REVIEW

Amniotic membrane transplantation for ocular surface reconstruction

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Abstract

The ocular surface epithelia function with the tear film to provide clear vision and comfort, and to serve as the first line of defense. It is therefore important to understand how ocular surface health is maintained and how ocular surface failure occurs. Furthermore, it is timely to summarize new information concerning the mechanisms of action and the clinical uses of amniotic membrane transplantation for ocular surface reconstruction. Therefore, a review has been conducted of findings published since 1995.

When appropriately processed and preserved, amniotic membrane as a native matrix can be used as a graft to restore conjunctival surfaces following removal of lesions such as pterygium, tumor, scar, symblepharon, and conjunctivochalasis. It can also be used as a graft to restore corneal surfaces with limbal stem cell deficiency. For partial limbal deficiency, amniotic membrane transplantation alone is generally sufficient, while for total limbal deficiency limbal stem cell transplantation with or without corneal transplantation is required. When used as a graft or patch, amniotic membrane can facilitate healing of persistent corneal ulcers and recurrent corneal erosion, and reduce corneal haze following keratectomy.

Reported data indicate that amniotic membrane transplantation facilitates rapid healing with recovery of a normal epithelial phenotype in the epithelium by prolonging the life span of progenitor cells, and reduces inflammation, vascularization, and scarring in the stroma. Other potential uses await further exploration.

Key words: *Amniotic membrane, Ocular surface diseases, Ocular surface reconstruction, Limbal stem cells*

Ocular surface health

Anatomically, the ocular surface encompasses the entire mucosal epithelial lining bordered by the skin at the superior and inferior eyelid margins. Histologically, this epithelial surface covers two major structures - the cornea and conjunctiva. The primary function of the ocular surface is to provide clear vision during open eye conditions. Therefore, the ocular surface has to be covered by a stable tear film. The intimate relationship between the ocular surface epithelia and the preocular tear film ensures ocular surface health. Recently, five important concepts have been summarized to explain how an organized and effective defense system achieves this goal:[1]

1. Ocular surface health is ensured by an intimate relationship between ocular surface epithelia and the preocular tear film.
2. A stable tear film is inherently maintained by external adnexae.

3. The intact protective mechanism is controlled by effective neuroanatomic integration.
4. Corneal epithelial stem cells are located at the limbus.
5. Ocular surface epithelial cell function is supported by stromal fibroblasts and basement membrane matrix.

The surface epithelia and the tear film function together to prevent dryness, discomfort and microbial infection when the eye is open. The tear film is composed of meibum lipids produced by meibomian glands; aqueous tear fluids produced primarily by lacrimal glands; and mucins produced by ocular surface epithelial cells. Hydrodynamically, tears are spread into a film to cover the entire ocular surface through frequent eyelid blinking. The external adnexae and eyelids are essential for achieving a stable tear film. Both the compositional and hydrodynamic aspects of the protective tear function are integrated with the ocular surface epithelia via two neuroanatomic reflexes, which are instigated by the first branch of the trigeminal nerve (afferent sensory input) and by the parasympathetic and motor branches of the facial nerve (efferent output), respectively.

The ocular surface epithelia are both protected by and active in forming a stable tear film. This notion is supported by the fact that conjunctival goblet cells secrete gel-forming mucins, one of the important components of the tear film.[2] In addition, both corneal and conjunctival non-goblet cells express membrane mucins on the superficial epithelial cells for fluid retention.[3,4] Furthermore, the non-keratinized corneal and conjunctival epithelia express different keratins to those of the normally keratinized epidermis.[5-7] Taken together, these findings suggest that both mucin expression and non-keratinization comprise the key epithelial phenotype endowing ocular surface epithelia with wettability.

