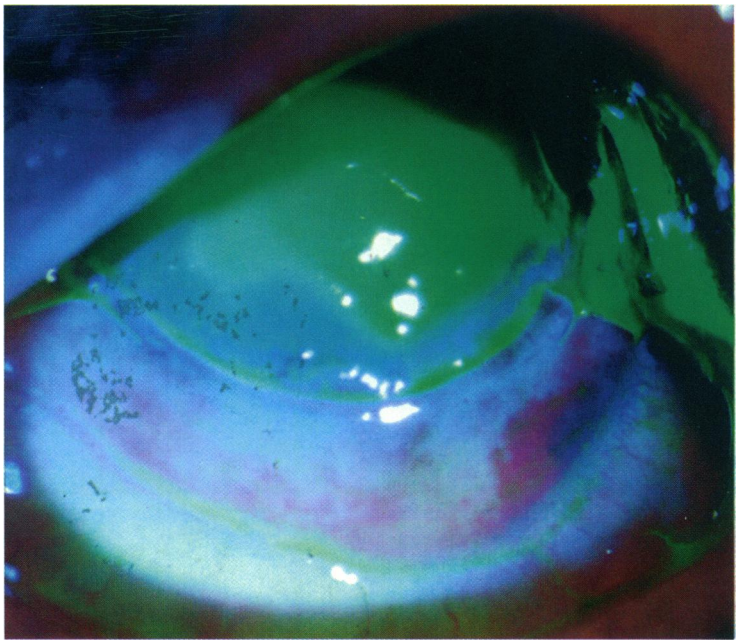


FIGURE 5B
Case 8. Two days following KLAL crescent technique. Early epithelial healing can be seen at peripheral cornea.

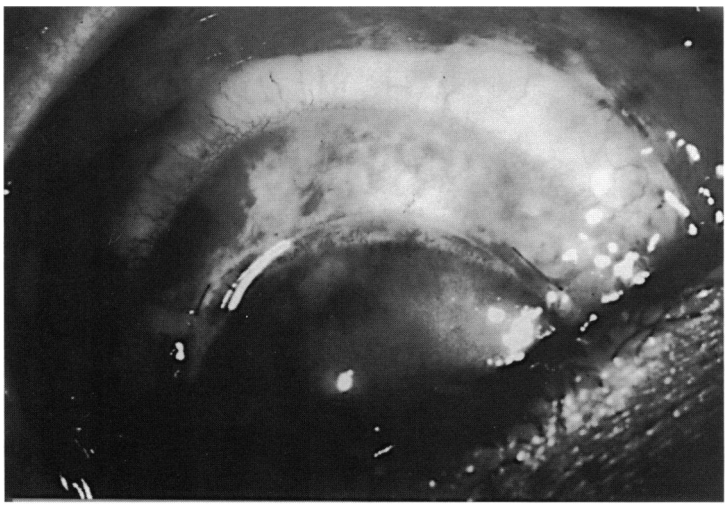


FIGURE 5C
Case 8. Superior crescent.

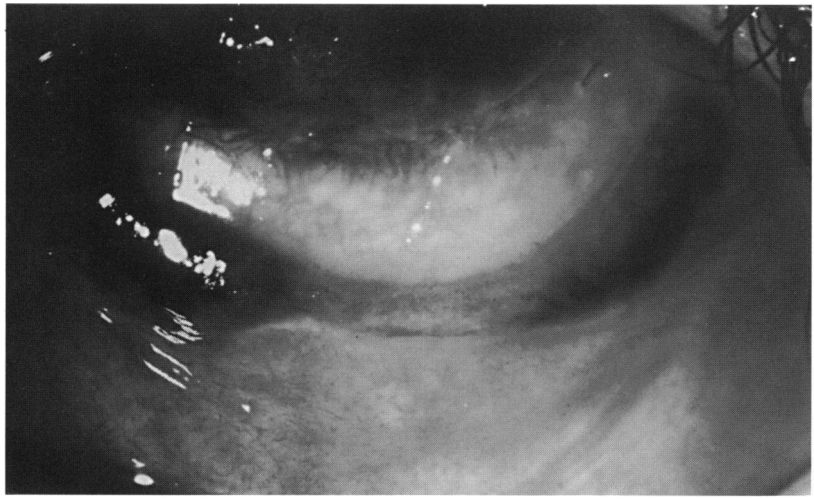


FIGURE 5D
Case 8. Inferior crescent 1 week postoperatively.

Eighteen eyes (72%) had a stable ocular surface following KLAL. Table VI compares success of ocular surface to the preoperative diagnosis and reveals that there are some differences between the groups (P=0.05). Patients with aniridia and those in the category "other" were most likely to

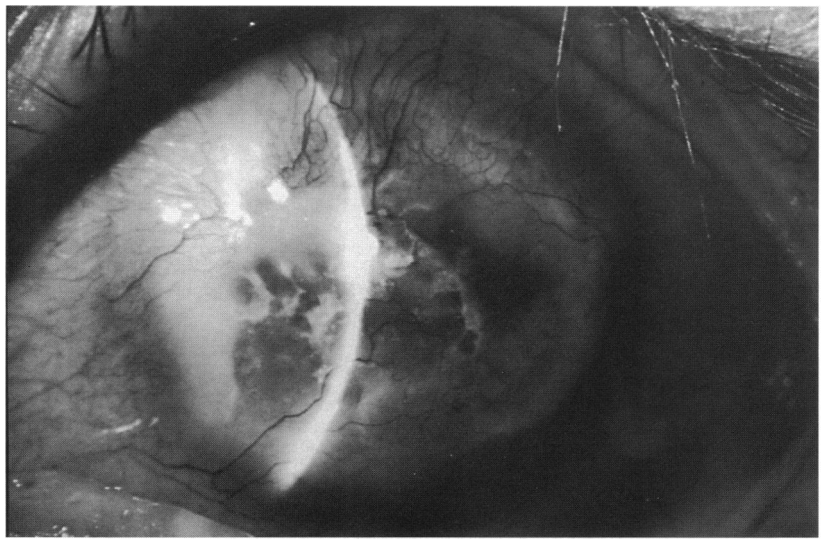


FIGURE 6A
Case 7. Preoperative appearance showing severe corneal scarring, neovascularization, and calcium and lipid degeneration following alkali burn 20 years previously.

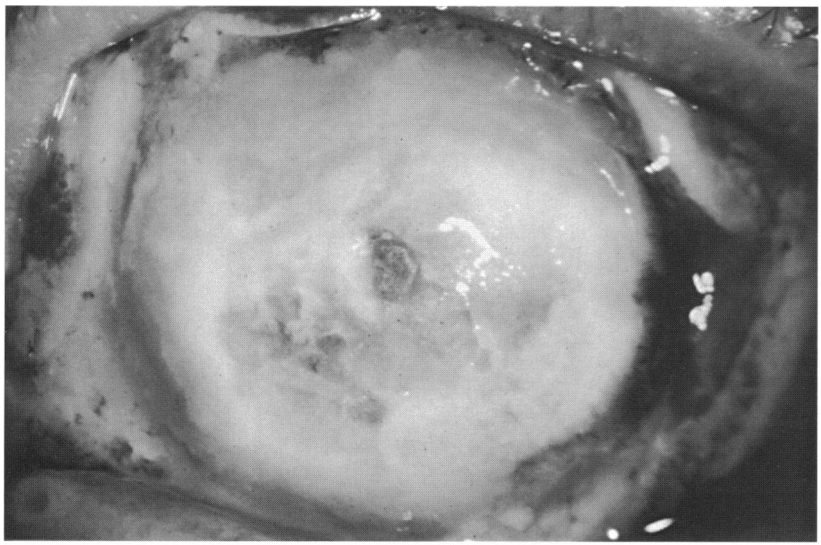


FIGURE 6B
Case 7. Five days after KLAL lenticule technique. Note somewhat transparent lenticules at recipient limbus.

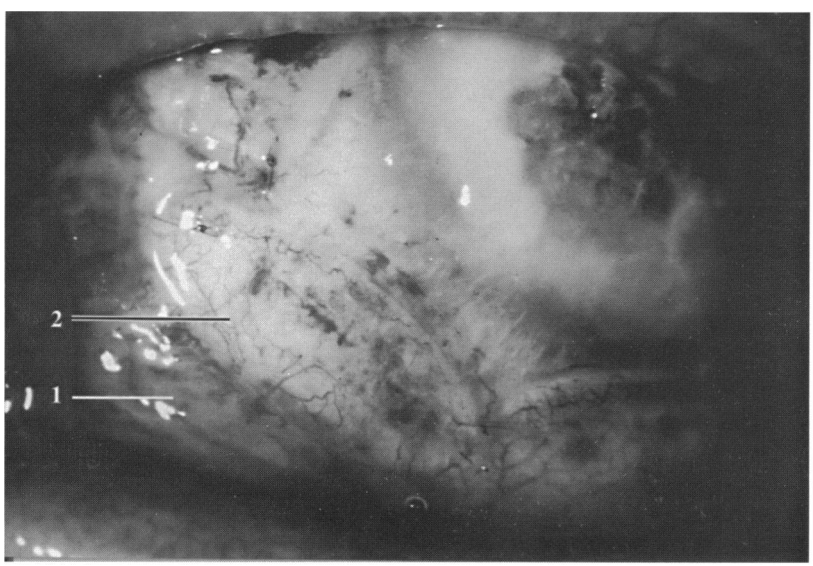


FIGURE 6C
Case 7. Recessed conjunctiva (1) adjacent to lenticule (2).

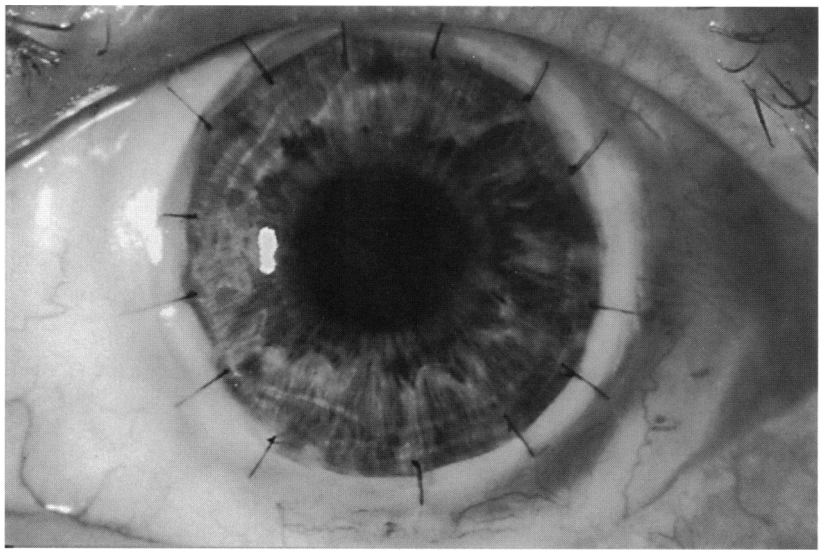


FIGURE 6D

Case 7. Penetrating keratoplasty at 1 year that was performed 3 months following KLAL. Note clear graft with healthy appearing epithelium. Visual acuity 20/25.

