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Changing Trends in the Definition and Diagnosis of Dry Eyes

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ONE OF THE PROBLEMS IN DEALING WITH DRY EYES is the lack of a precise definition. Until recently, the term *dry eye* implied tear volume deficiency, which is associated mainly with Sjögren syndrome.[1] The National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes in 1993 to 1994 sought to provide consensus definitions to assist in clinical trial development and communication and reported a global definition of dry eye: "Dry eye is a disorder of the *tear film* because of tear deficiency or excessive tear evaporation which causes damage to the interpalpebral *ocular surface* and is associated with *symptoms* of ocular discomfort."

The 1994 workshop also provided a classification for dry eyes and descriptions of diagnostic testing procedures.[2] However, the past decade has witnessed the emergence of a new understanding of the inflammatory basis of some forms of dry eye disease that surrounds an alteration of the composition of the tear film. Additionally, new types of dry eyes with a neurogenic component (such as dry eyes that occur after LASIK procedures) recently have been recognized. These new entities would not be included in the 1994 workshop definition for dry eyes.[3–5]

Diagnostic dry eye definitions and protocols still vary widely around the world. For instance, symptoms are not included in the diagnostic criteria of dry eyes in Japan, where only decreased tear secretion and stability and positive ocular surface staining are considered essential for the diagnosis of dry eyes.[6] Yet, evaluation of the presence and nature of symptoms might represent early evidence of ocular surface distress and may be the best way to monitor the effect of any treatment as far as the quality of life of any given patient is concerned.

On that front, the use of a symptom questionnaire might be beneficial because it may allow the grading of symptoms and may be repeatable for comparison purposes before, during, and after any given treatment.[7] Currently, many questionnaires exist, but no single questionnaire is good for all purposes. At this time, there is also no consensus about which symptoms correlate most closely with dry eyes or about which symptom questionnaire or combination thereof should be used in the evaluation of dry eye disease.

Likewise, practices of evaluating the tear stability differ worldwide. Although some clinicians have access to noninvasive diagnostic technologies such as the tear scope and define tear stability with noninvasive techniques, most practitioners use the fluorescein staining of tears and measure the tear film break-up time invasively.[8] Even on that simple front, some clinicians use dye-impregnated strips, and other clinicians prefer one drop of dye delivered through commercially available vials or double vital staining with a mixture of fluorescein and lissamine green or Rose Bengal dyes that are delivered to the conjunctival sac by the aid of micropipettes. Each technique delivers different volumes of dye to the tear film and results in different measured stability values. The recent development of fluorescein break-up time measurement with the dry eye test method may provide a breakthrough that permits accuracy and repeatability by delivering a measured dose of fluorescein into the tear film and may provide a testing method that is practical in the office setting.[9]

In relation to assessment of tear quantity, the Schirmer test still remains the "gold standard" and it is commonly accepted that ≤5 mm of wetting denotes tear deficiency when the test is performed without anesthesia. A comparable diagnostic cut-off value has not been agreed on for the Schirmer test with anesthesia. Of many concerns that could be raised regarding the Schirmer test, an unresolved issue relates to the commonly accepted cut-off value because tear secretion is affected by age. There is still no consensus on age-adjusted cut-off values for Schirmer testing.[8]

Several studies on dry eyes appear in the literature each year with variations in diagnostic methods that are not measuring the same parameter, which makes the evaluation and comparison of results difficult to evaluate.

It is clear that we also need new diagnostic markers of dry eye disease to make clinical diagnosis easier and to

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provide appropriate end points for clinical trials. It is important to understand where to place the new dry eye diagnostic technologies (such as improved tear osmolarity technology[10]) or biomarkers (such as adhesion molecules, inflammatory markers, cytokines, cytokeratins, or aquaporins), which have emerged as important over the past 10 years.[11,12] For instance, flow cytometry processing of conjunctival epithelial impression cytologic specimens has proved useful in the assessment of the ocular surface changes in Sjögren syndrome and may now be useful for large multicenter clinical trials.[13] The DR-1 tear film lipid layer interferometry appears to be a break-through development in the evaluation of tear stability and can assist in the analysis of the changes in thickness and the structure of the tear lipid layer.[14]

Likewise, a new tear stability analyses system that measures tear stability as a function of serial topographic indices that are measured at 1-second intervals during a 10-second period has demonstrated increases in the surface regularity index and the surface asymmetry index with reduced tear film stability in dry eyes of patients with Sjögren syndrome.[15] The focus of these new technologies seems to be shifting toward dynamic assessment of the tear film and dry eye conditions.

Taking into consideration the aforementioned discrepancies and variations in relation to definition and diagnosis of dry eyes and the changes that occurred over the past 10 years, it is time to organize another workshop. As an initial step, 47 recognized cornea and dry eye specialists who were chosen at random from the Association for Research in Vision and Ophthalmology membership database were sent a questionnaire to evaluate their current dry eye diagnostic practices, the details of which are given on the AMERICAN JOURNAL OF OPHTHALMOLOGY online site.

We think that such attempts will help in the evaluation of new diagnostic technologies and markers and in the determination of whether to include them into the realm of methods or tests that are ready for clinical prime time. Future dry eye workshops should also try to undertake the task of issuing briefing statements, an executive summary, a glossary of dry eye terminology, and guidelines for the interpretation of diagnostic data. We not only should examine methods of evaluating dry eye tests for clinical trials with Food and Drug Administration perspectives and guidelines but also make efforts to undertake the task of how to include symptoms in a revised definition of dry eyes and review of the data on existing symptom questionnaires that address the advantages and weaknesses of each instrument. New guidelines in relation to the use of the existing questionnaires for multiple purposes such as for case identification, the diagnosis of dry eye, and a description of change over time with treatment of dry eyes might provide invaluable assistance for future dry eye studies.

We anticipate that such efforts eventually will ensure that most dry eye practitioners and researchers are referring to well-described standards in relation to definition and diagnostics of dry eyes. Such efforts should be carried out more frequently and will pave the way to a better understanding of the pathogenesis of dry eyes and a better evaluation of specific treatment responses or at least to clarify the areas in which further prospective trials should be conducted.

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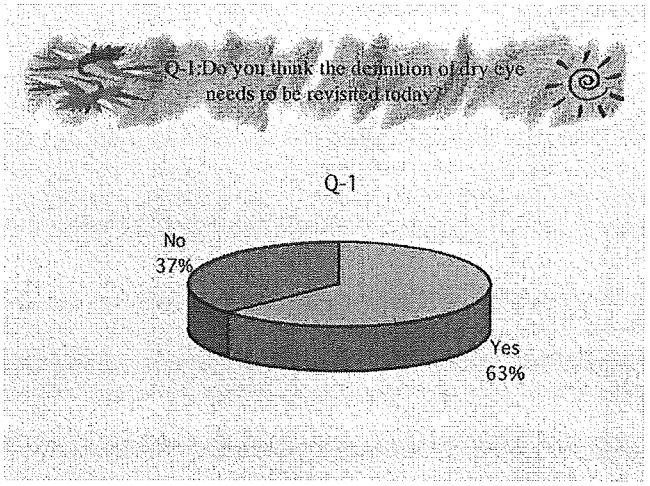

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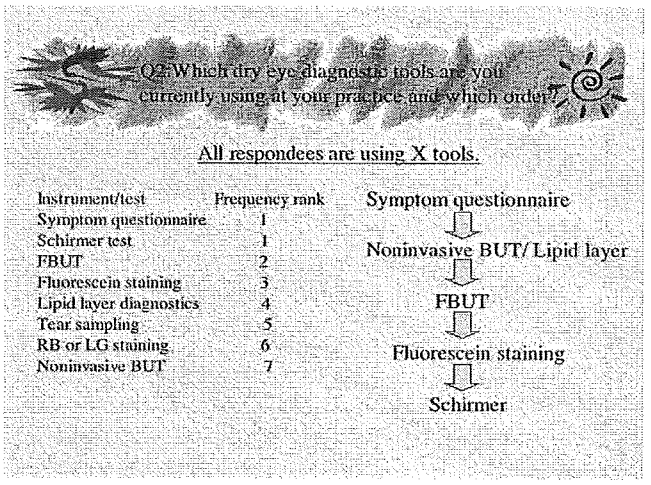

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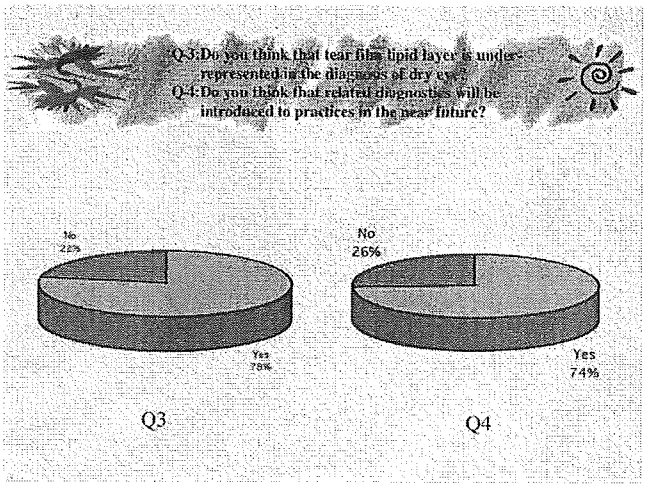

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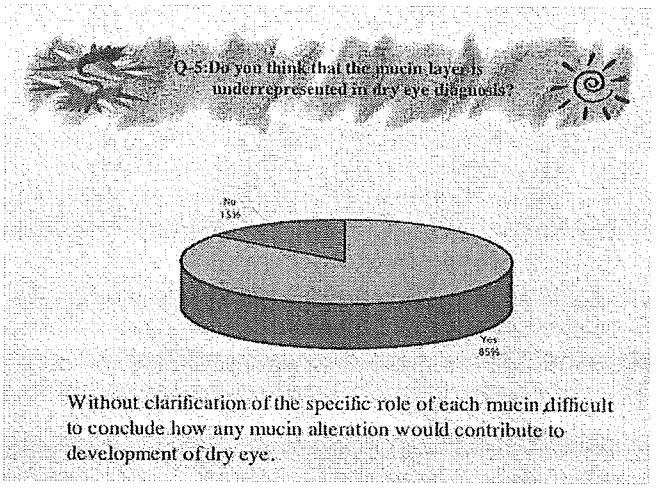