Normal terminal differentiation of the superficial epithelial cells is continuously supplied by proliferating progenitor cells as differentiation is coupled with cell death. One important advance in ocular surface epithelial cell biology is the recognition of epithelial stem cells as the ultimate source of epithelial regeneration.[8] The stem cells of the corneal epithelium have been identified at the limbus,[9] while the stem cells of the conjunctival epithelium remain to be determined although controversial reports show they are located at the fornix[10,11] or at the mucocutaneous junction of the lid margin.[12] Furthermore, increasing evidence has indicated that epithelial stem cells are regulated by the underlying stromal fibroblasts[8] and the basement membrane may play an important role in modifying epithelial-mesenchymal interactions, which indirectly affect epithelial functions. Collectively, these five concepts indicate that ocular surface health is controlled by extrinsic factors, which maintain ocular surface defense by providing a stable tear film, and by intrinsic factors, which regulate epithelial stem cells.

Ocular surface failure

Two major types of ocular surface failure have been identified by impression cytology. Squamous metaplasia is the process by which pathologic transition of normal non-keratinized ocular surface epithelia into keratinized epithelia occurs.[13] In the conjunctiva, squamous metaplasia is preceded by loss of goblet cells. Squamous metaplasia of the ocular surface can be caused extrinsically by an unstable tear film as a result of a poor ocular surface defense.[1,14] Intrinsically, it may be associated with various forms of cicatricial keratoconjunctivitis such as chemical burns, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, etc. The pathogenesis of squamous metaplasia caused by these diseases remains unclear and is highly correlated with intense stromal inflammation or loss of vascular supply as a result of scarring.[15] The second type of ocular surface failure is characterized by the replacement of the normal corneal epithelial phenotype with an invasive conjunctival epithelium in a process termed limbal (stem cell) deficiency.[16] Corneal diseases associated with limbal deficiency are all characterized by conjunctival epithelial ingrowth (conjunctivalization), vascularization, chronic inflammation, poor epithelial integrity manifested as irregular surface, recurrent erosion and persistent ulcer, destruction of the basement membrane, and fibrous ingrowth. Patients with limbal deficiency often suffer from severe photophobia and decreased vision, and are generally poor candidates for conventional corneal transplantation because only short-lived corneal transient amplifying cells are included. Furthermore, pre-existing corneal vascularization and inflammation increase the risk of allograft rejection.[8]

By means of impression cytology, limbal deficiency has been detected in a number of corneal diseases,[16] which can be divided into two major categories (**Table 1**). Diseases in category I are characterized by having a clear pathogenic cause, identifiable from the past history, which has destroyed the limbal stem cell population. The destruction can come from chemical or thermal injuries, Stevens-Johnson syndrome, multiple surgeries or cryotherapies at the limbal region, antimetabolite (5-fluorouracil) toxicity, contact lens wear or severe microbial infection; with many of these causes being iatrogenic in origin.[17] One case report has indicated that radiation therapy can cause temporary and reversible limbal deficiency.[18] The identification of limbal deficiency in this category justifies the transplantation of limbal stem cells.[19,20] Autograft limbal transplantation has been shown to restore the corneal surface more effectively than conjunctival transplantation. Clinically, limbal autografts have successfully reconstructed corneal surfaces for those patients with category I diseases, especially those with focal or unilateral limbal deficiency.[22-30] For patients with bilateral and diffuse limbal deficiency, corneal surface reconstruction relies on an allograft source of either HLA-matched living donors[27,28,31] or non-matched cadavers.[27,32-34] Because of potential allograft rejection, systemic use of cyclosporin becomes necessary and probably indefinite. For reasons that remain poorly understood, the efficacy rate for limbal allograft declines with time despite the use of cyclosporin.