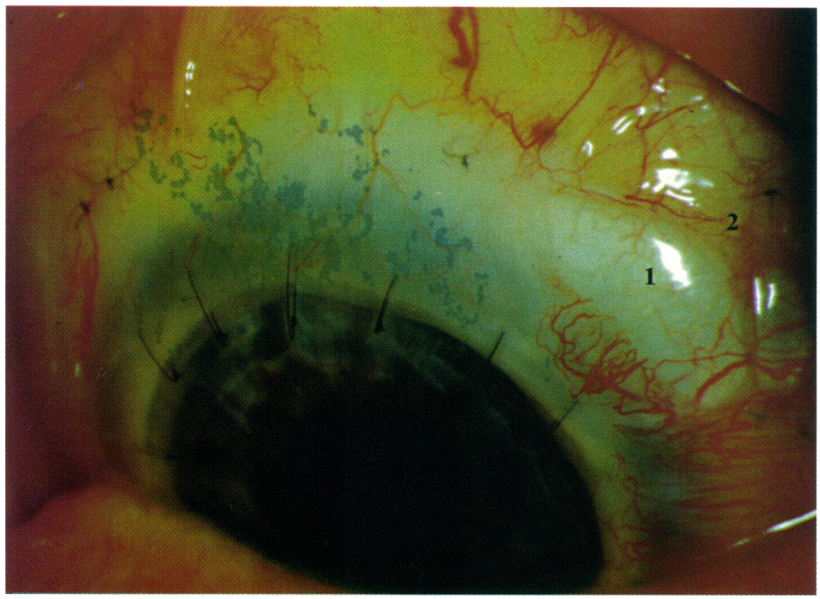


FIGURE 6E

Case 7. Superior lenticules 15 months after KLAL. Note noninflamed epithelium overlying lenticule (1) and injected conjunctiva posterior to lenticule (2).

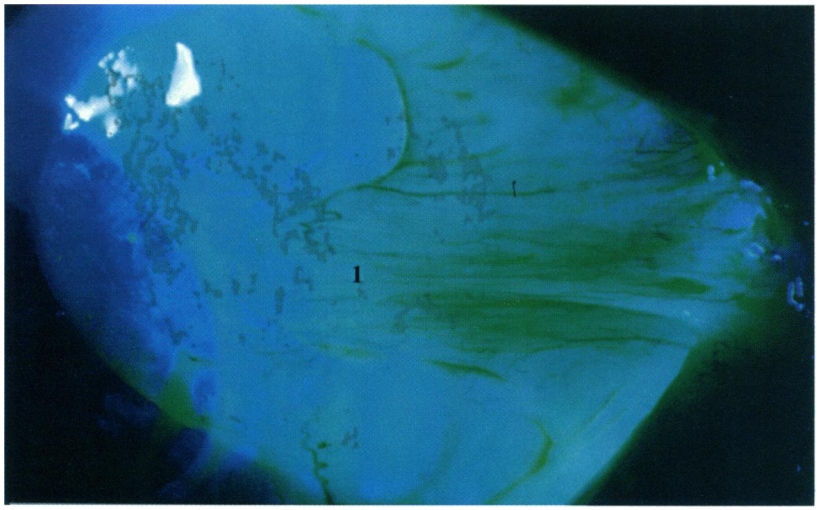


FIGURE 6F
Case 7. Late fluorescein staining of abnormal conjunctival epithelium between lenticules (1).

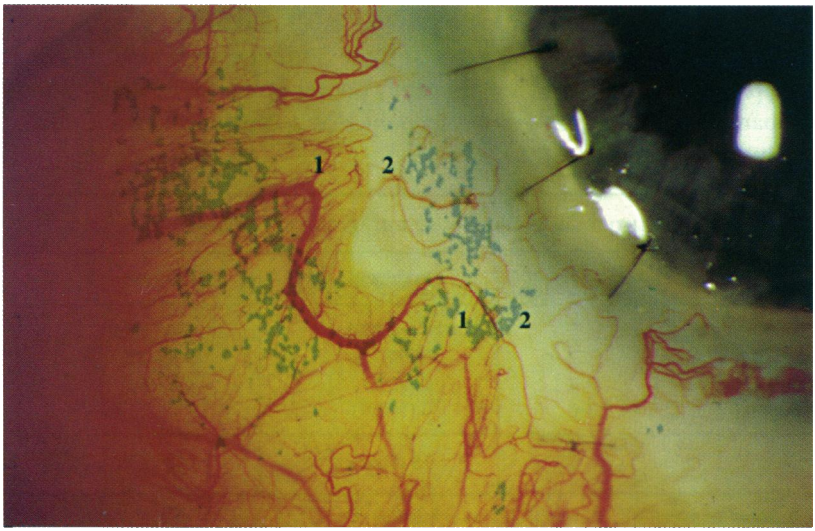


FIGURE 6G
Case 7. Nasal conjunctival injection (1) terminates at edge of lenticule (2).

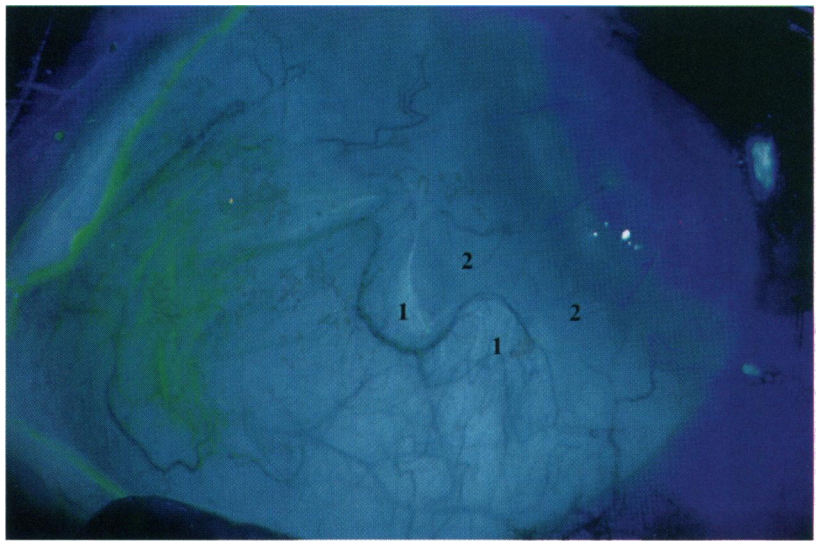


FIGURE 6H
Case 7. Fluorescein shows late staining of conjunctival epithelium (1) as compared with epithelium from lenticule (2).

develop a stable ocular surface following KLAL. Six of 7 eyes with aniridia and all eyes in the "other" category had successful results in this regard. Five of the 8 eyes that had experienced prior alkali injury developed a stable ocular surface. However, owing to the small sample sizes, no significant difference in the success rates of these 3 groups was found. In contrast, patients with SJS had a significantly poorer prognosis (P=0.03) when compared with the other groups, as only 1 of 4 eyes developed a stable ocular surface after KLAL.

TABLE VI: KLAL OCULAR SURFACE OUTCOME AFTER KERATOLIMBAL ALLOGRAFT

DIAGNOSIS	N	STABLE	FAILED
Alkali burn	8	5 (62.5%)	3 (37.5%)
Aniridia	7	6 (85.7%)	1 (14.3%)
SJS	4	1 (25%)	3 (75%)
Other	6	6 (100%)	0
Total	25	18 (72%)	7 (28%)

N, number of eyes; SJS, Stevens-Johnson syndrome.

Chi-square test (P=0.05)

Fluorescein staining of a stable ocular surface demonstrated no epithelial defects or extensive punctate staining. Most patients demonstrated late fluorescein staining of the conjunctiva indicative of abnormal host epithelium. Some patients exhibited late staining of the corneal epithelium (Fig 7), although not all of these patients progressed to surface failure.

A failed surface had at least one of the following findings: significant punctate staining; irregular, hazy, thickened epithelium; neovascularization; or recurrent epithelial defects. The time to surface failure was 2.6, 12.5, 4.4, 18.7, 4.5, 18.9, and 3.6 months, respectively, with a mean time of 9.3 months and a median of 4.5 months. Three of these cases exhibited late failure occurring after 1 year from KLAL. This type of failure showed a gradual progression of thickened irregular epithelium and late fluorescein staining (Fig 8).

Neovascularization of peripheral KLAL tissue was common. In addition, neovascularization of gap areas between KLAL tissue occurred (Fig 9). This effect was felt to be due to the fact that more gap areas existed in the lenticule cases than in the crescent ones.

The success of the ocular surface following KLAL was evaluated according to the presence or absence of preoperative conjunctival keratinization (Table VII). Seven of the 25 eyes had evidence of keratinization prior to KLAL. Five of these 7 eyes resulted in surface failure following

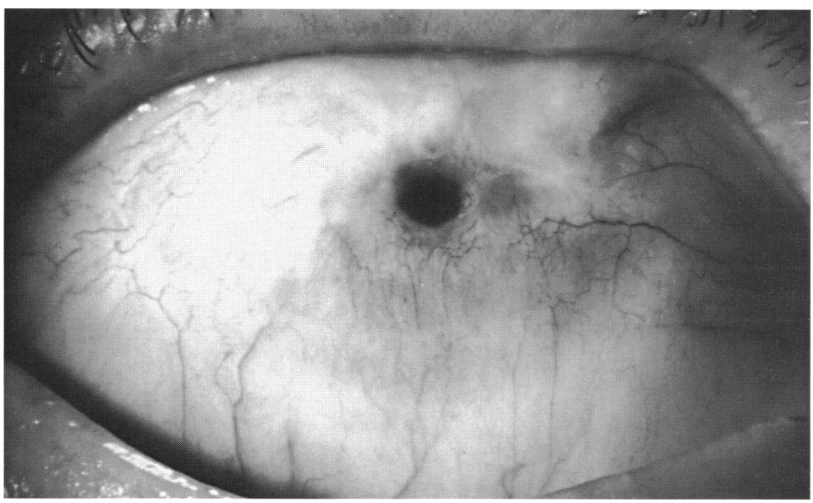


FIGURE 7A

Case 6 (Fellow of eye) Case 7. Preoperative appearance showing severe corneal scarring and thinning. Patient has a history of two failed penetrating keratoplasties due to limbal deficiency. Broad symblepharon is present superiorly.

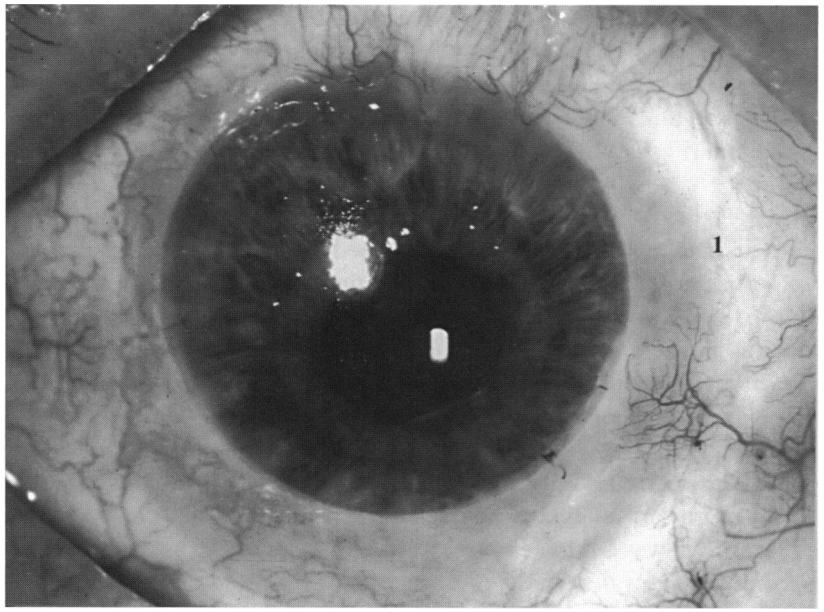


FIGURE 7B
Case 6. Appearance 27 months after KLAL and 24 months after penetrating keratoplasty. Lenticules have thinned over time and can barely be detected (1).