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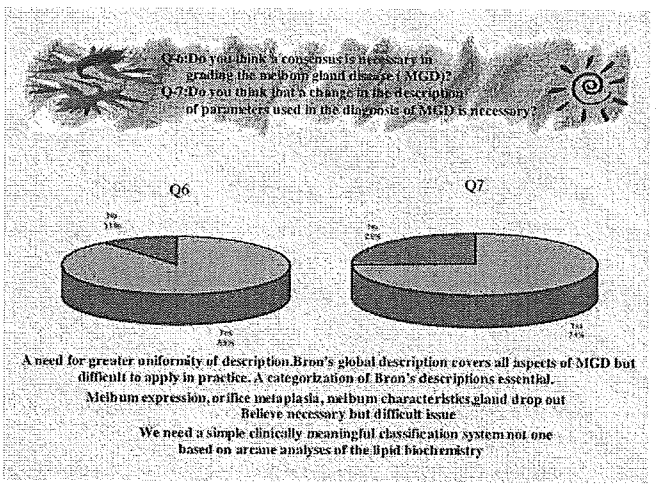

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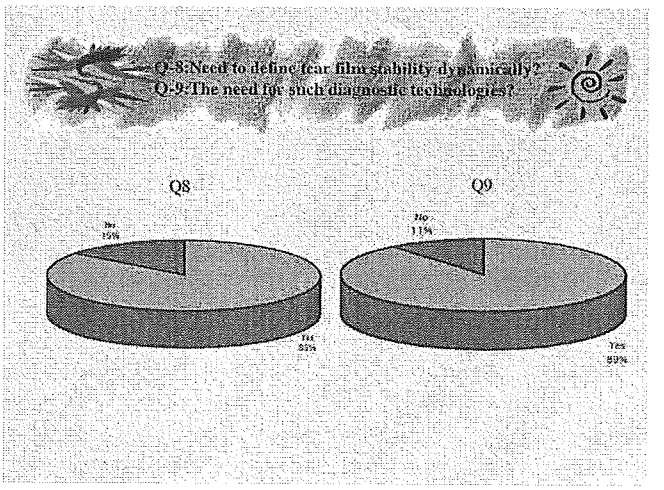

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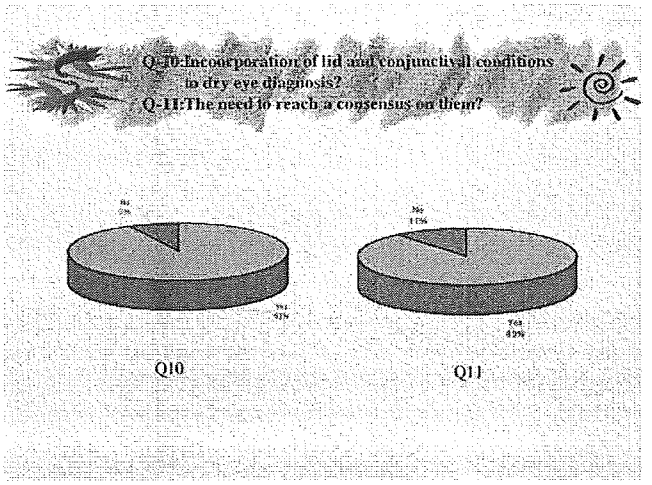

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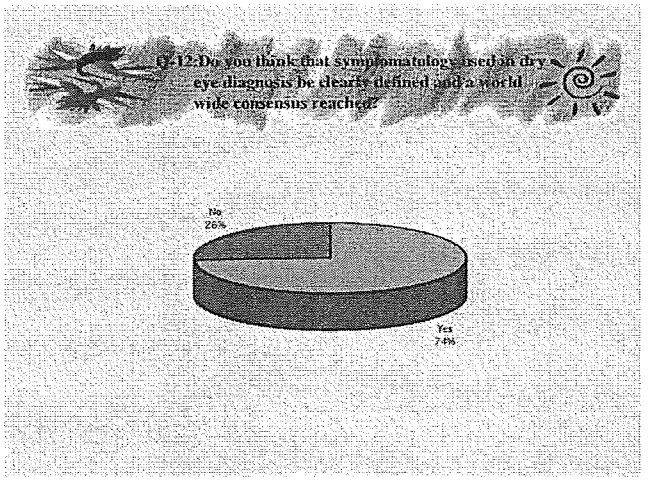

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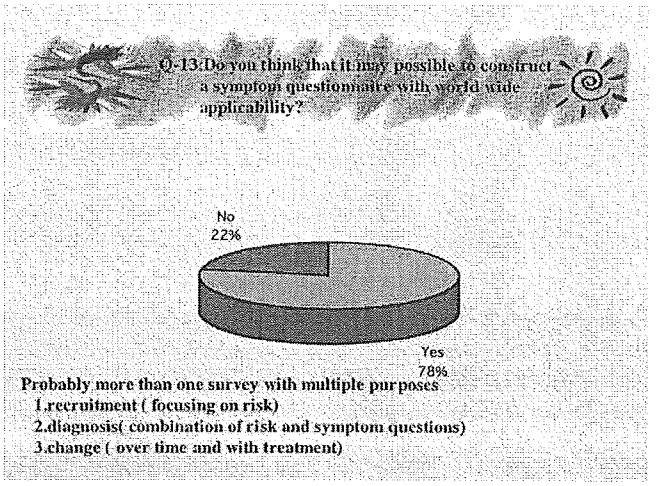

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Current Concepts in Ocular Surface Reconstruction

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ABSTRACT Diseases that affect the limbal stem cells are multifactorial and present with different stages of severity. The most important features to be considered in evaluating these patients include the degree of limbal stem cell loss, the extent of conjunctival disease, and the presence and etiology of ocular surface inflammation. Other important factors are tear film and eyelid abnormalities, keratinization of the ocular surface, laterality of the disease process, health and age of the patient. Careful consideration of all of these factors help tremendously in tailoring the most suitable method of treatment for each patient. The management of severe ocular surface disease has benefited from numerous advances in recent years. At one time, available techniques for visual rehabilitation consisted of superficial keratectomy, use of artificial tears, tarsorraphy as well as lamellar and penetrating keratoplasty. A lamellar or penetrating keratoplasty procedure resulted in a stable surface only for as long as the donor epithelium was present and once the epithelium sloughed off, the ocular surface failed due to conjunctivalization. The last few decades enjoyed the development and, especially, progress of new ocular surface reconstruction techniques such as amniotic membrane transplantation, limbal stem cell transplant procedures, transplantation of cultivated oral mucosal or limbal stem cell sheets. This review will briefly focus on the indications and methodology of each procedure and the currently available clinical data on the results of these procedures.

KEYWORDS ocular surface, limbal stem cell, keratoplasty, limbal transplantation, amniotic membrane

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Figures 8–13: Courtesy of Dr. Shigeto Shimmura, Tokyo Dental College Ichikawa General Hospital, Japan.

INTRODUCTION

Ocular surface reconstruction has recently become common terminology among corneal surgeons in the management of severe ocular surface disease, which is mostly refractory to conventional medical therapeutic modalities. The challenge in the field was especially initiated by the necessity to find a cure for patients with Stevens-Johnson syndrome (SJS), Ocular Cicatricial Pemphigoid (OCP), and chemical or thermal burns. The common problem shared by such patients is the depletion of the corneal epithelial stem cells, which are thought to be located in the limbal area surrounding the cornea, called Palisades of Vogt.[1] There is no doubt that standard penetrating keratoplasty (PKP) is a contraindication in surgical treatment of such patients. The transplanted corneal button

is inevitably replaced by invading vascularized tissue, further complicated by immunologic rejection and secondary glaucoma. PKP and lamellar keratoplasty in these patients have recently regained their status as a surgical tool, only in conjunction with limbal transplantation. Limbal transplantation, first described as an autologous procedure from the healthy fellow eye by Kenyon et al.,[2] is a means to restore corneal epithelial stem cells and the ocular surface in such diseased eyes. Keratoplasty is performed in conjunction with limbal transplantation when the underlying stroma is also diseased, since limbal transplantation can only restore the epithelium.

Ocular surface reconstruction in broad terms also refers to epithelium and stroma stabilizing procedures such as amniotic membrane transplantation, provision of sources of corneal epithelialization through transplantation of cultured corneal epithelial cells and reconstruction of the lid-ocular surface interface when necessary. Reconstructive efforts certainly include the fight against common problems in these patients, such as dry eyes through the use of artificial tears or punctal occlusion. This review will mainly focus on limbal and amniotic membrane transplantation procedures and current recommendations in ocular surface reconstruction.

Limbal Transplantation

Evolution of Keratolimbal Allograft

Keratolimbal allograft (KLAL) is a technique in which allogeneic limbal stem cells are transplanted to a recipient eye with severe ocular surface disease using the peripheral cornea as a carrier.[3,4] The technique has evolved considerably in recent years with changes in terminology as well (Table 1).

TABLE 1 **Classification for Epithelial Transplantation Procedures for Severe Ocular Surface Disease**

Procedure	Donor	Transplanted tissue
Limbal transplantation		
CLAU	Fellow eye	Limbus/conjunctiva
c-CLAL	Cadaveric whole globe	Limbus/conjunctiva
lr-CLAL	Living relative	Limbus/conjunctiva
KLAL	Cadaveric stored tissue	Limbus/cornea

CLAU—autologous conjunctival limbal transplantation.
c-CLAL—cadaveric conjunctival limbal allograft.
lr-CLAL—living-related conjunctival limbal allograft.
KLAL—keratolimbal allograft.