In contrast, the category II diseases do not have a clear past history but still exhibit a gradual loss of limbal stem cell function over time. It is noteworthy that category II diseases include such diverse causes as aniridia; keratitis associated with multiple endocrine deficiencies; neurotrophic keratopathy; peripheral inflammatory or ulcerative keratitis or limbitis; idiopathic keratopathy, or pterygium/

pseudopterygium (**Table 1**). Because limbal deficiency, at least that caused by radiation therapy, may be temporary and reversible,[18] clinical management should start with medical rather than surgical therapy, especially when the central cornea still retains functional transient amplifying cells (**Table 2**). Because of the unclear history associated with loss of limbal stem cell function, it appears that limbal stem cell function must be regulated by its stroma. Specifically, it is possible that limbal stem cell function can be modulated by developmental, hormonal, neuronal, vascular, and inflammatory factors in the limbal stromal microenvironment, and dysfunction of limbal stroma can lead to limbal deficiency. Furthermore, the identification of category II diseases also suggests that these pathologic insults can alter the stroma and indirectly affect epithelial function. Further studies are needed to explore the mechanism by which limbal stem cell function is regulated by the stromal micro-environment.

Table 1. Human corneal diseases characterized by limbal deficiency.

* Using impression cytology as a tool and the diagnostic criterion of conjunctivalization for limbal deficiency,[16] a host of corneal diseases has been identified to carry limbal deficiency. These diseases can be subdivided into two major categories of loss of limbal stem cells and dysfunctional limbal stroma. Modified with the permission of *Ophthalmology*.

I. Loss of limbal stem cell population due to destruction

- Chemical or thermal injuries
- Stevens-Johnson syndrome or toxic epidermal necrolysis
- Multiple surgeries or cryotherapies to the limbus (iatrogenic)
- Anti-metabolite (5-fluorouracil) toxicity
- Contact lens-induced keratopathy
- Severe microbial infection

II. Dysfunction of the limbal stem cell stromal micro-environment

- Aniridia (hereditary)
- Keratitis associated with multiple endocrine deficiency (hereditary)
- Neurotrophic keratopathy (neuronal or ischemic)
- Radiation-induced keratopathy
- Peripheral corneal inflammation and ulceration or chronic limbitis
- Pterygium and pseudopterygium
- Idiopathic

Table 2. Medical and surgical treatments for limbal deficiency.

* This algorithm is proposed according to the extent of limbal deficiency, the presence or absence of central TACs, and the depth of central corneal involvement.

Medical and Surgical Treatments for Limbal Deficiency

I. If limbal deficiency is partial and focal, and if sufficient corneal TAC are present on the central cornea,

1. Avoid further attrition of the remaining TAC
   - Avoid toxic medications, surgical debridement of the corneal TAC, and lamellar or penetrating keratoplasty
   - Consider preservative-free steroids, lubrication, bandage contact lens wear or scleral lens
2. Promote growth of remaining TAC and limbal SC
   - Consider debridement of invaded conjunctival epithelium
   - Consider amniotic membrane transplantation as a patch over the cornea but as a graft over the perilimbal sclera

II. If limbal deficiency is diffuse but corneal involvement is superficial, and if there are insufficient corneal TAC on the central cornea,

- Consider amniotic membrane transplantation with allograft limbal transplantation from cadavers or living related donors in conjunction with systemic cyclosporin A
- Consider amniotic membrane transplantation with autograft limbal transplantation if allograft fails and if the involvement is asymmetrical

III. If limbal deficiency is diffuse and severe, and if the corneal involvement is deep,

- Consider amniotic membrane transplantation first to restore perilimbal stromal environment
- Perform allograft limbal transplantation from cadavers or living related donors together with deep lamellar keratoplasty or penetrating keratoplasty in conjunction with systemic cyclosporin A

Amniotic membrane transplantation

Amniotic membrane, or amnion, is the innermost layer of the placenta and consists of a thick basement membrane and an avascular stromal matrix. Amniotic membrane transplantation used for reconstruction in different medical subspecialties has been described in the literature.[35] A live fetal membrane including both amnion and chorion was first used by de Rotth in 1940 as a graft for conjunctival surface reconstruction.[36] However, the reported result was not impressive, possibly due to the preparation method and the inclusion of chorion, and others did not follow suit. Based on the observation made by Brown,[37] who used rabbit peritoneum to cover a burned ocular surface and successfully promoted healing and prevented the spread of tissue necrosis, Sorsby *et al*[38,39] used processed human amniotic membrane as a patch for treating acute ocular burns, and reported impressive success in preventing symblepharon and corneal complications. For reasons still not clear to us, the use of amniotic membrane disappeared from the literature until 1995 when Kim and Tseng[40] reintroduced it for various ophthalmic uses. As described in this paper, encouraging results have since been reported by different investigators, presumably attributable to improved methods of processing and preservation. When appropriately processed and preserved, amniotic membrane can be used for a number of indications, either as a graft to replace the damaged ocular surface stroma matrix or as a patch to prevent unwanted inflammatory insults from gaining access to the damaged ocular surface.