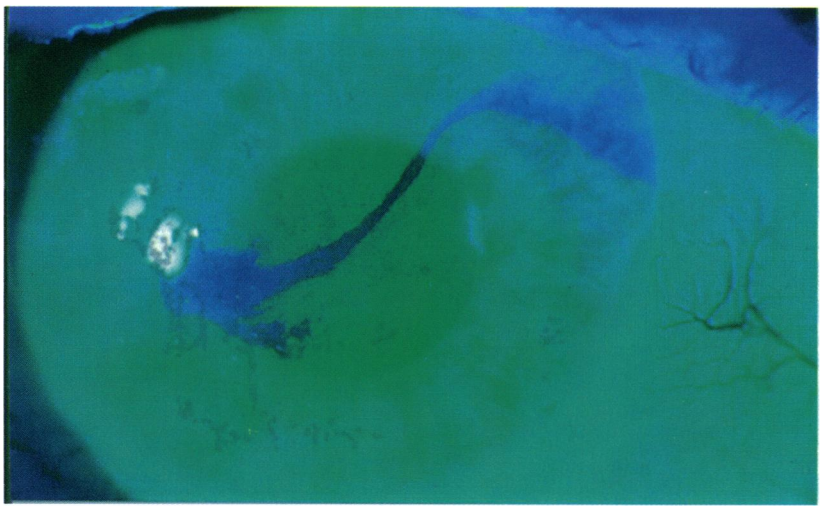


FIGURE 7C
Case 6. Fluorescein stain demonstrates late staining pattern of superior and inferior epithelium of penetrating keratoplasty. Note serpiginous area of no staining indicating centripetal movement of healthier stem cells from 2 o'clock limbus.

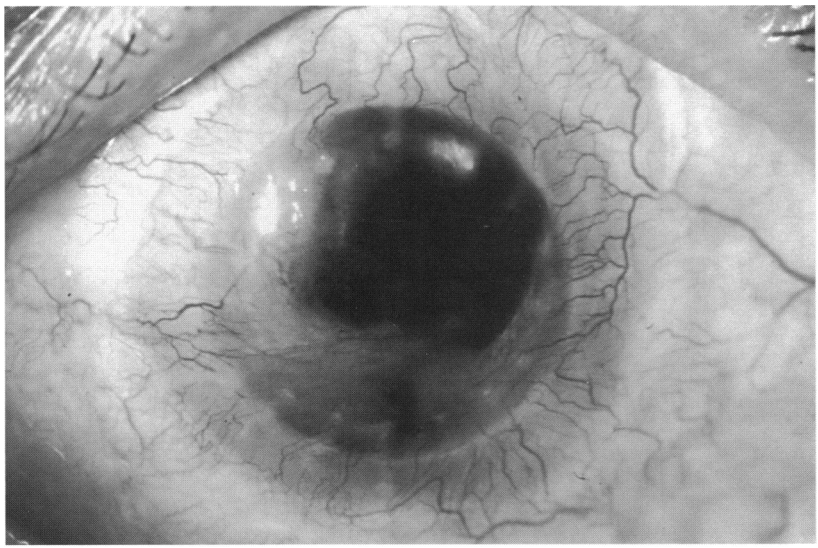


FIGURE 8

Case 12. Failed ocular surface in patient with aniridia and multiple surgeries who underwent KLAL followed in 3 months by penetrating keratoplasty. Note abnormal epithelial neovascularization and subepithelial fibrosis of penetrating keratoplasty.

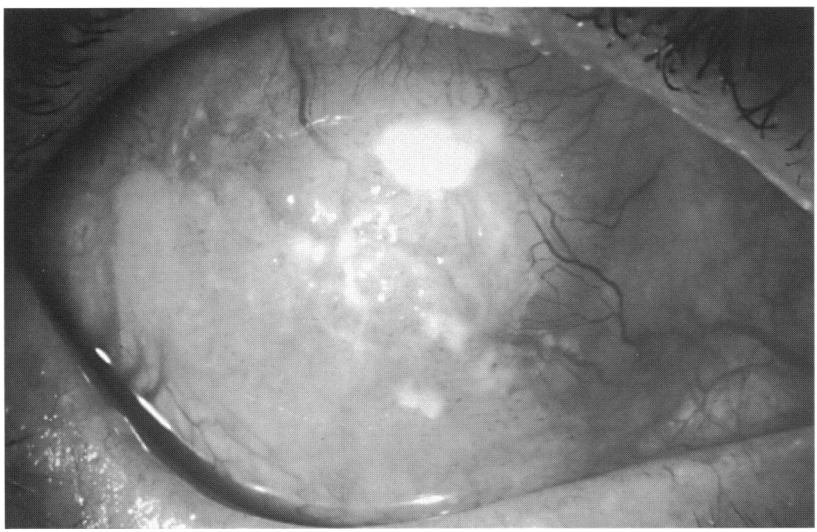


FIGURE 9A

Case 24. Preoperative appearance with severe conjunctival and corneal intraepithelial neoplasia.

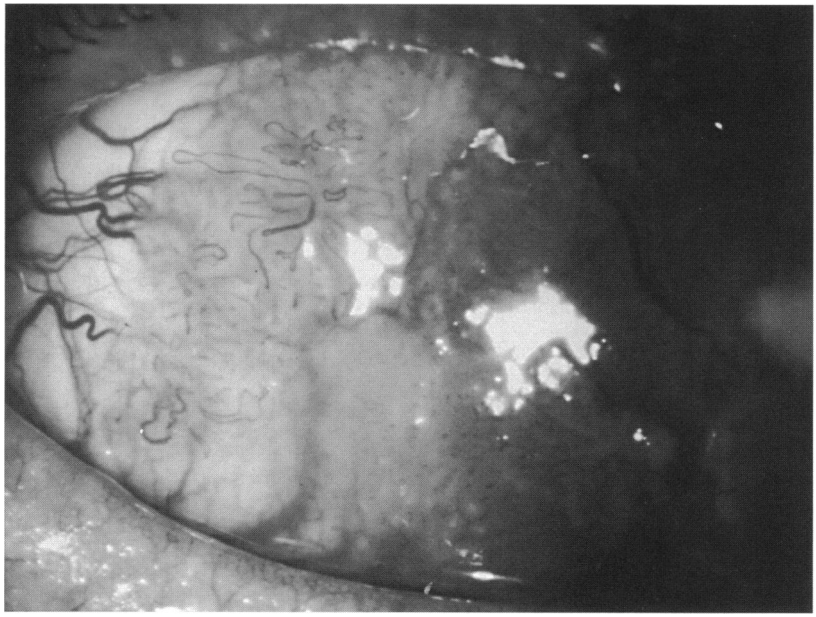


FIGURE 9B
Case 24. Extensive involvement of limbus and bulbar conjunctiva was present for 360 °.

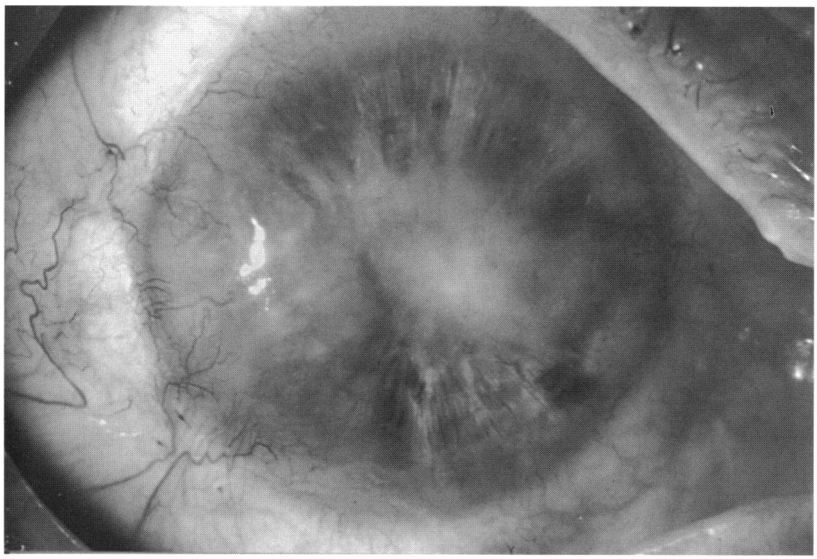


FIGURE 9C
Case 24. Six months after KLAL with stable ocular surface.

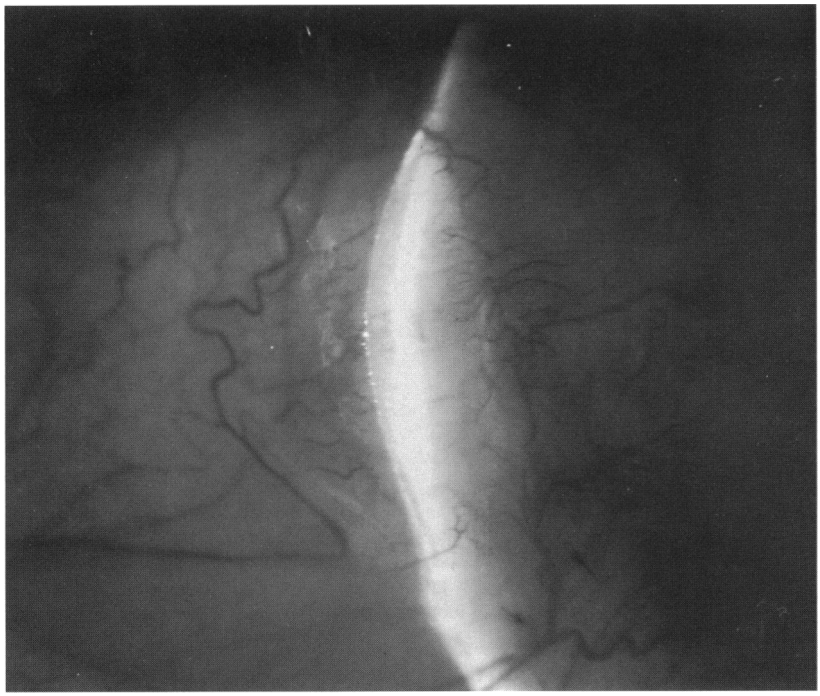


FIGURE 9D
Case 24. Transparent lenticule with normal epithelium.

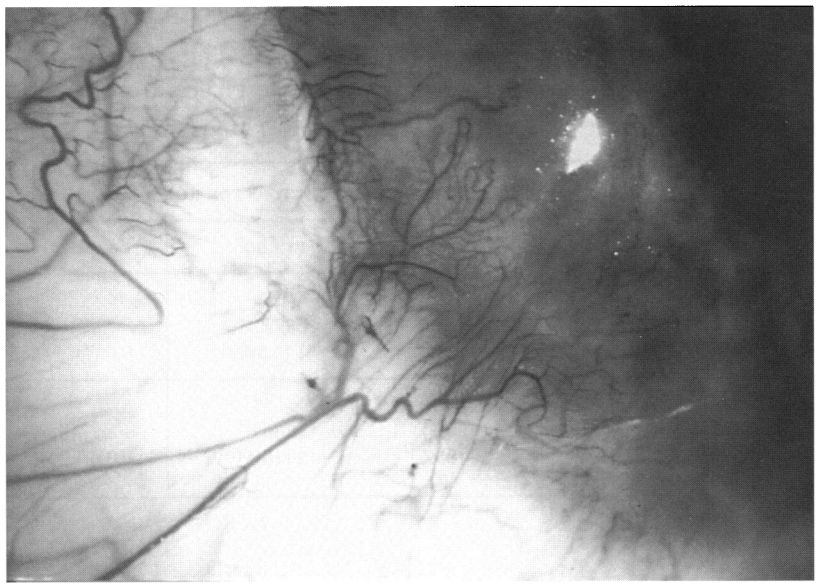


FIGURE 9E
Case 24. Note invasion of neovascularization in gap area between lenticules.