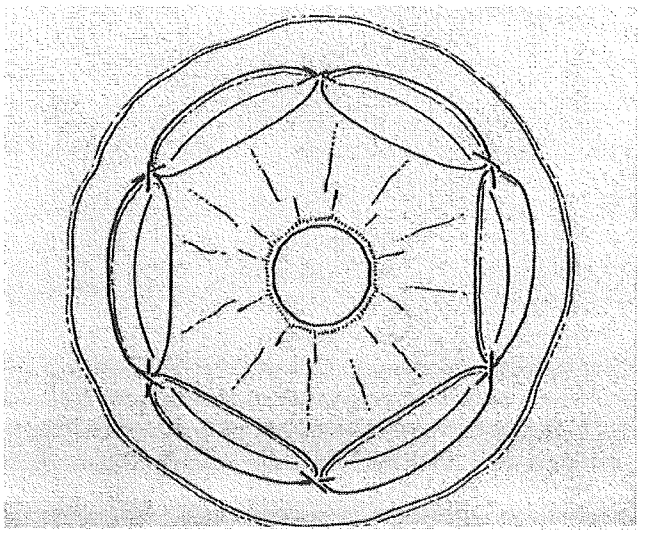

FIGURE 1 **Schematic representation of the keratoepithelioplasty procedure.**

Keratoepithelioplasty (KEP)

Thoft described a technique called keratoepithelioplasty (KEP) in 1984. This technique was the first allograft procedure for the management of severe ocular surface disease.[5] "Lenticules" of peripheral cornea were harvested from a cadaveric whole globe, placed evenly around the corneoscleral limbus, and sutured to the sclera (Figure 1). At that time Thoft did not refer to limbal transplantation in conjunction with his KEP procedure, since stem cell theory was not well known then, but it was possible that stem cells had been harvested along with the peripheral corneal lenticules. The idea behind keratoepithelioplasty was to provide epthelialization in cornea and prevent invasion and vascularization by the conjunctiva. Suggested indications for KEP are summarized in Table 2. Any vascular and fibrotic tissue covering the surface of the cornea should be excised during the procedure and corneal lenticules are sutured with 10/0 monofilament nylon sutures. Figures 2A–C summarize the application of this procedure in a patient with conjunctival intraepithelial neoplasia (Figures courtesy of Dr. Chie Sotozono, Kyoto Prefectural University, Dept. Ophthalmology, Kyoto, Japan).

In 1990, Thoft and co-workers modified the original KEP procedure to include limbal tissue with the peripheral cornea and thus described the first true KLAL.[6] Tsai and Tseng described a modification of Thoft's KEP technique in 1994.[7] A continuous annular ring of limbal tissue was harvested from a whole globe utilizing a suction trephine. The resultant keratolimbal ring was

TABLE 2 Indications for KEP

1. Persistent epithelial defects
2. Corneal epithelial stem cell failure/deficiency
   *Chemical and thermal burns
   *Toxic epitheliopathies
   *Congenital aniridia
   *SJS
   *OCP
3. Peripheral corneal ulcerations
   *Mooren ulcer
   *Rheumatoid arthritis, ulcerative colitis
4. Limbal tumors
   *Conjunctival intraepithelial neoplasia (CIN)
   *Squamous cell tumors
5. Corneal dystrophy
6. Recurrent pterygium

then subdivided and transferred to the recipient eye (Figure 3).

In 1995, Tsubota and co-workers reported the use of stored corneoscleral rims for limbal stem cell transplantation.[8] Storage afforded patients several days to coordinate surgery after acquisition of suitable donor tissue. Later on, Holland and Schwartz modified Tsubota's technique by using the stem cells from two stored corneoscleral rims instead of one. In this way, the potential number of transplanted limbal stem cells was doubled.[4,9] Another advantage of the latter procedure was that a continuous ring of keratolimbal crescents was placed around the recipient limbus, acting as a barrier for potentially invading conjunctival tissue. In 1996, Sundmacher, Reinhard, and co-workers presented their results of an alternative procedure, which they termed homologous penetrating central limbokeratoplasty.[10,11] In this procedure, a stored corneoscleral rim intentionally trephined off-center to create a 7.7–10.0 mm penetrating keratoplasty button. The donor graft is created in such a way that approximately 30–40% of the circumference of the graft contains limbal tissue. Patients undergoing this procedure might benefit from receiving a clear penetrating keratoplasty graft along with limbal stem cells in a single operation.

Indications of Keratolimbal Allograft Procedure

KLAL surgery is performed in order to treat severe bilateral ocular surface disorders secondary to limbal stem cell deficiency. It is also a surgical alternative for patients with unilateral disease who fear damage to the healthy fellow eye if used as a source of limbal stem cells. KLAL surgery may be the only choice for obtaining allogeneic tissue if there is no available or willing living relative. A KLAL procedure is ideally suited for disease entities that primarily affect the limbus with minimal or no involvement of the conjunctiva. Aniridia exemplifies the disease process that is probably best suited for KLAL.[12] For similar reasons, KLAL is the optimal procedure in most cases of iatrogenic limbal stem cell deficiency.[13] Most cases of iatrogenic deficiency, whether they

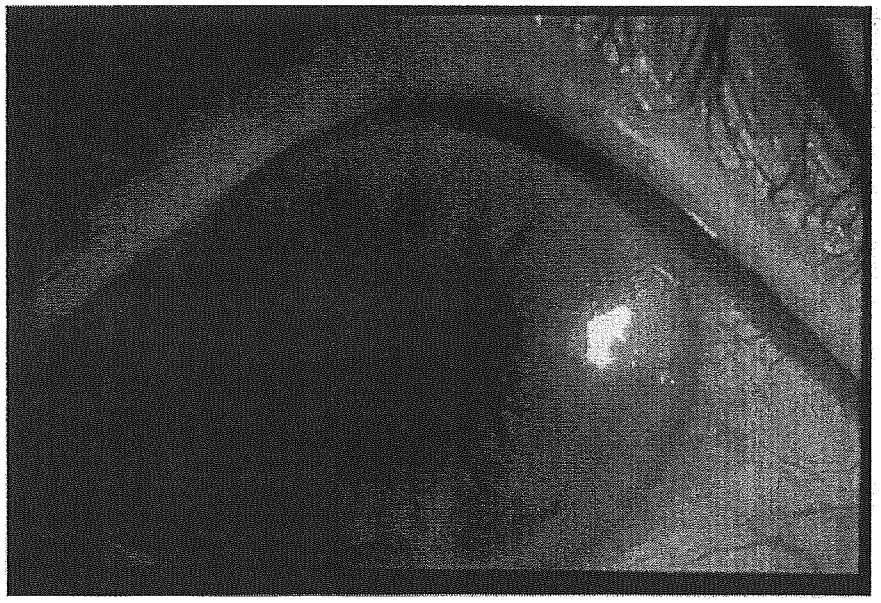

FIGURE 2A Anterior segment photograph of a patient with CIN.

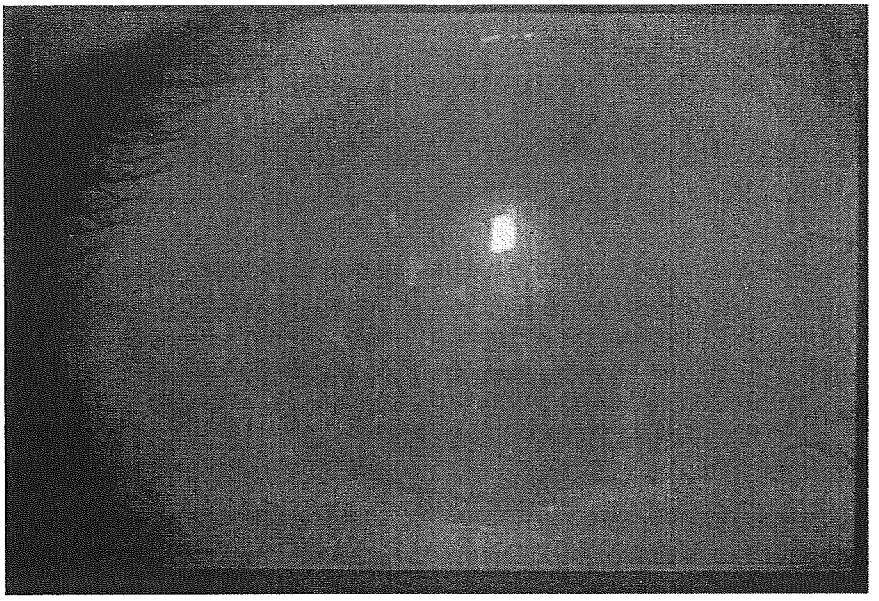

FIGURE 2B Fluorescein staining shows the corneal invasion by the dysplastic epithelium.

involve only sectoral or total limbal involvement, typically have reasonable normal conjunctiva. Patients with total limbal deficiency will require a 360-degree KLAL, while those with sectoral limbal deficiency may require only sectoral KLAL. Keratolimbal allograft may also be beneficial for patients with limbal stem cell deficiency with mild to moderate conjunctival involvement. Patients with chemical injuries may benefit from this procedure. Patients with mild SJS or OCP may also benefit from KLAL, and the chances of graft survival are highest if the inflammation can be controlled prior to surgery.

The success rate with KLAL decreases with increasing conjunctival inflammation. The most severe forms of ocular surface disease involve total limbal stem cell deficiency with active conjunctival inflammation (e.g., severe SJS, OCP, and recent chemical injuries).[13] In these cases, total limbal stem cell deficiency is complicated by conjunctival inflammation and scarring, decreased mucin and aqueous tear deficiency, and the potential

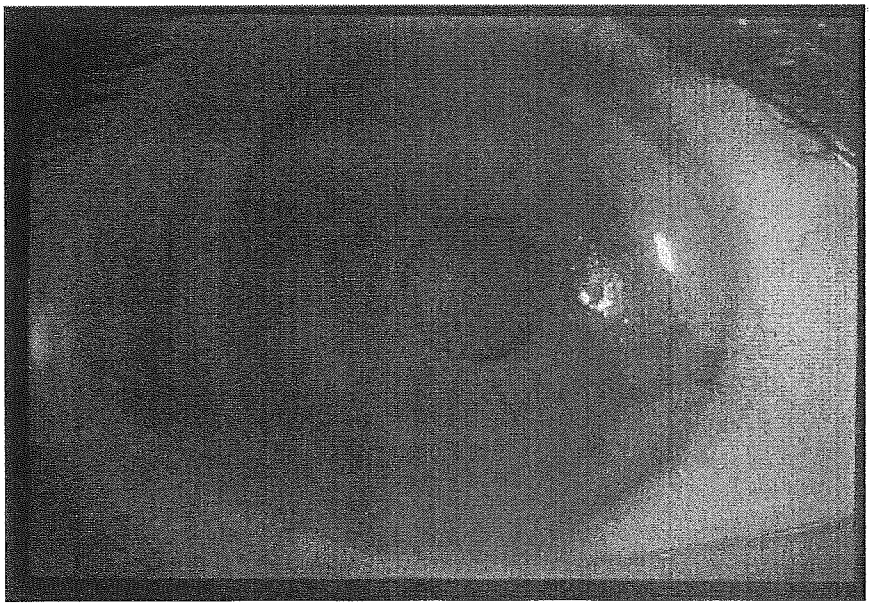

FIGURE 2C Anterior segment photograph of the ocular surface after KEP.