Recent reports indicate that the following factors may be potential mechanisms of action. The basement side of the membrane is an ideal substrate for supporting the growth of epithelial progenitor cells by prolonging the life span and maintaining clonogenicity.[41] This action explains why amniotic membrane transplantation can be used to expand the remaining limbal stem cells and corneal transient amplifying cells during the treatment of partial limbal deficiency,[42] and to facilitate epithelialization for persistent corneal epithelial defects with stromal ulceration.[43-45] In tissue cultures, amniotic membrane supports limbal epithelial cells grown from explant cultures, and the resultant epithelial cells/amniotic membrane can be retransplanted to reconstruct the damaged corneal surface.[46] The amniotic membrane can also be used to promote non-goblet cell differentiation of the conjunctival epithelium,[47] and conjunctival goblet cell differentiation is further promoted by co-culturing with conjunctival fibroblasts on the same side of the basement membrane.[41] This data supports the hypothesis that conjunctival goblet cell density is promoted following amniotic membrane transplantation *in vivo*.[48] The stromal side of the membrane contains a unique matrix

component that suppresses transforming growth factor (TGF-β) signaling and myofibroblast differentiation of normal human corneal and limbal fibroblasts.[49] This action explains why amniotic membrane transplantation reduces scars during conjunctival surface reconstruction,[50] prevents recurrent scarring after pterygium removal,[51] and reduces corneal haze following phototherapeutic keratectomy (PTK) and photorefractive keratectomy (PRK).[52,53] Although such an action is more potent when fibroblasts are in contact with the stromal matrix, a lesser effect is also noted when fibroblasts are separated from the membrane, suggesting that some diffusible factors as well as the insoluble matrix components in the membrane might also be involved. In line with this thinking, several growth factors have been identified in the amniotic membrane,[54] however, further studies are needed to resolve the exact mechanism of action. The stromal matrix of the membrane can also exclude inflammatory cells by stimulating rapid apoptosis,[52,55] and contains various protease inhibitors.[56] This explains why stromal inflammation is reduced after amniotic membrane transplantation[43,50] and corneal neovascularization is mitigated;[57] important actions for preparing the stroma to support limbal stem cells for transplantation either at the time or later.[42,58-60] This action also explains why keratocyte apoptosis can be reduced, preventing stromal haze in PRK.[52,55,61]

Table 3. Mechanisms of action and observed effects of amniotic membrane transplantation.

* TGF-β transforming growth factor-β

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 signaling system and myofibroblast differentiation of normal fibroblasts

Observed Clinical Effects

- Facilitate epithelialization
- Maintain normal epithelial phenotype
- Reduce inflammation
- Reduce vascularization
- Reduce scarring

Clinical aspects of amniotic membrane transplantation

Conjunctival surface reconstruction

The mechanisms summarized in **Table 3** help to explain why amniotic membrane transplantation can facilitate epithelialization, maintain normal epithelial phenotype (with goblet cells when performed on conjunctiva)[48] and reduce inflammation, vascularization and scarring. Based on these therapeutic effects, one hypothesis is that amniotic membrane transplantation can be used for conjunctival surface reconstruction to restore normal stroma and provide a healthy basement membrane for renewed epithelial proliferation and differentiation. The literature shows that amniotic membrane transplantation can be used to reconstruct the conjunctival surface as an alternative to conjunctival graft following removal of large conjunctival lesions such as pterygium,[51,60,62] conjunctival intraepithelial neoplasia and tumors,[50] scars and symblepharon,[50,63] and conjunctivochalasis.[50] These results indicate that the reconstructed area can be large as long as the underlying bed is not ischemic and the bordering conjunctiva has a normal epithelium and subconjunctival stroma. To address these difficulties, further studies are needed to determine whether reconstruction can be extended by transplanting amniotic membrane carrying *ex vivo* expanded conjunctival stem cells with or without normal conjunctival fibroblasts. Recently, amniotic membrane has been reported to augment the success of mitomycin C following glaucoma surgery.[64]