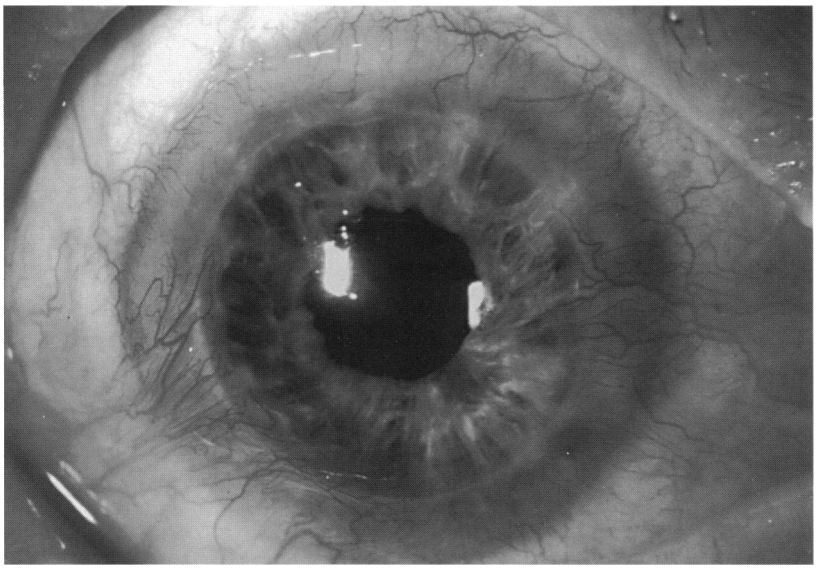


FIGURE 9F

Case 24. Penetrating keratoplasty 15 months after surgery. Note normal surface and clear graft.

KLAL (Fig 10). However, 16 of the 18 eyes that did not have preoperative keratinization developed a stable ocular surface (P=0.01).

The patients were also evaluated on the basis of reflex Schirmer testing for aqueous tear production. A Schirmer test was performed without anesthetic and was considered abnormal with a reading of 2 mm or less. Four of 5 eyes with an abnormally low Schirmer test failed to achieve a sta-

TABLE VII: OCULAR SURFACE RISK FACTORS FOR KLAL

RISK FACTOR	N	STABLE	FAILED	P-VALUE*
Keratinization				
Present	7	2 (28.6%)	5 (71.4%)	
Absent	18	17 (94.4%)	1 (5.6%)	0.007
Schirmer test†				
≤ 2 mm	5	1 (20%)	4 (80%)	
> 2 mm	20	17 (85%)	3 (15%)	0.012

N, number of eyes

* Fisher's exact test.

† Schirmer test without anesthesia at 5 minutes.

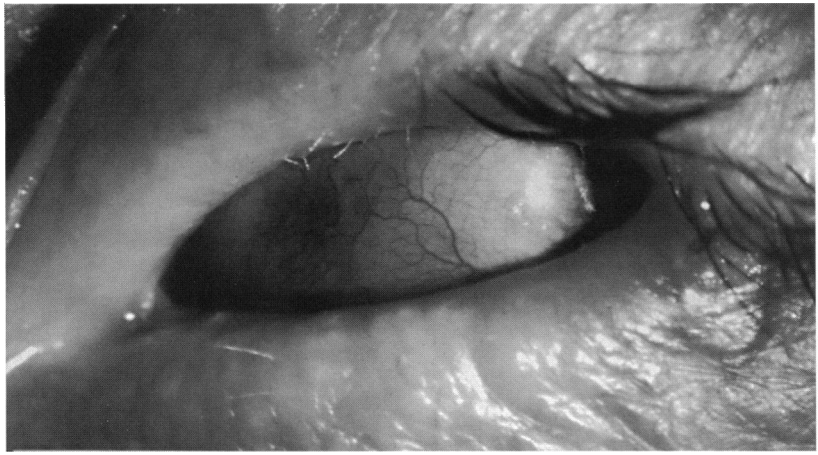


FIGURE 10A

Case 14. Preoperative appearance of patient with severe Stevens-Johnson syndrome and failed penetrating keratoplasty. Extensive symblepharon and keratinization are present.

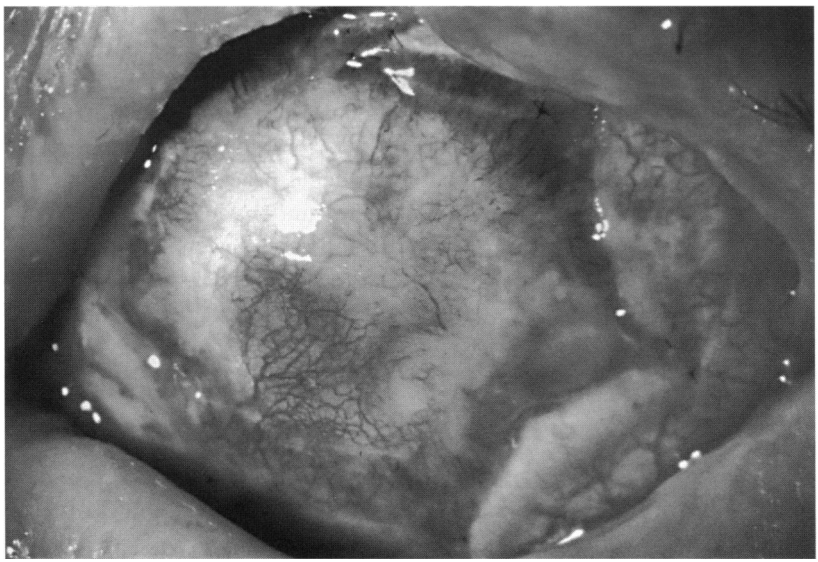


FIGURE 10B

Case 14. Five days after KLAL.

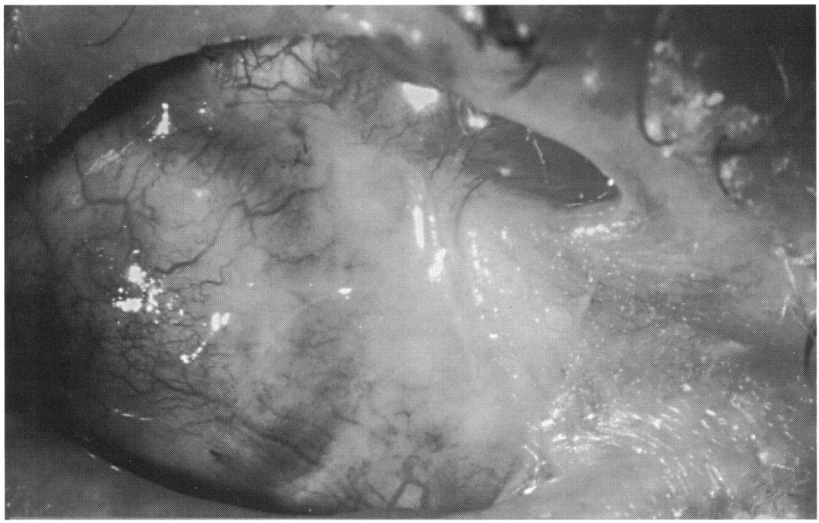


FIGURE 10C
Case 14. Failed ocular surface 3 months after KLAL with recurrence of symblepharon and keratinization.

ble ocular surface following KLAL. However, 17 of 20 eyes with a normal Schirmer test resulted in a stable ocular surface (P=0.01). Utilizing logistic regression, a combination of both the presence of keratinization and the abnormally low Schirmer test revealed that only the presence of keratinization was significant for the risk of ocular surface failure (P=0.01).

The two types of KLAL procedures performed were compared. The success of the ocular surface for the lenticule procedure was compared with the crescent procedure. Eighteen eyes underwent the lenticule procedure, and 12 of these (66.7%) were successful. Seven eyes underwent the crescent procedure, and 6 of these eyes achieved a stable ocular surface (85.7%). The difference between the two groups was not significantly different.

KLAL REJECTION

Ten eyes exhibited signs of epithelial rejection with acute injection adjacent to the graft and secondary neovascularization of the graft. All reactions occurred within the first 6 months postoperatively. One eye, case 24, exhibited an epithelial rejection line over the KLAL tissue (Fig 11). All cases of rejection were treated with intense topical or oral corticosteroids.

In all cases the acute signs resolved. The outcome of the ocular surface in those patients revealed that seven were stable and three failed.

OUTCOME OF PATIENTS WITH PERSISTENT EPITHELIAL DEFECT

Ten eyes of 10 patients underwent KLAL because of a persistent epithelial defect. In each case, the epithelial defect was unresponsive to lubrication, punctal occlusion, therapeutic soft contact lens, and tarsorrhaphy. All 10 patients had resolution of the epithelial defect following KLAL in 3 to 25 days. In the majority of patients, there was a marked reduction of inflammation following the healing of the epithelial defect (Fig 12). Eight of 10 patients developed long-term stable ocular surfaces. Two patients developed late surface failure; however, in no patients did the epithelial defects recur.

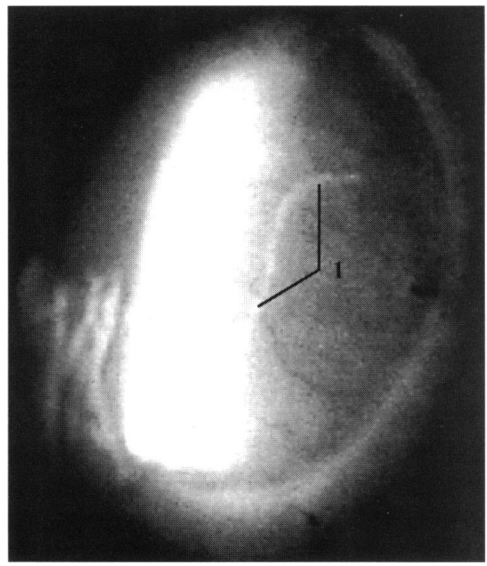


FIGURE 11

Acute epithelial rejection 3 months after KLAL. Epithelial rejection line (1) is present on lenticule.

FORNIX RECONSTRUCTION

Fourteen eyes of 16 patients had significant symblepharon formation, with a score of 2 or more, prior to KLAL. Three patients, cases 2, 22, and 23, had recurrent ankyloblepharon. A total score of symblepharon was applied to each patient before and after KLAL. A significant reduction in symble-

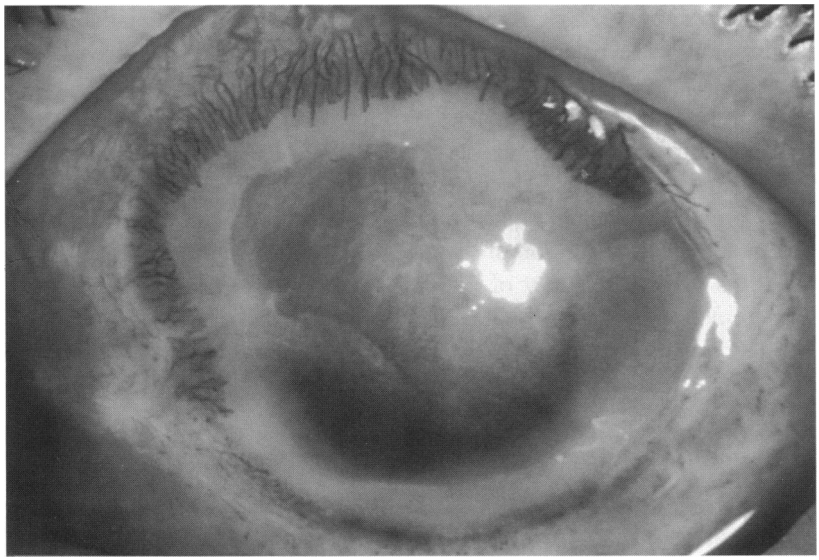


FIGURE 12A

Case 19. Severe bilateral alkali burn. Two months following injury patient has persistent corneal necrosis and nonhealing epithelial defect.