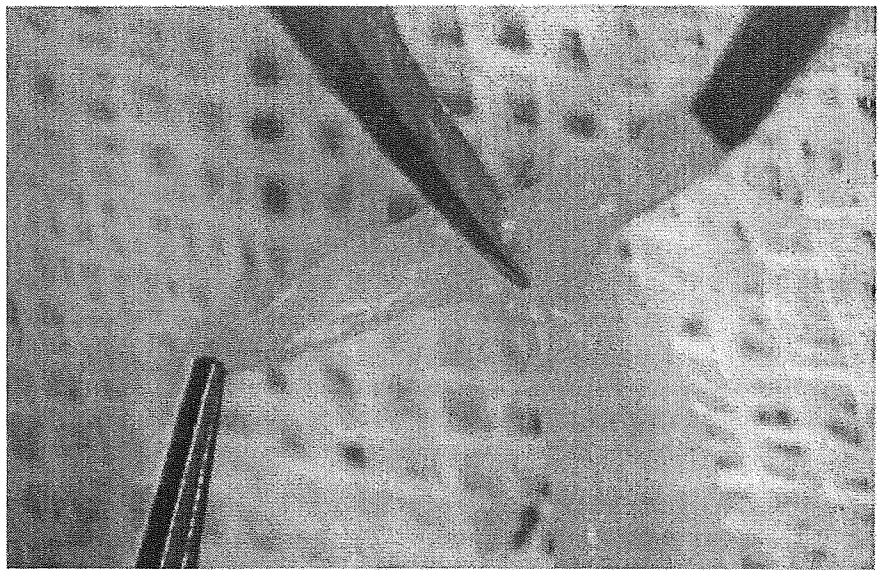

FIGURE 3 **Dissection and preparation of limbal tissue for KLAL.**

for keratinization of the ocular surface. Because KLAL does not provide healthy conjunctiva, one might consider living-related conjunctival limbal allograft if a living donor is available (Table 1).

Preoperative Considerations

One major threat to the success of any ocular surface reconstruction procedure, including KLAL, is the lack of a healthy and stable tear film.[14] If a proper tear film layer is not present, it must be restored by correcting lid abnormalities, neurotrophic exposure, and severe aqueous tear deficiency. This is especially true for those with decreased reflex tearing. Eyelid abnormalities, such as lagophthalmos, misdirected lashes, and malpositioned or keratinized lid margins, should be reconstructed either prior to, or at the conclusion of, KLAL. Great care must be taken when performing KLAL in patients with abnormal or absent blink, since persistent epithelial defects may develop with risk of subsequent scarring and infection.

Severe aqueous tear deficiency with decreased reflex tearing is another relative contraindication to KLAL because these patients lack essential tear components such as vitamin A and epidermal growth factor (EGF).[15–18] If KLAL is undertaken in such patients, successful surface rehabilitation will be maximized if autologous serum is applied regularly following KLAL. The success of KLAL is decreased in eyes with significant keratinization of the ocular surface, as well.[19] It has been recognized that conjunctival epithelium is derived from a stem cell population[20] that is separate from that of the limbus.[21] Therefore, KLAL alone is not sufficient to correct eyes with diffuse keratinization caused by concomitant loss of both epithelial stem cell populations. It remains to be determined if simultaneous transplantation of both types of epithelial stem cells may ameliorate this difficult situation. When uncontrolled, severe inflammation is another poor prognostic factor for KLAL. For instance, severe inflammation limits the success in acute chemical burns in humans undergoing conjunctival limbal autograft.[2,22] Although the exact mechanism remains unclear, inflammatory cytokines such as interferon gamma can upregulate Fas or HLA class II antigen and encourage the epithelium to undergo apoptosis in acute chemical burns.[23] Upregulation of HLA class II antigens in the context of inflammation may augment immune sensitivation leading to allograft rejection. These data support the notion that the success of keratolimbal allograft is hampered by uncontrolled inflammation and that suppression of inflammation is an important strategy for improving the outcomes. The fact that suppression of inflammation is beneficial following KLAL is supported by the favorable results observed when amniotic membrane transplantation (AMT) is used in conjunction with KLAL in inflamed eyes.[24] AMT has been shown to suppress inflammation, facilitate epithelialization, and prevent cicatricial complications in acute chemical and thermal burns and will be discussed later in the chapter.

Surgical Techniques

The purpose of performing KLAL is to provide healthy limbal stem cells to the recipient host limbus. Stem cells lie in a narrow, fragile portion of the limbus and must be delivered attached to a more robust carrier tissue. The use of a peripheral corneoscleral tissue allows for safe transfer and secures attachment of the stem cells to the recipient limbus.

Corneoscleral Ring Technique of Tsubota

In this technique, tissue from one donor corneoscleral rim is used instead of two (Figure 4). The donor tissue is prepared from the conventionally processed corneoscleral button obtained from the eye bank. Theoretically, the potency and capability of the limbal epithelial stem cells should be greater in a younger donor. However, there are reports suggesting that the stem cell reserve may extend to much older donors.[25] In our practice, the mean storage time after enucleation is six days and a preliminary study showed that the limbal epithelial stem cells remained viable within such a period of storage time.[26] It is of interest to note that inflammatory cells, such as Langerhans cells, become depleted after several days' storage, suggesting that a theoretical advantage of the longer storage period may, in fact, be beneficial.

The corneal buttons are preserved in Optisol GS^{TM} (Chiron Technolas, Irvine, CA) as per routine eye bank procedure. It remains to be determined whether this storage medium is ideal for preserving corneal

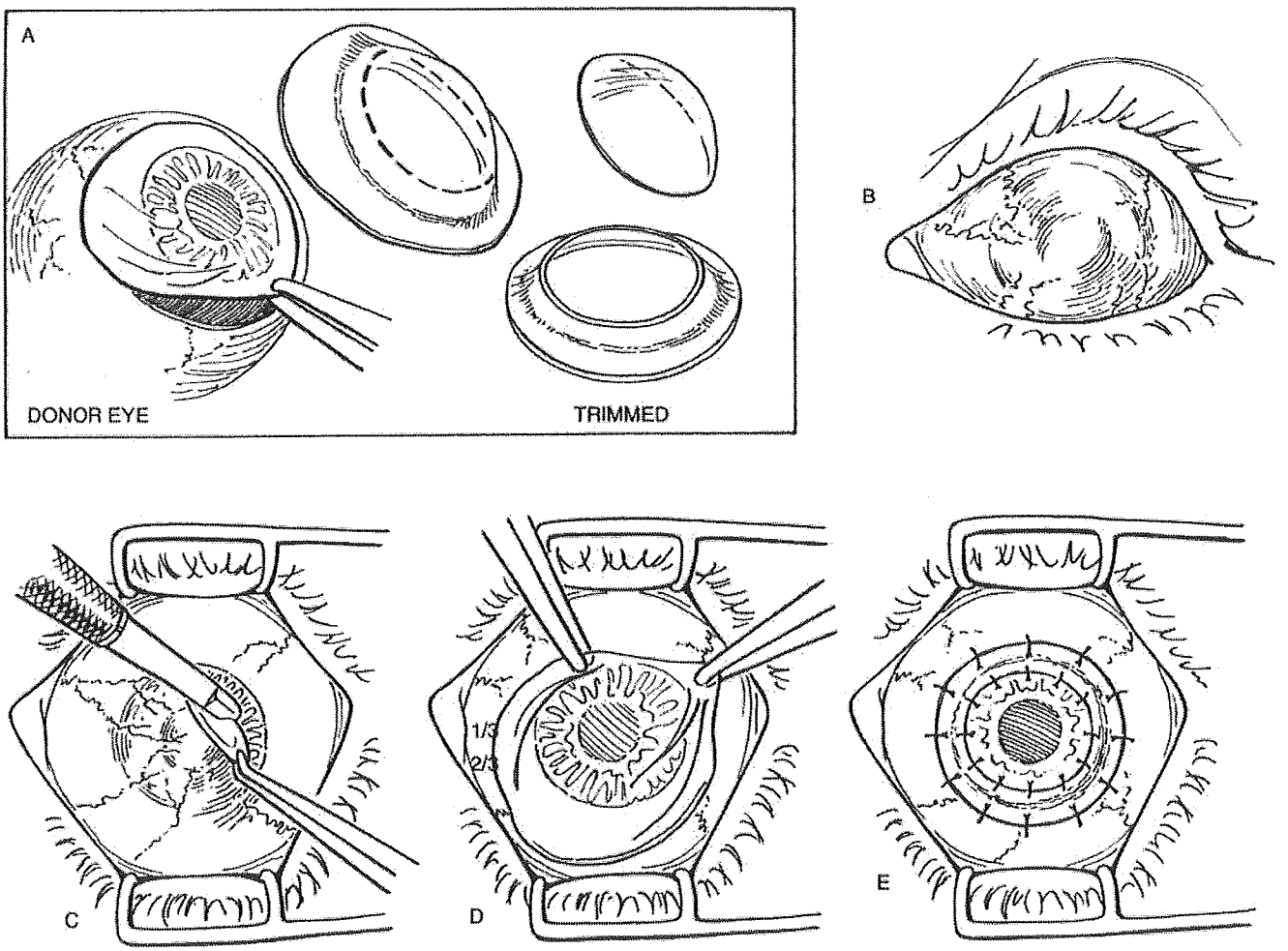

FIGURE 4 KLAL procedure (Tsubota's Corneoscleral Ring method). A: Corneascleral rim is harvested. During surgery, central cornea is removed with a trephine, creating a central button for penetrating or lamellar keratoplasty and a corneascleral ring for the stem cell transplant. B: Removal of the abnormal epithelium over the recipient ocular surface. C:360^0 peritomy. D: Amniotic membrane patch sutured to sclera. E: Suturation of the ring-shaped limbal tissue to the peripheral cornea and limbus. A penetrating or lamellar keratoplasty may be performed.

epithelial stem cells. The central cornea of the corneoscleral rim is excised with a 7.5 mm trephine. An Iowa cutting press is used, and the tissue is placed epithelial-side-down in the standard fashion used for cutting a corneal button for routine keratoplasty. Scissors are used to dissect the excess peripheral scleral tissue, leaving approximately 1 mm of sclera peripheral to the limbus. The posterior one-half to two-thirds of the ring is then removed by lamellar dissection using a sharp, rounded, steel crescent blade. These steps are performed under the operating microscope and usually require the aid of an assistant. Unnecessary tissue must be removed as much as possible leaving thin, ring-shaped limbal tissue containing corneal epithelial stem cells. In the past, we saved the central corneal button to perform PKP simultaneously with KLAL. We now know that simultaneous PKP with KLAL is associated with a high rejection rate of the corneal graft despite the use of systemic cyclosporine and suggest PKP to be performed after KLAL.[24] Holland et al.[12] advocate performing a PKP no sooner than 3 to 4 months following KLAL, and we try to adhere to this rule if tissue is available.