Corneal surface reconstruction

New strategies for treatment of limbal deficiency include the use of amniotic membrane transplantation and limbal stem cell transplantation.[42,58,59] The former is intended to restore the damaged limbal stromal environment, and the latter to restore the limbal stem cell population. Our recent clinical experience shows that this combined approach is effective in treating limbal deficiency according to the following parameters: the extent of limbal deficiency; the presence or absence of the central corneal transient amplifying cells (TAC); and the depth of central corneal involvement.[42] The proposed algorithm of clinical medical and surgical management is summarized in **Table 2**.

One major advantage of amniotic membrane transplantation is that partial limbal deficiency can now be improved without the use of limbal transplantation.[42] This result, first observed in rabbit studies,[40] indicates that patients with partial limbal deficiency can now be treated without the long term use of oral cyclosporin. The second advantage is the extremely low incidence of limbal allograft rejection when systemic cyclosporin is given concomitantly with amniotic membrane transplantation as the first stage in the procedure to restore the limbal stromal environment. This effect is presumably due to the restoration of a non-inflamed limbal stroma. **Figure 1** illustrates how amniotic membrane transplantation is performed together with a limbal allograft, for corneal surface reconstruction. Difficulty remains, however, for those patients who have severe limbal deficiency requiring concomitant transplantation of corneal grafts, which have a rejection rate of more than 50%.[42]

Amniotic membrane can also be used to treat corneal surface diseases, either as a temporary or permanent graft or patch. Experimentally, when used as a patch on a temporary basis, this membrane has been shown to reduce corneal haze following PRK or PTK,[52,61] an effect verified in human patients.[53,62] When used as a graft or a patch, amniotic membrane can promote healing of persistent corneal ulcers from various causes, including neurotrophic keratopathy caused by various underlying etiologies.[43,44,62] This

approach is superior to conjunctival flaps or tarsorrhaphy as it preserves a cosmetically more acceptable appearance. A recent multi-center trial shows that amniotic membrane transplantation can be used to treat symptomatic bullous keratopathy caused by aphakia, pseudophakia or failed corneal grafts in patients with pain, recurrent erosion and infection. It should be noted that the ocular surface defense should be restored prior to or at the same time as corneal surface reconstruction by amniotic membrane transplantation with or without limbal stem cell transplantation. These measures include punctal occlusion or application of serum drops for severe aqueous tear deficiency,[58] plastic correction of lid margin and lash problems, and tarsorrhaphy or refractory exposure. Severe dry eyes, diffuse keratinization and stromal ischemia remain difficult to overcome, if not contraindications. Further exploration of the mechanism of action should uncover more applications.

Case report

A 78-year-old man with atopic keratoconjunctivitis presented with itchy eyes and corneal problems for the past 15 years. He had previously been treated with topical medication and had received lid surgery on two occasions. Four years ago, he was administered a course of topical cyclosporin A and experienced relief of symptoms. Nevertheless, both eyes exhibited symblepharon, loss of inferior fornix, and corneal pannus formation extending into the central cornea in both eyes, which was more pronounced in the left eye than in the right eye (**Figures 1A** and **1B**, respecively). He subsequently underwent extracapsular cataract extraction and intraocular lens implantation in the right eye and his vision in this eye improved to 20/200, while the vision in the left eye deteriorated to hand movement only. He underwent amniotic membrane transplantation for fornix reconstruction and limbal allograft transplantation in the left eye, together with administration of systemic cyclosporin A and 1% non-preserved methylprednisolone and Ocuflox. Epithelial defects created by surgery included disruption of the entire corneal surface, which healed after three weeks. The fornix deepened, the conjunctival surface became smooth, and the corneal surface was smooth without vascularization four months later (**Figures 1C** and **1D**). His vision improved to 20/200, but was primarily limited by a dense cataract.