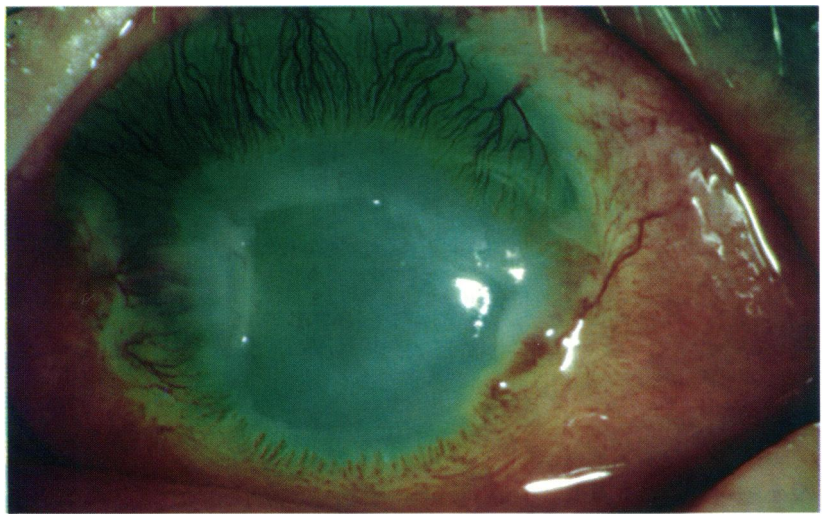


FIGURE 12B

Case 19. Severe bilateral alkali burn. Three months after injury corneal necrosis worsens with no evidence of epithelial healing.

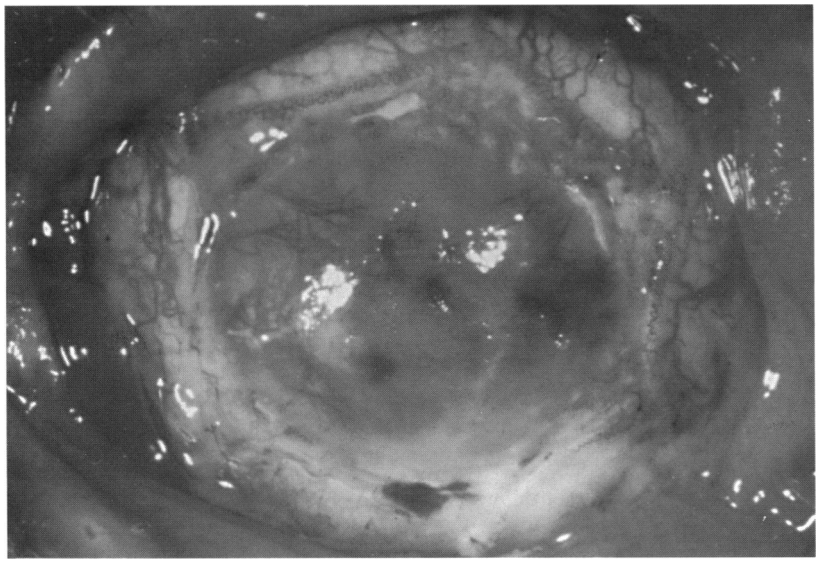


FIGURE 12C
Case 19. Severe bilateral alkali burn. KLAL performed 3 months after injury. Lenticules are visible at recipient limbus.

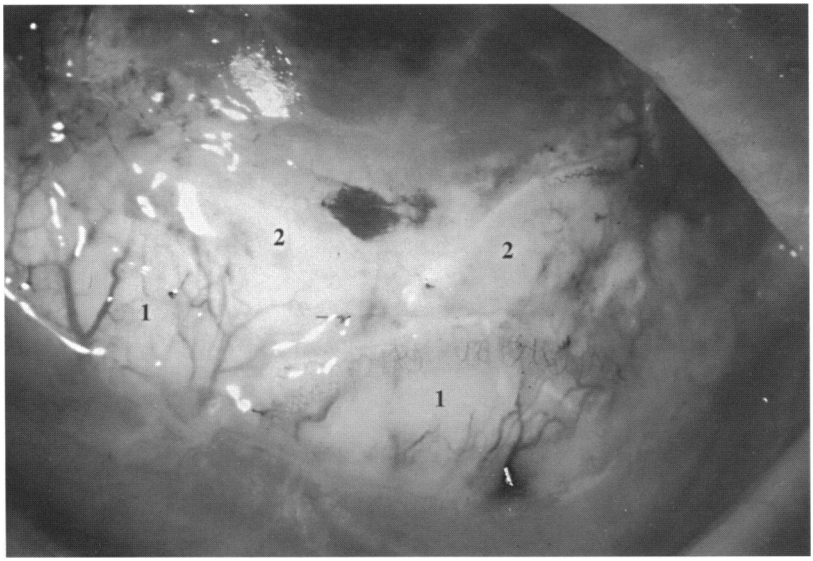


FIGURE 12D
Case 19. Severe bilateral alkali burn. A second row of lenticules (1) is placed inferiorly, below the more limbal lenticules (2), to prevent symblepharon.

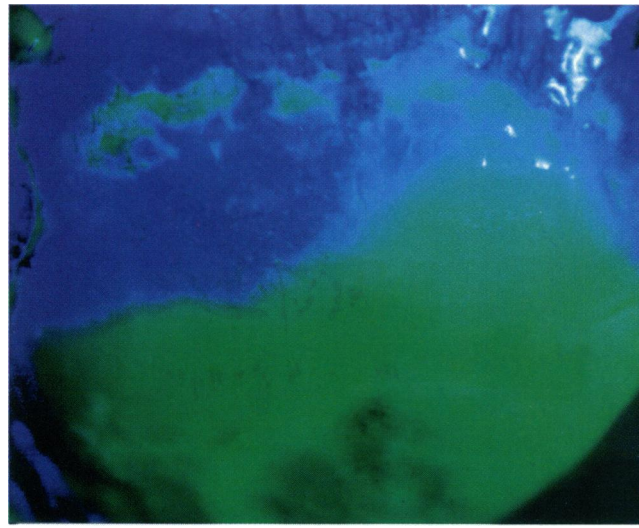


FIGURE 12E

Case 19. Severe bilateral alkali burn. Four days after KLAL, epithelium is moving centrally off lenticules.

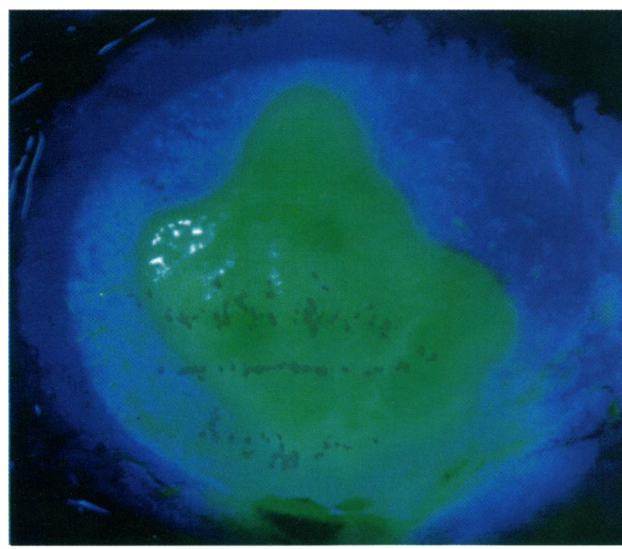


FIGURE 12F

Case 19. Severe bilateral alkali burn. Six days after KLAL, the epithelial defect is almost healed. Pattern of new epithelium corresponds to lenticule position.

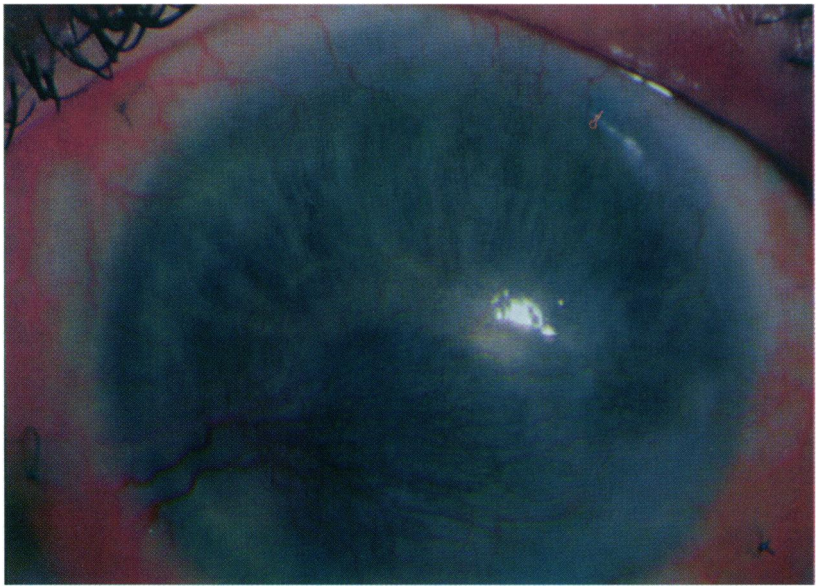


FIGURE 12G
Case 19. Severe bilateral alkali burn. Three months after KLAL, ocular surface is stable. Cornea has cleared considerably, with regression of neovascularization and epithelial healing.

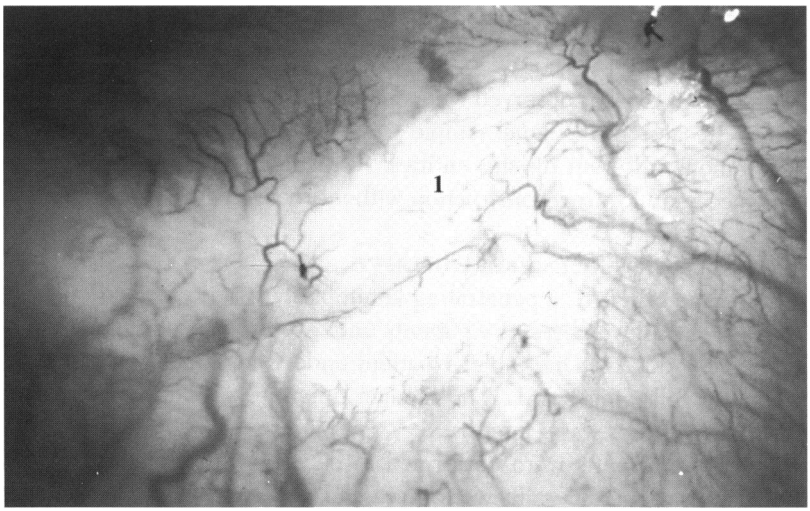


FIGURE 12H
Case 19. Severe bilateral alkali burn. Lenticule of KLAL (l) 3 months postoperatively.