Preparation of the Recipient Eye

Superficial keratectomy and 360-degree limbal peritomy are initially performed. Whenever possible with inflamed eyes, AMT is performed prior to placement of the KLAL ring. Amniotic membrane has been used as a substrate in restoring the ocular surface when the underlying stroma tissue has been destroyed.[27–29] We noted that AMT, either before or during KLAL, might also facilitate epithelialization and reduce inflammation and scarring. During limbal transplantation, amniotic membrane is dissected from the chorion and placed on the ocular surface with the epithelial side facing outward. The membrane is then secured to the eye with eight 9-0 silk sutures. The ocular surface is covered as much as possible, with exception of the palpebral conjunctiva.

Placement of the Donor Tissue

The ring-shaped limbal tissue is sutured to the recipient limbal areas with interrupted sutures. Four 10-0 nylon sutures are routinely used to secure the central corneal portion. Nine-zero silk for 8-0 Vicryl sutures are used to suture the scleral portion of the limbal graft to the scleral bed. The host conjunctiva is not sutured, but is allowed to adhere to the posterior aspect of the graft of its own accord. At the conclusion of this procedure, a penetrating or lamellar keratoplasty may be performed using tissue from the same corneoscleral button. Figures 5A–E show preparation of recipient bed, amniotic membrane and limbal transplantation and PKP performed four months afterward in a SJS patient.

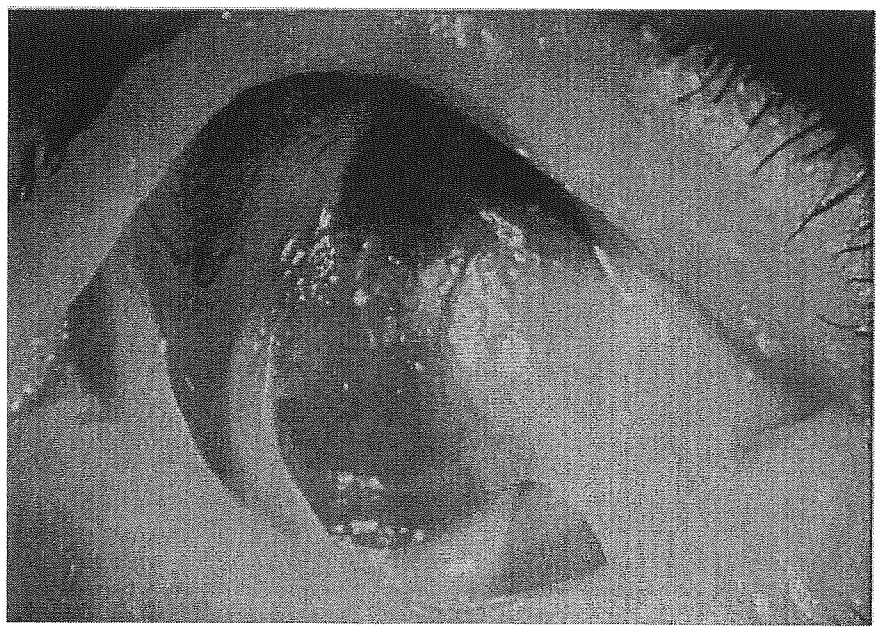

FIGURE 5A Conjunctivalized ocular surface and symblepharon in a SJS patient.

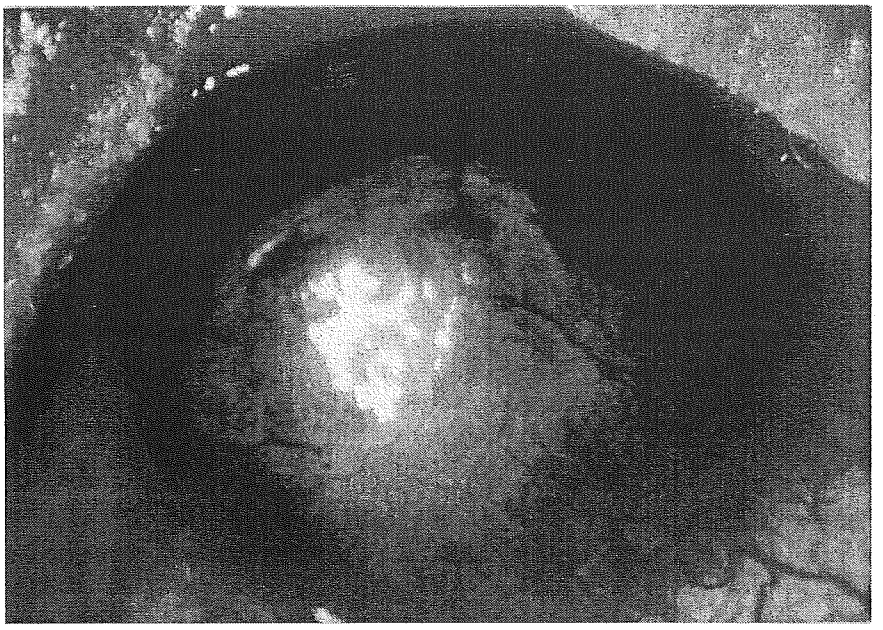

FIGURE 5B Excision of fibrotic tissues.

Postoperative Care

The key to successful KLAL lies in the postoperative care. Because limbal stem cells are more antigenically active than are central corneal stromal and endothelial cells, the risk of postoperative failure secondary to rejection is much more likely following KLAL than it is following PKP. In fact, all patients undergoing KLAL benefit from systemic immunosuppression consisting of corticosteroid, azathioprine and cyclosporine in order to decrease the risk of graft failure secondary to rejection. The immunosuppression protocol that we use in our patients is summarized in Table 3. It should be remembered that frequent checks of renal and hepatic functions as well as blood pressure are essential during immunosuppression with steroids and cyclosporin.

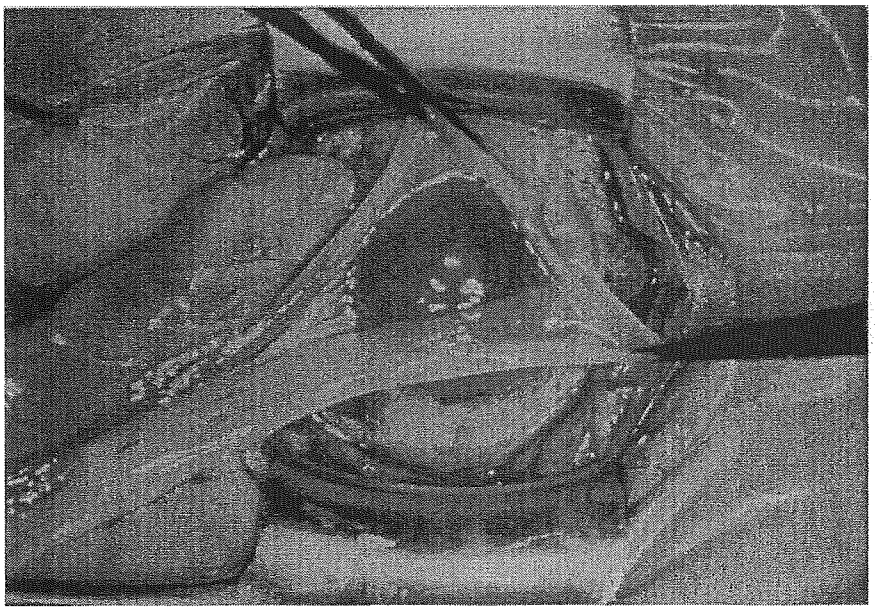

FIGURE 5C Preparation of amniotic membranes.

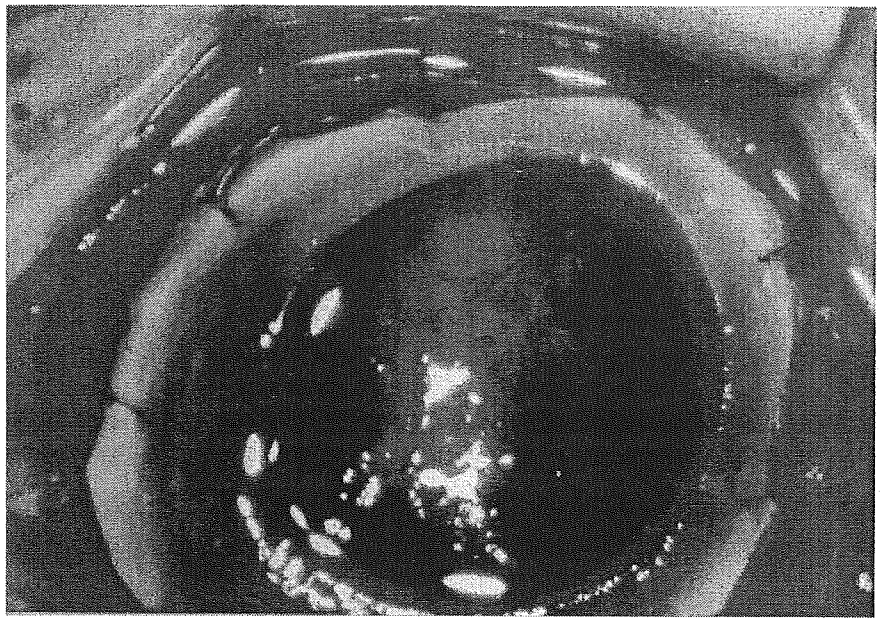

FIGURE 5D AM and limbal transplantation.