Epinephrine 1:1000 was given at the start of the surgical procedures for vasoconstriction. The globe remained fixed downwards upon insertion of the speculum due to symblepharon and fornix shortening (**Figure 2A**). A peritomy incision was made through the upper limbus. The subconjunctival fibrovascular scar tissue was removed (**Figure 2B**), and the entire corneal pannus was removed.

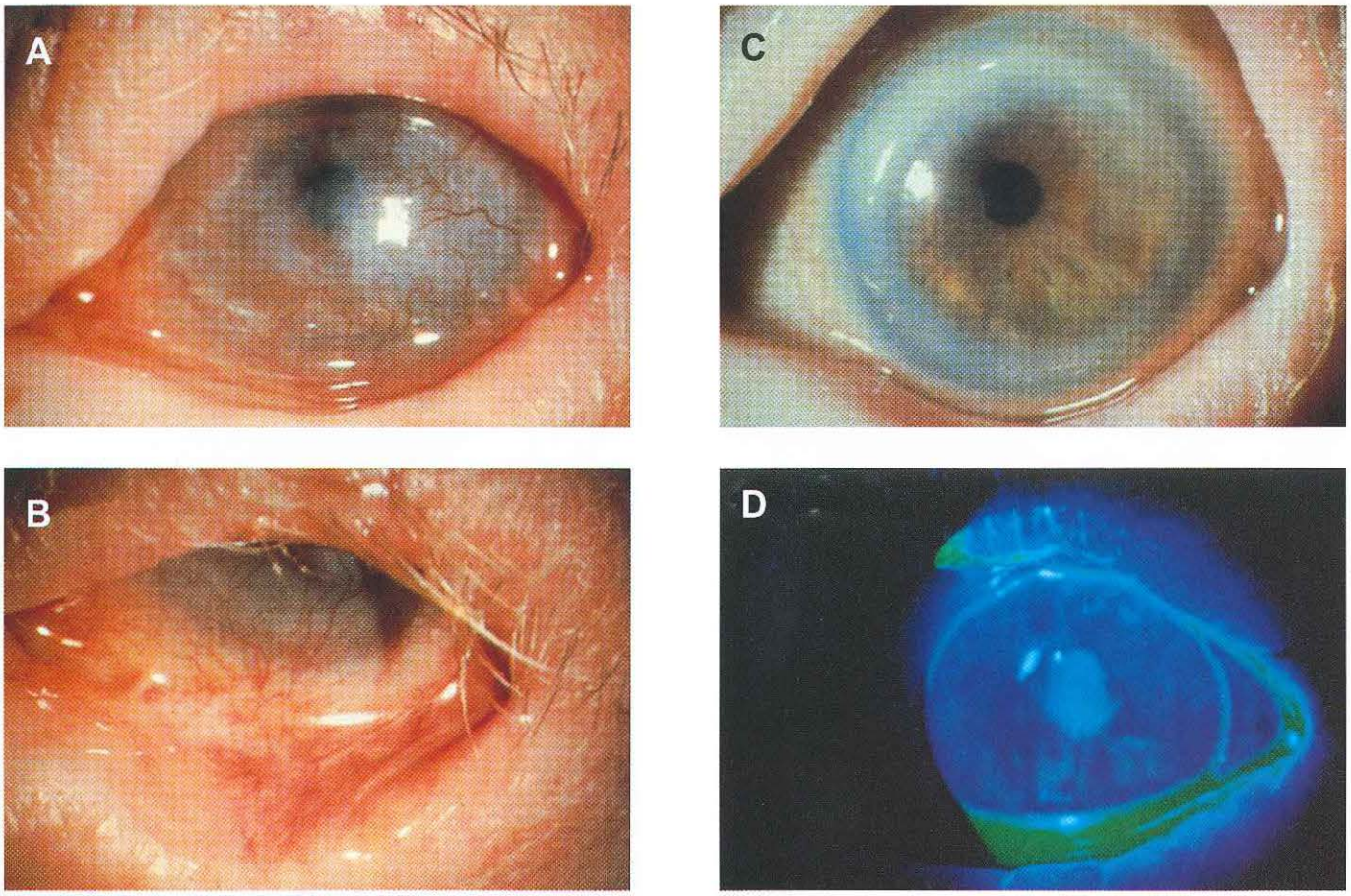
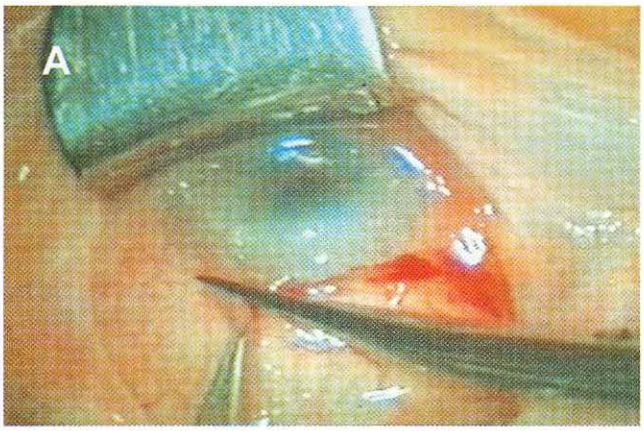