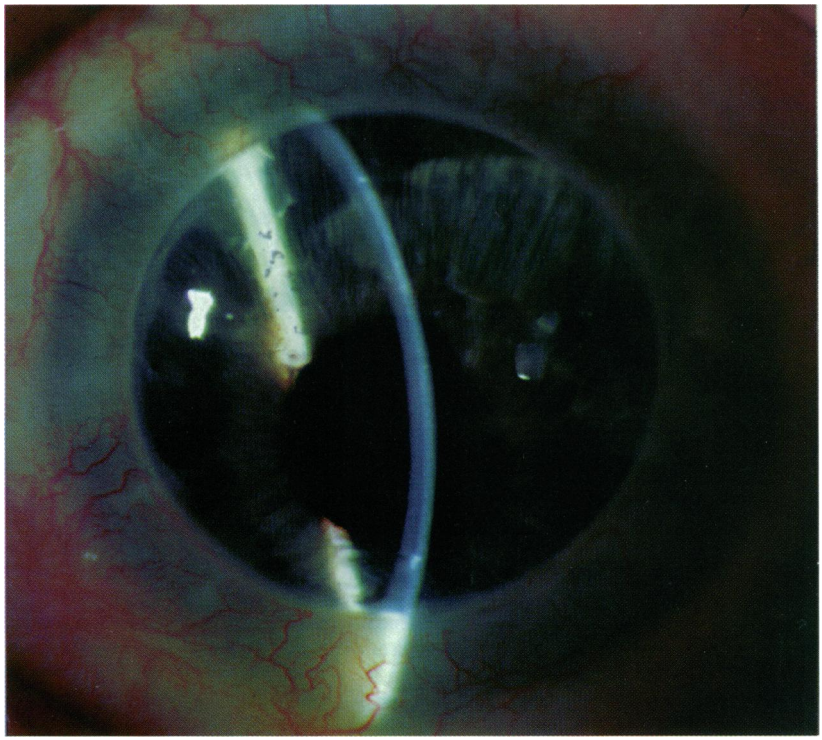


FIGURE 12I
Case 19. Severe bilateral alkali burn. Penetrating keratoplasty 7 months after KLAL.

pharon ($P<0.01$) was observed when all patients were looked at as a single group. Because of the small sample sizes, no significant differences were seen among different disease entities. All patients with ankyloblepharon had successful fornix reconstruction without recurrence (Table VIII).

SUCCESS OF SUBSEQUENT KERATOPLASTY

At the time of KLAL a penetrating keratoplasty was performed on 1 eye of 1 patient. Eleven eyes of 9 patients underwent penetrating keratoplasty following KLAL. One eye of 1 patient underwent lamellar keratoplasty 3 months following KLAL. The median time interval between surgeries for these 12 cases was 4 months .

Six of the grafts were performed in patients with a history of alkali burn. Four of these 6 grafts were successful. One graft failed secondary to endothelial rejection, while another failed secondary to late ocular surface failure. Two grafts were done in aniridia patients. One graft remained clear

TABLE VIII: MEAN SYMBLEPHARON SCORE BEFORE AND AFTER KLAL

DISEASE	N	PRE-KLAL (SD)	POST-KLAL (SD)
Alkali burn	8	3.8 (2.1)	2.0 (1.9)
Aniridia	7	0 (0)	0 (0)
SJS	4	4.0 (0.8)	3.5 (1.9)
Other	6	2.3 (2.5)	0.7 (1.2)
Total	25	2.4 (2.3)	1.36 (1.9) P<0.01*

N, number of eyes; KLAL, keratolimbal allograft; SJS, Stevens-Johnson syndrome.

*Wilcoxon signed-ranks test.

(Fig 13), and the other graft failed owing to recurrence of ocular surface disease. Three grafts were performed in SJS patients. All three grafts failed owing to recurrence of the ocular surface disease. Two patients receiving grafts were in the "other" category. One patient (case 20) had limbal failure due to multiple surgeries and atopic disease. His graft failure was secondary to endothelial rejection. The other patient (case 24) had severe conjunctival intraepithelial neoplasia, and his graft has remained clear.

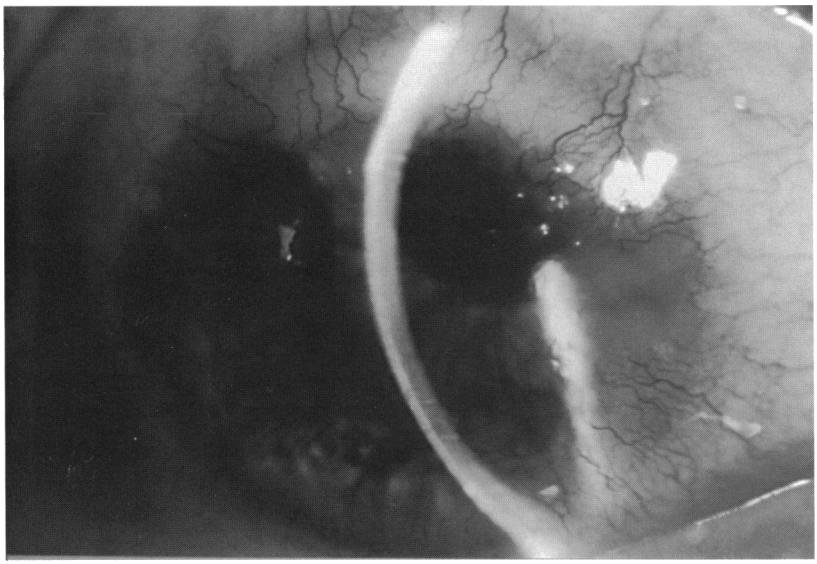


FIGURE 13A

Case 16. Preoperative appearance of patient with aniridia. Corneal epithelium is thickened and irregular, and superficial neovascularization is present.

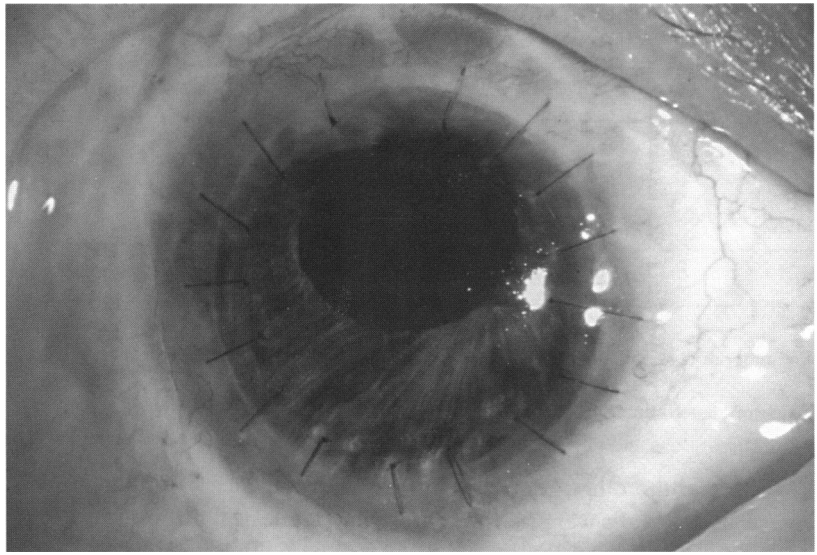


FIGURE 13B
Case 16. Clear penetrating keratoplasty with normal epithelial surface 3 years after surgery.

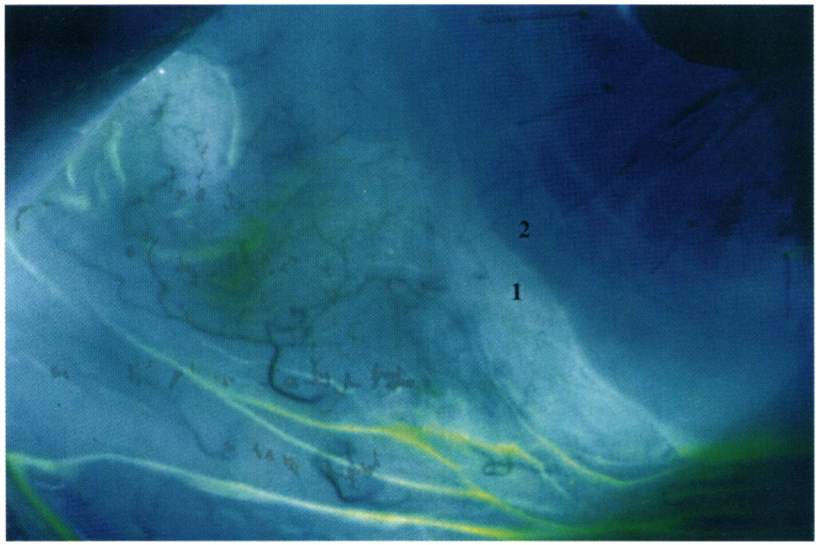


FIGURE 13C
Case 16. Fluorescein stain reveals late staining of conjunctiva (1) and no staining of limbus (2).

An evaluation of keratoplasty procedures reveals that 6 grafts (46%) were successful, while 2 grafts (15%) failed because of endothelial rejection and 5 grafts (38%) failed owing to recurrence of ocular surface disease. Of these 5 grafts, 4 of them had revealed keratin present prior to KLAL.

VISUAL ACUITY

Preoperative visual acuity was determined for each patient and ranged from light perception to 20/80. The final visual acuity was obtained at the last examination. For 13 eyes, this final value represents visual acuity following penetrating or lamellar keratoplasty in addition to KLAL. For 12 eyes, this value represents visual acuity following KLAL only.

If an improvement of at least two lines of Snellen visual acuity was considered significant, 15 of 25 eyes (60%) achieved improvement (Table IX). Nine eyes had no significant change, and 1 eye exhibited worse vision. The range of final visual acuity in those patients with improved vision was 20/25 to 20/300, with 13 of these patients achieving 20/80 vision or better. Of these 15 eyes, 5 had undergone penetrating keratoplasty, and 1 had undergone lamellar keratoplasty following KLAL. Twenty eyes had an initial visual acuity of 20/200 or worse. Nine of these eyes obtained better than 20/200 vision following KLAL, with a range of 20/25 to 20/150.

TABLE IX: IMPROVEMENT IN VISUAL ACUITY AFTER KLAL

DIAGNOSIS	N	SIGNIFICANT IMPROVEMENT IN VA*	NO SIGNIFICANT IMPROVEMENT IN VA
Alkali burn	8	4 (50.0%)	4 (50.0%)
Aniridia	7	5 (71.4%)	2 (28.6%)
SJS	4	1 (25.0%)	3 (75.0%)
Other	6	5 (83.0%)	1 (17.0%)
Total	25	15 (60.0%)	10 (40.0%)

N, number of eyes; VA, visual acuity; SJS, Stevens-Johnson syndrome; KLAL, keratolimbal allograft

*Greater or equal to 2 lines of improvement in visual acuity, from initial vision to final vision. Includes those patients who underwent keratoplasty.

CYCLOSPORINE A

Patients were evaluated on the basis of use of CsA. Nineteen patients received topical CsA in the postoperative period. Four of these patients received oral CsA in addition to topical CsA. Thirteen of these 19 eyes achieved a stable ocular surface, and in 6 of the 19 eyes, the ocular surface failed.

Six eyes did not receive any CsA following KLAL. Five of these 6 eyes achieved a stable ocular surface, while the ocular surface failed in 1 eye. No statistically significant difference was seen between the 2 groups.

CONJUNCTIVAL IMPRESSION CYTOLOGY FOLLOWING KLAL

Nine of the 25 eyes (alkali burn, 3; SJS, 3; aniridia, 2; and conjunctival intraepithelial neoplasia, 1) underwent conjunctival impression cytology. This test was performed an average of 12 months after KLAL (range 1 to 20 months). Seven of 8 eyes showed normalization or improvement by mean cytology grade when compared with the untreated fellow eye of the preoperative state. One monocular alkali burn patient did not have a fellow eye or preoperative cytology for comparison but appeared to have normalization of the surface. One patient with SJS did not show improvement.

Evaluation of the 3 patients with SJS revealed an increased number of polymorphonuclear neutrophils (PMNs) in the recipient eye, nonoperated fellow eye, and on the preoperative test (Fig 14). All three of these patients underwent subsequent penetrating keratoplasty and eventual surface failure. The patients with diagnoses other than SJS showed little or no PMNs in their specimens (Fig 15). Six of these patients subsequently underwent penetrating keratoplasty, and 5 of these grafts have remained clear with stable surfaces.