In evaluating the success of KLAL, it is important to consider the length of follow-up. When examining a patient with a healthy-appearing ocular surface 3–4 months after PK, it may very well be that one is observing the transplanted adult epithelial cells. In these cases, it may be a year or more before the limbal stem cells are called upon to repopulate the ocular surface. Thus, one cannot pass judgment on the success of KLAL until the patient is at least one year after limbal transplant surgery.

Amniotic Membrane Transplantation

In the English-language literature, De Rotth first used a live fetal membrane including both amnion and chorion in 1940 as a graft for conjunctival surface reconstruction.[30] In 1995, Kim and Tseng[27] reintroduced amniotic membrane (AM) for ophthalmic uses. In a rabbit model they showed that 40% of corneas with total limbal deficiency could be reconstructed by

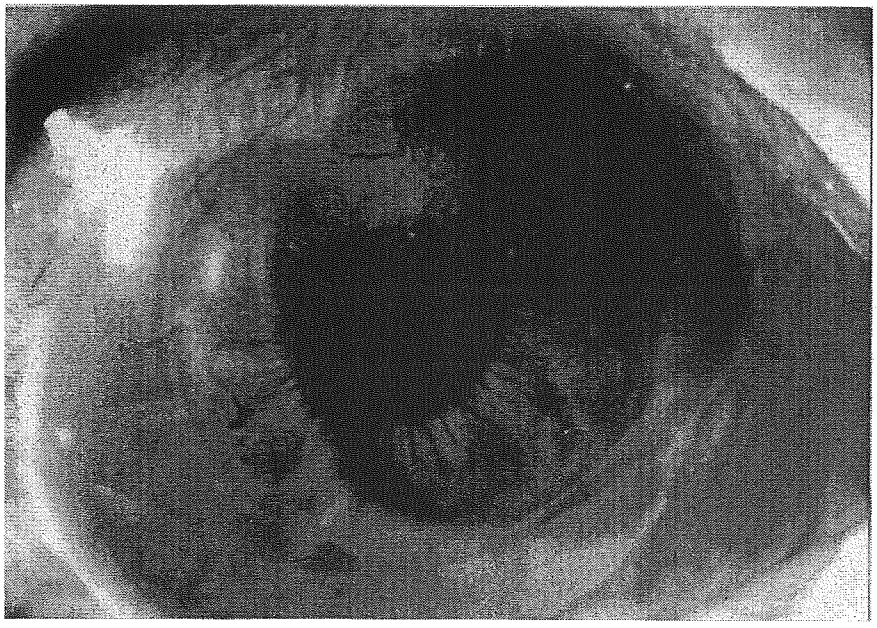

FIGURE 5E PKP performed 4 months later.

TABLE 3 Immunosuppression After KLAL

Patients below 70 years of age
*i.v. betamethasone 8 mg/day the day after surgery for 4 days and oral tapering afterward
*i.v. cyclosporin A 3 mg/kg for 1 week, orally afterward trying to keep a serum trough level at 100 ng/mL for 2–3 weeks, initiation of tapering with discharge
Patients above 70 years of age
*i.v. betamethasone 4 mg/day the day after surgery for 4 days and oral tapering afterward
*i.v. cyclosporin A 2 mg/kg for 1 week, orally afterward

TABLE 4 Action Mechanisms and Observed Effects of Amniotic Membrane Transplantation

Action Mechanisms
• Prolong life span and maintain clonogenicity of epithelial progenitor cells
• Promote non-goblet cell epithelial differentiation
• Promote goblet cell differentiation when combined with conjunctival fibroblasts
• Exclude inflammatory cells with anti-protease activities
• Suppress TGF-β signalizing system and myofibroblast differentiation or normal fibroblasts
Observed Clinical Effects
• Facilitate epithelialization
• Maintain normal epithelial phenotype
• Reduce inflammation
• Reduce vascularization
• Reduce scarring

replacing the conjunctivalized surface with a preserved human AM.

The AM constitutes the inner wall of the fetal membranes, and consists of a single layer of epithelium with an underlying stroma rich in extracellular matrix providing heparin sulfate proteoglycans, laminin, and collagens, especially collagen 4 and 7, which are important for basement membrane integrity. AM has proven to be an integral part of ocular surface reconstruction, acting as a replacement for damaged basement membrane, and as a patch offering temporary protection of the ocular surface.

Several mechanisms are believed to be involved in the anti-inflammatory effects of AMT. Pathology of AM collected from patients often reveals inflammatory cells trapped within the stroma of AM tissue.[31] AM also contains various growth factors such as EGF, TGF alpha, KGF, HGF, bFGF, and TGF-beta.[32] However, since these growth factors are expressed by AM epithelium, it is unknown whether they play a key role in clinical situations using preserved AM. Prevention of scarring is another feature of AMT, which may be used to prevent symblepharon formation in cicatricial disease.[33,34] This can be explained by the direct interaction of AM on corneal fibroblasts by down-regulating the expression of TGF-beta receptors in these cells, inhibition of myofibroblastic differentiation, reducing unwanted keratocyte apoptosis and synthesis of new extracellular matrix components.[35]

Action mechanisms and observed effects of amniotic membrane transplantation are summarized in Table 4.[36–40] The AM is usually used following cryopreservation, but fresh AM seems to work as well.[41] AM can easily be obtained from seronegative mothers undergoing routine Cesarean sections. Recently, amniotic membrane biotissue grafts procured during cesarean sections have become commercially available as shown in Figure 6 (Bio-Tissue, Miami, USA). The low cost associated with procuring fresh tissue has made AMT a popular procedure. For transplantation, AM acts as a basement membrane allowing the migration of epithelial cells over areas of bare sclera, and can avert impending perforation of the cornea. A convincing report by Chen et al.[42] shows the efficacy of AMT as a substrate in the treatment of neurotrophic ulcers of the cornea where 70% of patients in his series healed by AMT after a mean follow-up of 18.8 months. The success of AMT in neurotrophic ulcers leads one to speculate that humoral factors of AM origin may also be involved in the healing process. Figures 7A–C show successful AMT in a patient with herpetic neurotrophic ulcer.

Segments of AM can also be used as a filling in localized stromal deficiencies, even when accompanied by perforation.[43,44] Small segments of AM in such cases are stuffed under an overlying layer of AM that acts as a basement membrane. This procedure can also be done with the use of surgical adhesion glue.[45] The possibility of patching a perforation with AM can save the eye in many ways. Institutions without the immediate availability of donor tissue can buy time before performing a therapeutic keratoplasty.[33] AMT can also circumvent a primary therapeutic keratoplasty. Surgical indications for AMT are presented in Table 5.

Preparation of Preserved Membranes and Surgical Procedures

Human placentas are obtained under sterile conditions from planned, uneventful Caesarean sections

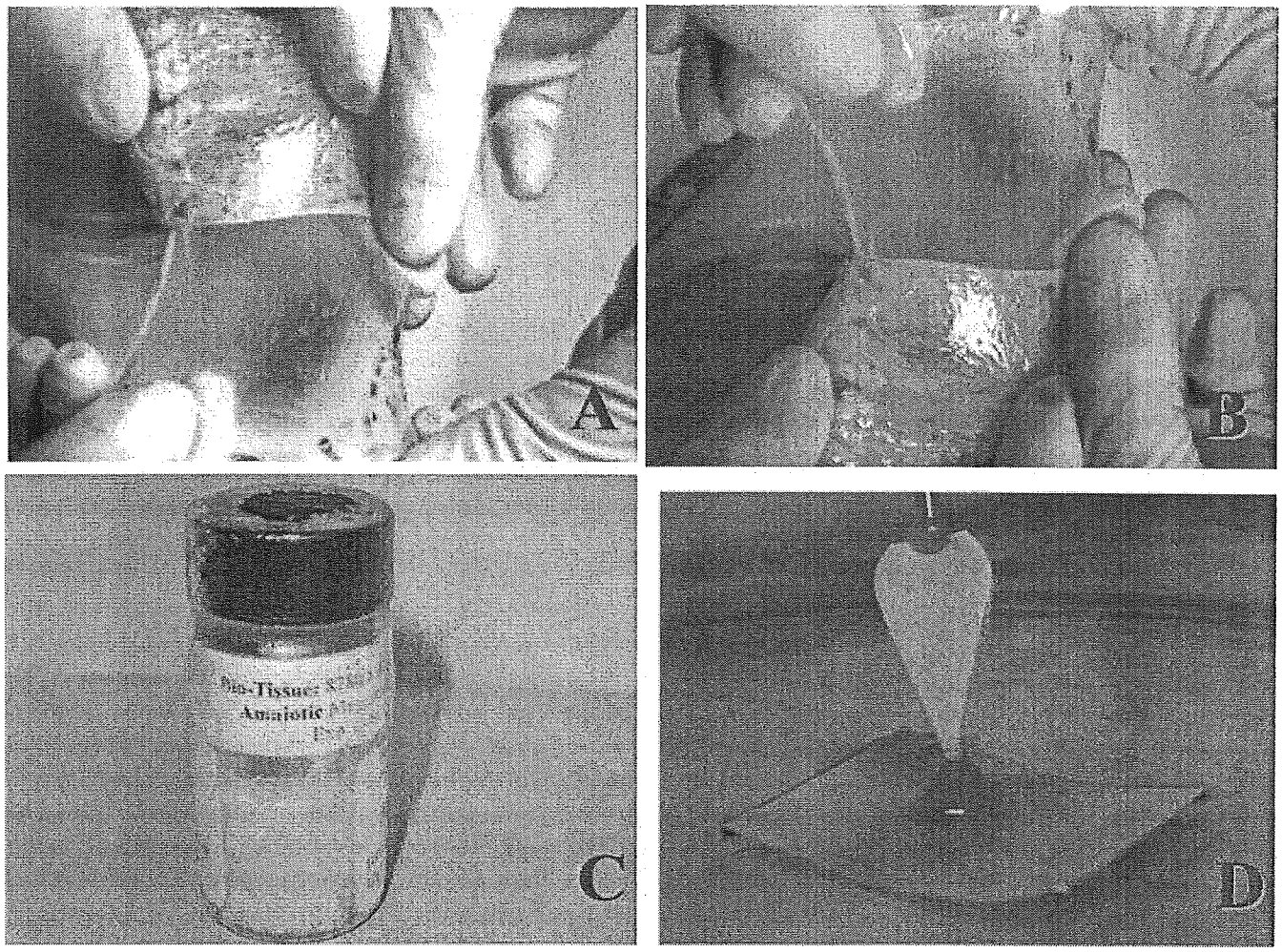

FIGURE 6 **(A–B) Procurement of AM, separation of amnion from chorion. (C) Preparation of frozen vial stored Bio-Tissue amniograft. (D) Thawing the amniograft and checking the stromal side with merocel sponge.**