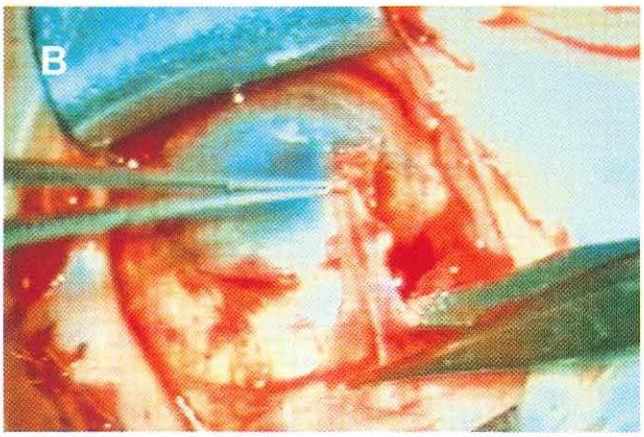
Figure 1. The preoperative appearance showed an inferior symblepharon (A) and cornea haze with pannus (B). Six weeks after amniotic membrane transplantation and limbal allograft transplantation the eye became stable with a smooth corneal surface (C), without fluorescein staining four months later (D).

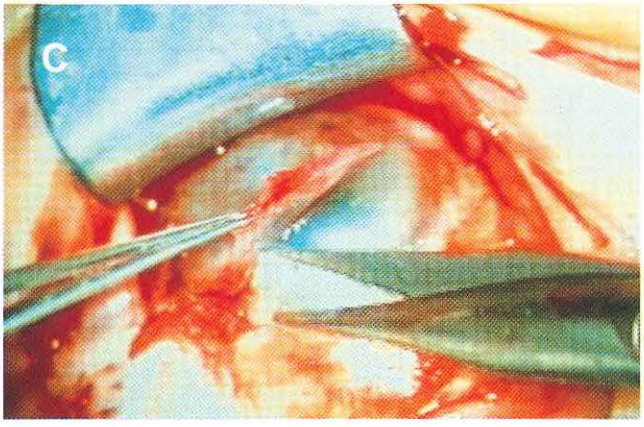
through the plane identified via blunt dissection (**Figure 2C**). This resulted in denuded corneal and perilimbal sclera up to 5 to 7 mm from the limbus (**Figure 2D**).