DISCUSSION

In this thesis we propose a classification of the surgical procedures of epithelial transplantation for severe ocular surface disease. This classification is based on the source of donor tissue (self, relative, cadaver) and the tissue transplanted (conjunctiva, limbus, cornea). The surgical procedures can be assigned to one of the following categories: conjunctival autograft (CAU), conjunctival allograft (CAL), conjunctival limbal autograft (CLAU), cadaveric conjunctival limbal allograft (c-CLAL), living related conjunctival limbal allograft (lr-CLAL), or keratolimbal allograft (KLAL). This classification system eliminates the confusion resulting from the multiple titles for the same procedures and provides an anatomic basis for the

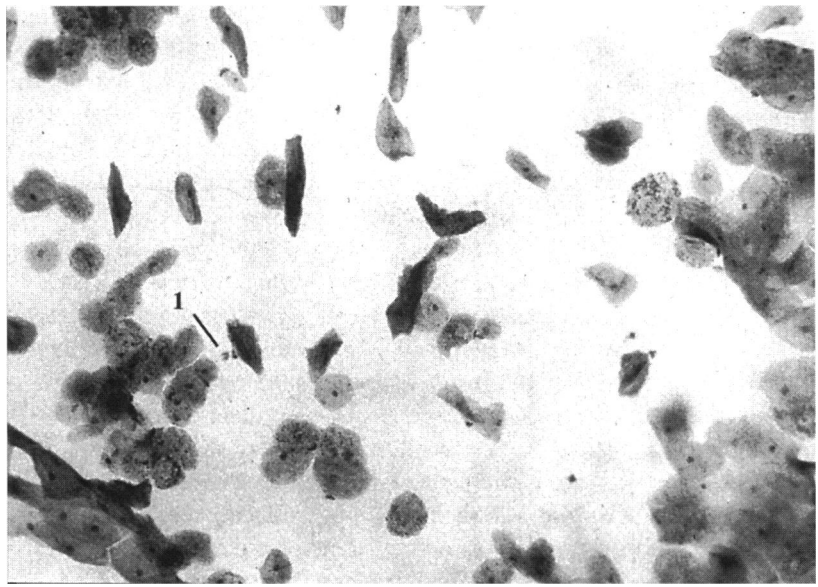


FIGURE 14

Case 17. Conjunctival impression cytology from patient with Stevens-Johnson syndrome taken 6 months after KLAL. Epithelial cells are large with pyknotic nuclei. Keratinization and PMNs (1) are present (PAS-hematoxylin, original magnification x100).

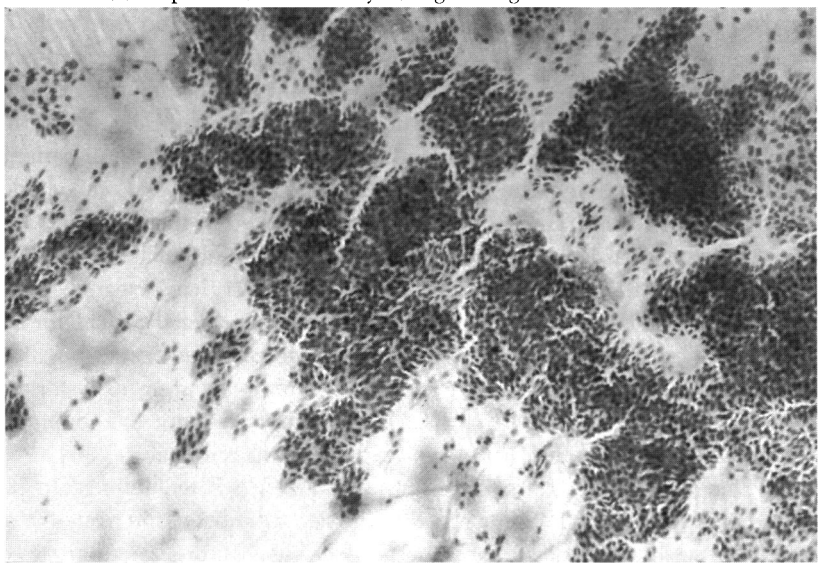


FIGURE 15

Case 17. Conjunctival impression cytology from patient with aniridia taken 4 months after KLAL. Normal appearing conjunctival epithelium with small epithelial cells and relatively large nuclei. No keratinization or PMNs are seen (PAS-hematoxylin, original magnification x100).

terminology. With this classification it should be easier and more accurate to compare past and future studies of epithelial transplantation .

In this thesis, we also report our experience with KLAL for the management of severe ocular surface disease. These data demonstrate that this procedure is a useful technique to improve the ocular surface of a group of patients with otherwise poor prognoses. Twenty-five eyes of 21 patients with severe limbal deficiency underwent the procedure.

One of the indications for the procedure was the presence of a persistent epithelial defect unresponsive to conventional treatment. KLAL was very successful in the management of these patients. Ten eyes underwent surgery for this indication, and in all 10 eyes the epithelial defect healed. In addition, 8 of these 10 eyes developed a successful surface long-term and in none of the eyes did the epithelial defect recur.

Evaluation of the KLAL procedure in terms of stability of the ocular surface revealed a successful outcome in 18 of the 25 eyes. Successful surface meant minimal to no epithelial staining, reduction or absence of neovascularization, and absence of epithelial inflammation and defects.

KLAL was successful in those patients who required fornix reconstruction. Recurrence of symblepharon following severe ocular surface disease can be a problem. Simple lysis of the scar tissue typically results in immediate re-formation. Other tissues such as oral mucosa and hard palate have been successfully used, but these do not provide stem cells for the cornea. CAU and CLAU are both very successful for fornix reconstruction[60,44]; however, these procedures are not options in patients with bilateral disease. In the present study, 16 eyes had clinically significant symblepharon and 3 patients had ankyloblepharon formation. All patients achieved a reduction in the symblephara, and following KLAL, ankyloblephara did not recur. The surgical strategy employed in these patients was to cover the areas lacking in epithelium with a new epithelial surface. The bulbar side of the symblepharon was recessed rather than resected, so the symblepharon tissue could be used to provide an epithelial surface for the palpebral conjunctiva. KLAL tissue was used to cover, as best as possible, all exposed bulbar surfaces. In addition to supplying stem cells for the corneal surface, it appears that KLAL may supply cells for the conjunctival surface, since recurrence of symblepharon was minimal.

The KLAL procedure in this study utilized two techniques. In the lenticular technique, patients received the keratolimbal tissue from a fresh globe in which lenticules of peripheral cornea and limbus were harvested. This technique is similar to Thoft's revised keratoepithelioplasty procedure.[50] However, patients in this study received lenticules from two globes, as opposed to one, in an effort to provide more limbal stem cells. A disad-

vantage to the lenticule technique for KLAL is that the tissue must be utilized within 24 to 36 hours because it is harvested from a whole globe and not preserved in tissue media. In addition, two globes are required in order to obtain enough tissue to cover the recipient limbus in a contiguous fashion.

The crescent technique for KLAL used in this study was modified from that of Tsubota and associates.[52] This procedure utilizes a corneoscleral rim for limbal tissue. There are two major logistic advantages to this procedure when compared with the lenticule procedure. First, tissue may be stored in corneal storage media, thus giving extra time prior to surgery. The extra time is important, as patients may live long distances from the location of the surgeon. Second, donor testing for potential infectious diseases, which is done on all donor tissue, can cause delays and therefore eliminate whole globes as donor tissue owing to the time constraints. Although in this study we used both lenticule and crescent tissue within 36 hours, it was not always possible for screening tests to be completed within this time frame. Tsubota's group utilized tissue stored for an average of 5 days. Their pathologic examination of stored tissue demonstrated viable limbal epithelium for 25 days, providing evidence that KLAL donor tissue may be viable in corneal storage media for several days.

In the present study, no statistically significant difference was observed in the success of the ocular surface between the two procedures. However, the crescent technique utilized one donor corneoscleral rim as opposed to two globes for the lenticule technique. Because ideal donor tissue for KLAL is difficult to obtain owing to the strict criteria of young age, short storage time, and excellent epithelium, a procedure that uses one globe instead of two is desirable. An additional potential advantage of the crescent technique is that it may be possible to tissue match future patients for KLAL with improvements in storage media for the epithelium and efficiency in tissue typing.

There were 7 patients whose ocular surface failed after KLAL. Patients with SJS had a significantly poorer result when compared with the alkali burn, aniridia, and "other" groups. Patients with aniridia and patients in the "other" category (three with multiple surgeries, 1 with conjunctival intraepithelial neoplasia, 1 with acid burn) had the best outcome. It is conceivable that these patients display a better prognosis because the extent and severity of their disease is less than what is typically seen in SJS. The latter group of patients has not only corneal epithelial problems but also chronic conjunctival inflammation and scarring.

An important risk factor associated with surface failure has been iden-

tified in this study. Patients with keratinization had a significantly poorer result than patients without such keratinization. All 4 patients with SJS had keratinization, and of this group, 3 had surface failure following KLAL.

Aqueous tear production was another useful parmeter to predict outcome. Patients with a Schirmer test of 2 mm or less at 5 minutes without anesthesia had a significantly poorer prognosis. However, using logistic regression, the combination of both keratinization and low Schirmer testing demonstrated that only keratinization was a significant risk factor. It is possible that an abnormally low Schirmer test is also an independent risk factor, but our study did not have any patients with a low Schirmer test without keratinization to support this theory.

Four patients failed early (in less than 5 months) and 3 patients failed late (after 1 year). Two of these late failures were after 18 months. There are several possibilities for ocular surface failure in our patients, including absence of stem cells, failure of stem cells, and rejection. It is possible that in some patients limbal stem cells are not transplanted or if they are, they are not viable. It may be that the storage, either refrigeration for whole globes or refrigeration and cornea storage media for corneoscleral rims, may not sustain limbal stem cells. If viable limbal stem cells are not transplanted, then suprabasal corneal epithelium cells, which are TDC and not stem cells, may migrate and repopulate the ocular surface. This epithelial migration can give the appearance of a successful procedure; however, TDC do not have the ability to proliferate as stem cells do, and late failure would result. This situation is no different than that for routine keratoplasty for ocular surface disease, in which the short-term results are usually acceptable, but the long-terms results are dismal.

It is also possible that viable stem cells may be functional early in the postoperative period, but added stress to the stem cells, such as a keratoplasty procedure, may contribute to stem cell demand and push the surface into failure. Chen and associates[61] reported that corneas with partial limbal deficiency that were further challenged with removal of the corneal epithelium progressed to have conjunctivalization of the surface. This study suggests that when a compromised surface with partial limbal stem cells is challenged, the stem cell reserve may not be able to respond. Chen postulates that TAC may have a different role in epithelial healing in that the TAC may be primarily responsible for migration and mitosis during the healing phase. This increased demand on the TAC may trigger limbal stem cell activation to generate more TAC.

Immunologic rejection of the KLAL may be responsible for surface failure. Acute epithelial rejection was identified in 10 patients. This reaction occurred in the early postoperative course in all patients. All patients

were treated with topical and systemic corticosteroids, resulting in resolution of the inflammation. It is a concern that acute rejection may be associated with surface failure either early or late; however, in this group of patients it did not appear to affect the outcome, as 7 of the 10 cases had stable surface. This success rate was similar to the success rate for all our patients (72%).

Thoft and Sugar[62] reported 3 patients undergoing "keratoepithelioplasty" (KLAL) who experienced an epithelial rejection reaction and subsequent surface failure. They postulated that the keratolimbal tissue, which contained a higher concentration of vascularized endothelial cells and Langerhans' cells, may have served as antigen presenting cells. Therefore, there may have been a higher degree of immunoreactivity than the original technique of keratoepithelioplasty, which contained peripheral corneal but not limbus (KAL). The investigators also postulated that in this type of epithelial transplant procedure, inclusion of limbal tissue, while possibly advantageous from a stem cell point of view, might have been counterproductive from an immunologic viewpoint. However, it was unclear in Thoft's original procedure of keratoallograft (without limbus) what the source of future epithelial cells may have been, since peripheral corneal epithelial cells do not have the capacity to proliferate.