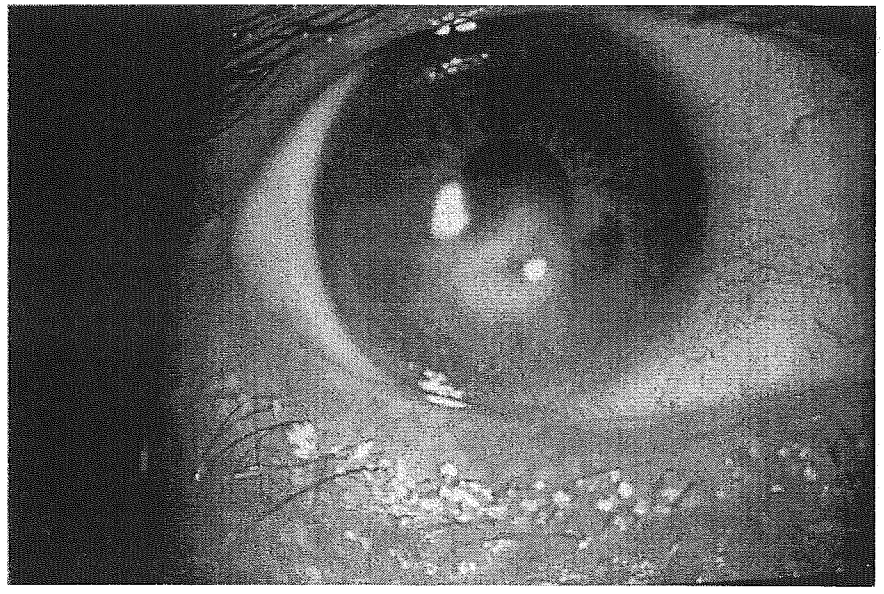

FIGURE 7A **Ocular surface in a patient with herpetic neurotrophic corneal ulcer.**

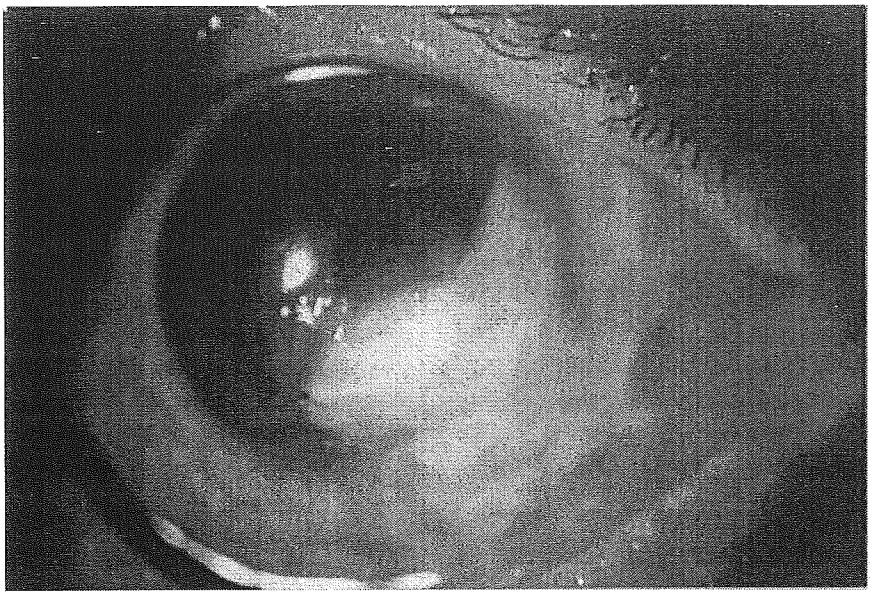

FIGURE 7B AMT procedure in the same patient(filling and partial patch).

performed because of anatomic considerations. A written consent should be obtained from the mothers to harvest the amniotic membrane. Maternal blood should be screened for antibodies against syphilis, human immunodeficiency virus, and hepatitis virus types B and C. The placentas are then washed free of blood clots with balanced saline solution containing 50 μg/ml of penicillin, 50 μg/ml of streptomycin, 100 μg/ml of neomycin, and 2.5 μg/ml of amphotericin B. The AM is separated from the rest of the chorion by blunt dissection. The membranes are flattened and sutured with the epithelium surface up onto nitrocellulose filter papers. The membrane with the paper is placed in sterile vials containing Optisol-GS (Bausch & Lomb, CA, USA) and glycerol at a ratio of 1:1 (volume/volume). Some other washing protocols use dimethylsulfoxide and phosphate buffered saline solutions. The membranes should not be released for use after a second serologic testing for HIV and hepatitis viruses from the donor, performed six months after donation, proved negative. The membranes should be defrosted by warming the vials to room temperature for 10 min

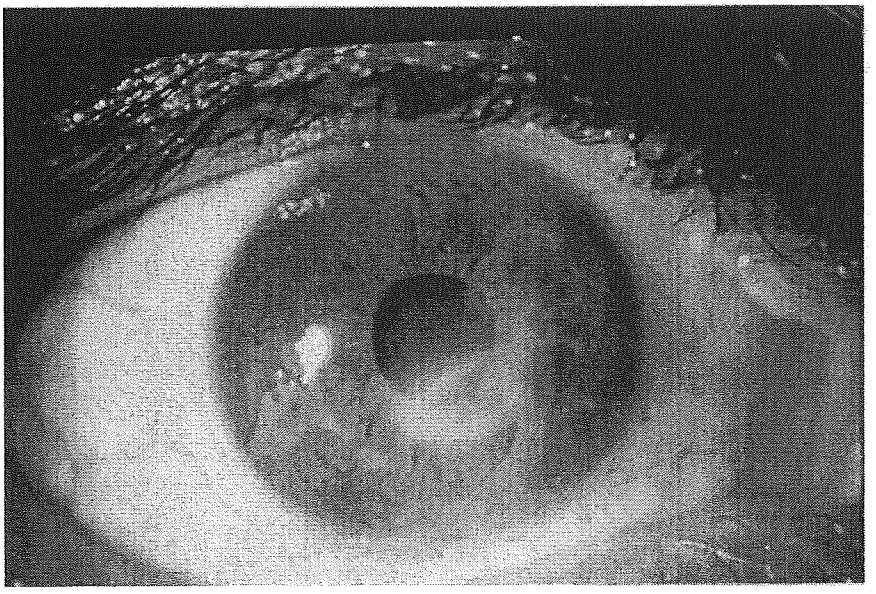

FIGURE 7C Note the wound healing with stromal gain 1 year after AMT procedure.

TABLE 5 Surgical Indications for Amniotic Membrane Transplantation

As a Graft
- Pterygium
- Conjunctival defects after removal of large lesions or scars
- Symblepharon lysis
- Conjunctivochalasis
- Bleb leakage or revision
- Scleral melt
- Lid reconstruction
- Orbital reconstruction
- Persistent corneal epithelial defect with or without ulceration
- Partial limbal stem cell deficiency
- Total limbal stem cell deficiency (with limbal transplantation)
- For chemical burns, Stevens-Johnson syndrome
- Painful bullous keratopathy with erosion
- Band keratopathy

As a Patch
- Acute stage of chemical or thermal burns, Stevens-Johnson syndrome
- Preventing scar after PRK or PTK
- Refractory or recalcitrant inflammatory or ulcerative keratitis: HSV, HZO, and vernal

As a Carrier for Expanding Epithelial Stem Cells Ex Vivo
- Limbal stem cell deficiency states

immediately before use.[46] Alternatively, commercially available Amniografts can also be used.

Amniotic Membrane Transplantation

Surgery is usually performed under topical or retrobulbar anesthesia. The base of the ulcer is debrided with a microsponge and fine forceps, and the poorly adherent epithelium adjacent to the edge of the ulcer is removed up to the area where the epithelium is adherent. The AM is removed from the storage medium, peeled from the nitrocellulose filter paper, transferred to the recipient eye, and fitted to fill up the ulcer bed and cover the defect by trimming off the excess edges. This stroma-side down is then secured to the edges of the ulcer by interrupted 10/0 nylon sutures and the suture knots are buried. The decision for transplanting more than one layer of amniotic membrane should be made by a careful assessment of preoperative and intraoperative ulcer depth with the aid of slit-lamp biomicroscopy and surgical microscope. More than one layer of AM is used if the ulcer is deep, and in those instances, the bottom layers are left unsutured as a filling. The second AM layer is transplanted as a basement membrane (amniotic membrane graft). Depending on the aqueous tear status and the eyelid blinking function, a third AM layer can be transplanted as a cover (amniotic membrane patch). When AM is used as a patch, this is performed by placing the AM over the cornea and extending it beyond the limbus with the basement membrane side facing down and suturing it with 10/0 interrupted nylon sutures over the perilimbal area.[46]

Postoperative Care

Before epithelialization, the patients are followed weekly and are treated with topical antibiotics and nonpreserved steroid eye drops three to four times a day. After epithelialization is completed, the antibiotic eye drops can be discontinued but the former is gradually tapered off over 3–4 months. Patients with dry eyes should be prescribed topical preservative-free artificial tears or autologous serum drops six to eight times a day.

Perhaps one of the most promising uses of AM is in the treatment of acute chemical and thermal injury of the ocular surface. Early epithelialization and inflammation control can prevent scarring, symblepharon formation, as well as total stem cell loss during the chronic phase. AMT within two weeks of injury suppressed inflammation and promoted early epithelialization in mild to moderate disease states according to recent reports.[47,48] AMT alone is not sufficient in severe disease where a subsequent limbal transplant may be required. However, limbal transplant in an inflamed eye is at a higher risk of failure than in a quiet eye,[49] and AMT as a patch is a useful means of reducing inflammation in preparation for limbal transplants. Another aspect that has made AMT an attractive surgical tool is the lack of any immunologic rejection. However, the possibility of transmitting an unknown pathogen cannot be ruled out, and there is at least one case report.[50]

Current Topics

Transplantation of Human Limbal Epithelium Cultivated on AM

Despite the current enthusiasm, a study from our department of the long-term prognosis of AM transplantation plus stem cell transplantation using allograft demonstrated that long-lasting ocular surface reconstruction was achieved in less than half of the cases.[26] Persistent epithelial defect of the cornea was the most common postoperative complication, developing in

60% of the cases despite meticulous postoperative management of the epithelium. To overcome this difficulty, in vitro cultivation of the corneal epithelium on the AM was introduced. When cultivated corneal epithelium is transferred with AM, the corneal surface is expected to epithelialize instantly. The question of whether the prognosis for this method would be improved by using a different cultivation protocol is intriguing. Although multilayered epithelium with a basement membrane-like structure was obtained by the current cultivation method, other techniques, such as the use of feeder fibroblasts or airlifting, might improve structural integrity. We observed that some cultivated epithelium sloughed off immediately after surgery, suggesting that adhesion of cells to the AM was not firm enough. Further studies are needed to achieve optimal culture conditions.

Deep Lamellar Keratoplasty Versus Penetrating Keratoplasty in Ocular Surface Reconstruction

Archila and associates first reported deep lamellar keratoplasty (DLKP) in 1985.[51] By replacing diseased tissue with healthy donor cornea, improvement in visual acuity can be obtained, while the risk of endothelial rejection or intraocular complication can be lowered. In addition, more donor corneas can be used in DLKP since the procedure does not require a healthy donor endothelium. This is an important issue in countries where donor corneas are lacking. There have been a number of reports indicating favorable visual outcome after DLKP;[52–57] however, no appropriate comparison between PKP and DLKP has been done. We carried out the first randomized prospective clinical trial of DLKP versus PKP and the results shed some insights on this issue.[58]

Briefly, in DLKP, recipient corneal stroma is trephinated 1/2–2/3 in depth using Hessbarg-Barron trephine and additional stroma is excised by lamellar surgical knives as shown in Figure 8 (Ultrasharp microsurgical knives, No. 681.01 and 681.25, Grieshaber, Switzerland). Intrastromal injection of either air or balanced saline is performed to facilitate stromal dissection as demonstrated in Figure 9.[59] As dissection of deep stromal tissue proceeds, aqueous humor is aspirated through small limbal incision to lower the intraocular pressure. Typically, the central Descemet membrane of 5 to 6 mm in diameter is exposed (Figure 10). Donor corneas are trephined using Barron donor punch with a diameter 0.25 mm larger than the recipient eye. The donor endothelium and the Descemet membrane are dissected using toothed forceps (Ultrafine notched forceps F240W, Inami, Tokyo, Japan) and spring-type micro-scissors (S-551C, Inami, Japan) as shown in Figures 11 and 12. The donor buttons are secured by a single continuous 10-0 nylon suture (Figure 13).

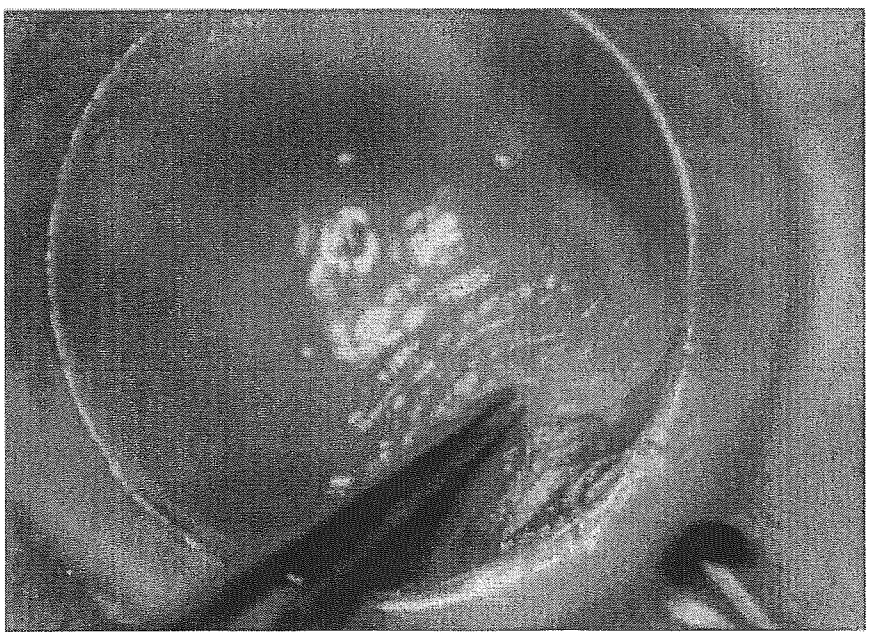

FIGURE 8 DLKP procedure:Trephination of recipient corneal stroma 1/2–2/3 in depth using Hessbarg-Barron trephine and excision of additional stroma by lamellar surgical knives.

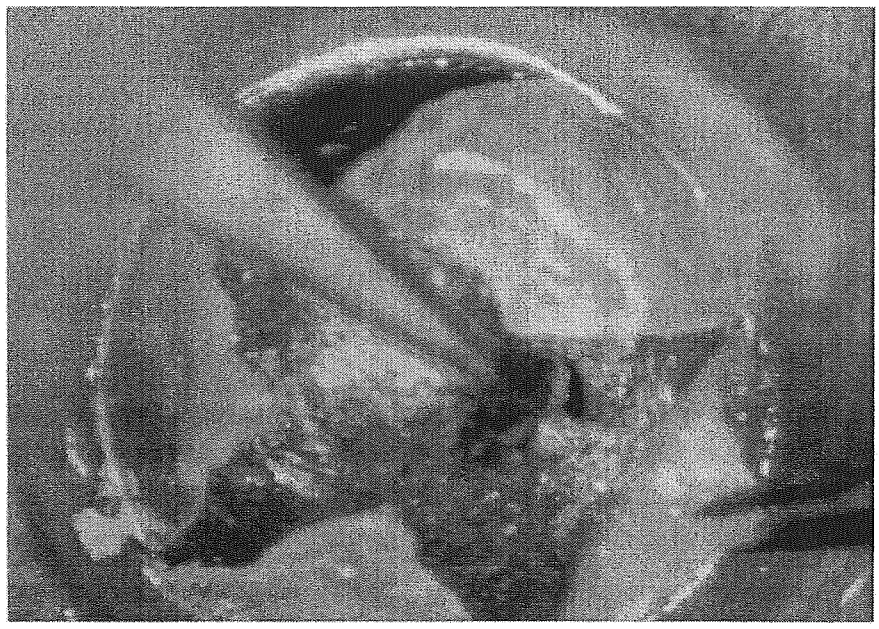

FIGURE 9 Further stromal dissection after intrastromal injection of either air or balanced saline.

In our study, best corrected visual acuity after DLKP was comparable with PKP.[58] However, recovery tended to take longer after DLKP than PKP, which has also been reported by Amyem and Anwar[55] in keratoconus. Patients who had DLKP in one eye and PKP in the other often prefer PKP, claiming the DLKP eye "sees less clearly." The preference of PKP over DLKP may be due to either delayed recovery of corrected visual acuity or stronger astigmatism in the DLKP group. There were several aspects showing clear advantages of DLKP over PKP. Corneal endothelial density stabilized after six months in DLKP. In contrast, continuous loss of endothelial cells was noted in PKP, which is in good accordance with previous reports.[51] We found a significant difference in endothelial density between DLKP and PKP groups at 24 months and it is probable that

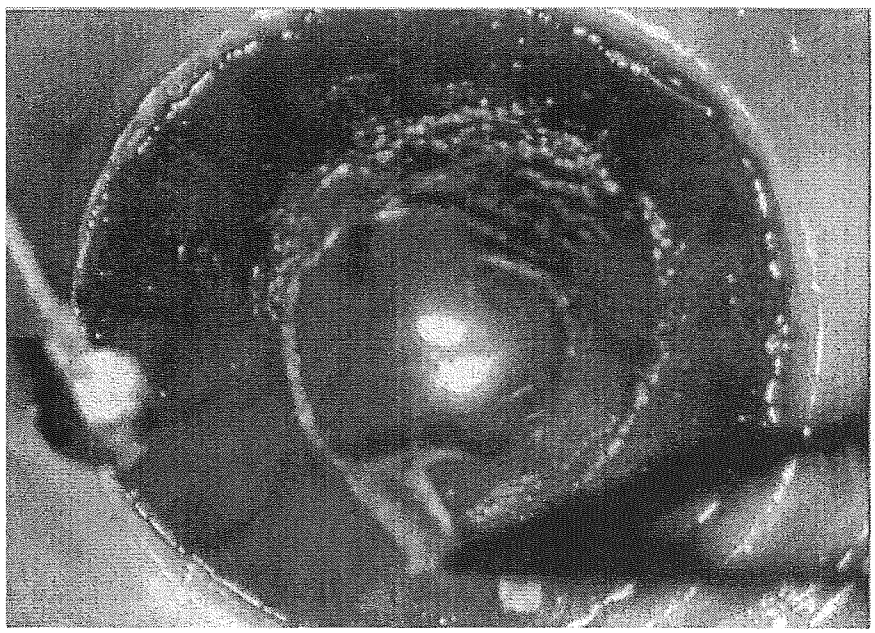

FIGURE 10 Exposure of the central Descemet membrane of 5 to 6 mm in diameter.