Chronic rejection of KLAL is also a possibility for those patients with late surface failure. Patients with severe ocular surface disease typically exhibit chronic inflammation of the ocular surface. Rejection of penetrating keratoplasties may occur months to years after surgery. Therefore, it would not be unusual for surface inflammation in these patients to be a precursor of chronic, low-grade rejection. As previously stated, conjunctival impression cytology revealed that the SJS patients had an abnormal amount of PMNs detected, indicating more inflammation than the other patients. The SJS group also had a significantly poorer result.

COMPARISON OF KLAL TO OTHER EPITHELIAL TRANSPLANTATION PROCEDURES

The results of our KLAL patients can be compared with other epithelial transplantation studies. Because KLAL is an allograft, it is not reasonable to compare this study with those with autografts. Autografts obtained from the fellow eye have the advantage of eliminating the problem of immunologic rejection. Moreover, fewer anti-inflammatory medications are needed in these patients. Anti-inflammatory medications can slow epithelial healing and, when given topically, can cause epithelial toxicity.

Evaluation of the reported CAU procedures reported show that 29 of 32 eyes (92%) attained a stable surface (Table II). Kenyon and Tseng's report of CLAU revealed excellent results, with 20 of 21 eyes (95%) achieving a stable surface and all 7 subsequent keratoplasties in these eyes being successful.[56] The other report of CLAU by Jenkins and associates[21] did not have as good a result. However, in this study, the fellow eyes that served as donors were not free of epithelial disease.

CAU and CLAU are successful procedures and have significantly changed the methods of managing severe unilateral ocular surface disease. CAU is useful for managing unilateral primary and secondary pterigia, symblephara, and cicatricial conjunctival disease not due to limbal stem cell deficiency. Strong evidence supports the case for the value of limbal stem cell; therefore, if ocular surface disease is unilateral and due to stem cell deficiency, CLAU is indicated.

Bilateral disease must be managed by allografts, and our results can be compared with other reports of allografts. Our KLAL results can be compared with the 3 other published studies of KLAL by Turgeon and Thoft,[50] Tsai,[51] and Tsubota.[52] First, considering ocular surface outcome, 18 of 25 (72%) eyes in our study achieved a stable surface, compared with a combined 11 of 17 (65%) in the other studies. Tsai did not report on ocular surface stability. Improvement of two or more lines of visual acuity was seen in 15 of 25 eyes (60%) in our study compared with 16 of 21 eyes (76%) in the others. These differences were not statistically significant

Comparison of our patients with the other allograft procedures, c-CLAL, lr-CAL, and lr-CLAL of Pfister,[7] Kwitko,[54] and Kenyon,[55] respectively, is warranted. Results of the ocular surface outcome revealed that a combined 18 of 21 eyes (86%) developed a stable surface, compared with 72% of our patients. Thirteen of 21 eyes (62%) showed two or more line improvement in vision compared with 60% in our study. Neither of these comparisons was significantly different.

Of interest is the success rate of SJS among the various allograft procedures. When combining our KLAL patients with the other published results, only 2 of 7 eyes developed a stable surface. One of these patients with a reported stable surface was described by Turgeon and Thoft[50] and had only 6 months' follow-up. In examining the combined data for lr-CAL and lr-CLAL for SJS, 10 of 10 eyes developed a stable ocular surface. Eight of these eyes were reported by Kwitko,[54] who utilized a technique that does not transplant limbal tissue (lr-CAL).

These results may indicate that diseases such as SJS, inflammatory disorders of the conjunctiva that lead to limbal deficiency, may be better managed with a conjunctival technique. There are several factors that may

lead to the better results. First, the tissue for CAL and CLAL are obtained from living relatives, while KLAL tissue is not. Kwitko concluded that either the HLA identity or haplo identity was of benefit in reducing the risk of graft rejection. In Kenyon's study[55] rejection in the HLA compatible or incompatible patients was not detected.

Second, it is also possible that the transplanted conjunctival tissue may yield another benefit in addition to providing epithelial cells for the cornea. The transplanted conjunctival tissue may supply normal goblet cells, which are compromised in the recipient conjunctiva in severe cases of SJS. Another benefit may be the re-formation of normal limbal anatomy, including normal blood vessels. It has been proposed that the limbus continues a stem cell niche, and that the presence of blood vessels in the limbus is vital to this function.[14] Blood vessels provide for increased levels of nutrition in addition to interaction with blood-derived cytokines.

A prospective trial to evaluate the success of lr-CLAL and KLAL would be beneficial. Important issues to be answered include the importance of tissue typing in lr-CLAL. Also, if there were not a good match in lr-CLAL, would it be more beneficial to perform KLAL that requires only one operation, instead of the two that the lr-CLAL procedure requires? Also, the role of immunosuppression in all of the allograft procedures needs to be defined. It is likely that all patients receiving the epithelial allografts would benefit, and the risks of immunosuppression need to be considered.

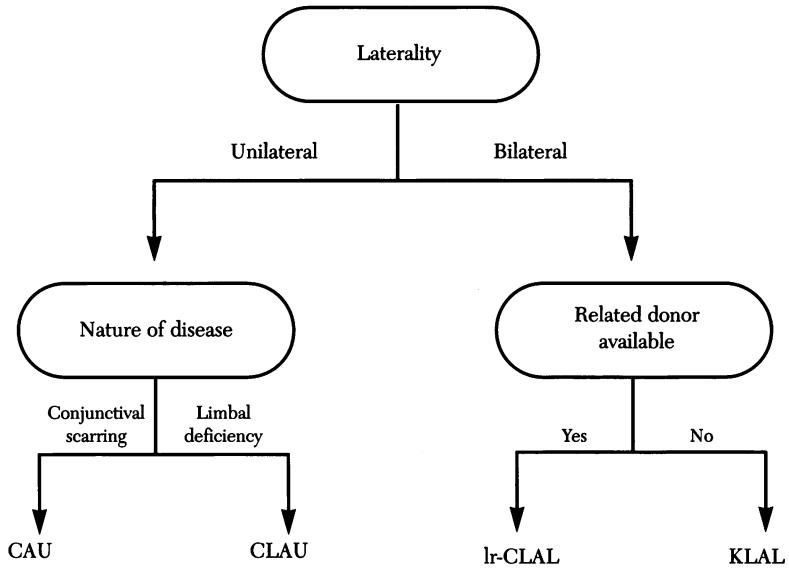
RECOMMENDATIONS FOR EPITHELIAL TRANSPLANTATION PROCEDURES

Clinicians managing patients with severe ocular surface disease have several options. It is important that the clinician recognize and understand the concept behind limbal stem cells. If the stem cell concept is not taken into consideration, standard treatment options are often implemented with poor results.

Indications for epithelial transplantation include cicatrizing conjunctival disease and limbal deficiency. Patients with limbal deficiency may benefit from epithelial transplantation for the management of a persistent epithelial defect of the cornea, to improve decreased vision due to an irregular corneal surface, or to stabilize an ocular surface prior to keratoplasty.

The first consideration is whether the condition is unilateral or bilateral (Table X). If unilateral, the autograft procedures hold the best prognosis, because they eliminate the issue of rejection. If the disease process involves conjunctiva only and does not have a limbal deficiency compo-

TABLE X: RECOMMENDATIONS FOR EPITHELIAL TRANSPLANTATION PROCEDURES



CAU, conjunctival autograft; CLAU, conjunctival limbal autograft; lr-CLAL, living related conjunctival limbal allograft; KLAL, keratolimbal allograft.

nent, then the recommended procedure is a CAU.

If the condition results from a unilateral limbal deficiency, then the procedure of choice is a CLAU. This procedure has an excellent prognosis compared with the allograft procedures. Because it provides conjunctiva in addition to limbal stem cells, it is beneficial for patients with conjunctival inflammation. Because it is an autograft, it eliminates the need for immunosuppression. One major concern is the risk for the fellow eye, which acts as the donor. A recent study has shown that partial removal of full-thickness limbal zone will compromise the donor surface.[63] When a large corneal epithelial defect was subsequently introduced in these eyes, a clinical picture consistent with limbal deficiency occurred. In addition, it has been shown that even the removal of partial-thickness limbal epithelium could cause a milder form of limbal deficiency and abnormal corneal epithelial wound healing.[64]

We feel the risk of donor epithelial problems is low, especially if less than 6 clock hours of limbal tissue and a moderate amount of conjunctiva are removed from the donor eye. We limit the use of CLAU to those patients without disease in the donor eye. We have not seen complications in the donor eyes of our patients, and this is supported by the fact that Kenyon, in his report of the largest series of CLAU, did not discover problems either. [56]

Autograft procedures are limited primarily to unilateral disease. The patients with the greatest need are those with bilateral disease. These patients require an allograft to manage ocular surface disease due to limbal deficiency. The clinician may choose from three procedures in order to restore the ocular surface: the CAL, CLAL, and KLAL.

Since most disease entities resulting in bilateral disease affect the limbus, limbal transplantation procedures are more beneficial than conjunctival transplant alone. CLAL tissue may be obtained either from a cadaver or a living relative. The c-CLAL procedure has only two published cases and appears to have significant disadvantages when compared with lr-CLAL and KLAL. First, cadaveric conjunctival tissue is difficult to handle and often is not available on donor globes. Second, the survival of cadaveric conjunctiva on whole globes in storage media has not been documented. Third, the survival of this tissue has not been studied. Therefore, at this time, the two procedures most appropriate for bilateral ocular surface disease are the lr-CLAL and KLAL.

Reviewing published studies and our data demonstrates that if possible, a lr-CLAL should be the first procedure considered for bilateral severe ocular surface disease due to limbal deficiency. The best tissue match possible should be sought. In addition, systemic immunosuppression should be used in the absence of medical contraindications. This procedure appears to be advantageous over KLAL in cases with conjunctival inflammation.

For patients with bilateral disease without a related donor, KLAL should be performed. This procedure is available to all patients, as cadaveric donors are the source of the transplanted tissue. KLAL has been shown to be successful in our study as well as in Thoft's,[50] Tsai's,[51] and Tsubota's.[52] From our data, it may be inferred that KLAL should not be performed on patients with keratinization and possibly not on those with a low Schirmer test. The recommendation of KLAL for SJS should also be made with caution, as these patients had the poorest results in these studies. In Tsubota's study, as well as in our study, KLAL tissue preserved in corneal storage media was utilized. Future studies should evaluate the

length of time limbal stem cells are viable in storage media.

CONCLUSION

Patients with severe ocular surface disease due to limbal deficiency are some of the most challenging in ophthalmology. Epithelial transplantation procedures have significantly improved the success rate of the management of these patients. Classification of these procedures to provide a uniform terminology among clinicians and researchers will be beneficial for an accurate comparison and discussion of the multiple procedures with multiple terms now employed .

KLAL is a successful epithelial transplantation method. Its advantages are that it is useful in the management of bilateral and unilateral disease. Since the donor tissue is from a cadaver, KLAL is an option for all limbal deficiency recipients. In addition, with corneal storage there is the potential for tissue typing. Consideration of lr-CLAL should be made prior to KLAL, since this procedure appears to be advantageous in those patients with conjunctival inflammation and limbal deficiency.

Important issues regarding epithelial transplantation remain to be studied. The role of systemic immunosuppression for both matched and unmatched allografts should be established. The importance of HLA and ABO typing needs to be defined. Significant progress has been made in the management of severe ocular surface disease; however, continued studies are needed to improve the success in treating these patients.

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