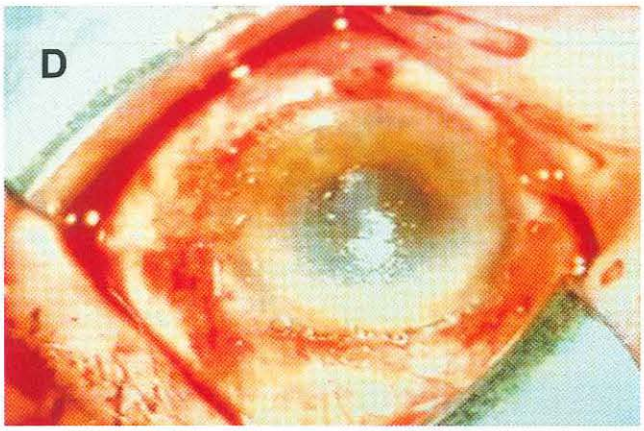
The limbal graft was prepared as follows: the donor cornea was removed from the storage medium and the central corneal button was removed by an 8 mm trephine with the epithelial surface facing upwards. Excessive scleral tissue was removed (**Figure 2E**). The remaining corneoscleral ring was then protected with insertion of Healon. The graft was turned with the corneal endothelium facing upwards and the posterior two-thirds of the stroma was removed (**Figure 2F**). The corneal margin and the scleral margin were both tapered off by scissor trimming of additional stroma (**Figures 2G** and **2H**). The finished corneolimbal lamellar graft was then protected on the epithelial side with additional Healon.

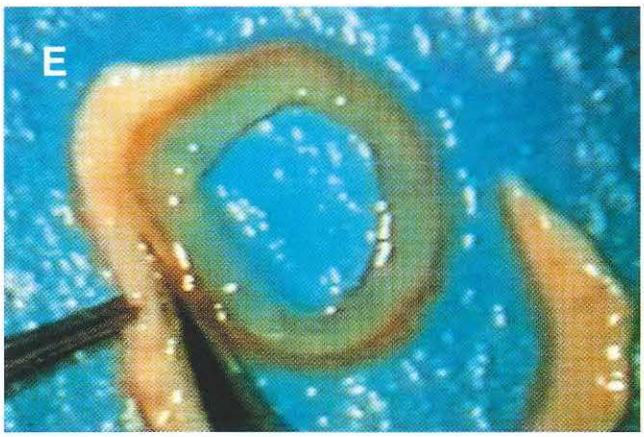
The amniotic membrane was removed from the storage medium, peeled off from the nitrocellulose filter paper and laid to cover the denuded ocular surface (**Figure 2I**). The membrane was sutured to the conjunctival edge at the bulbar sclera close to the fornix with interrupted 10-O Vicryl sutures with episcleral fixation (**Figure 2J**). The donor corneolimbal graft was then placed over the cornea (**Figure 2K**) and sutured to the sclera and the membrane with interrupted 10-O Vicryl sutures with episcleral fixation (**Figure 2L**) and to the cornea, as necessary, with interrupted 10-O nylon sutures or combined with a continuous running suture.

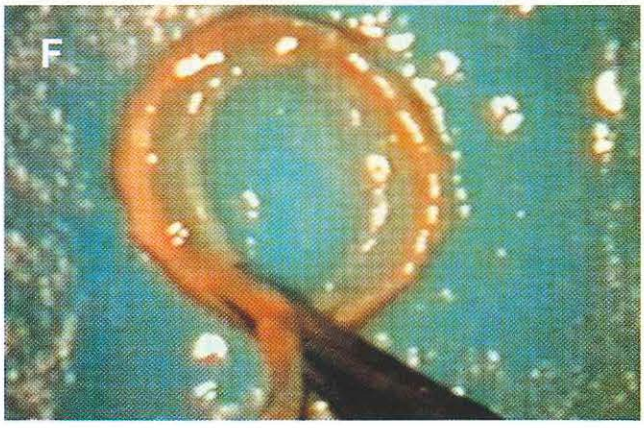












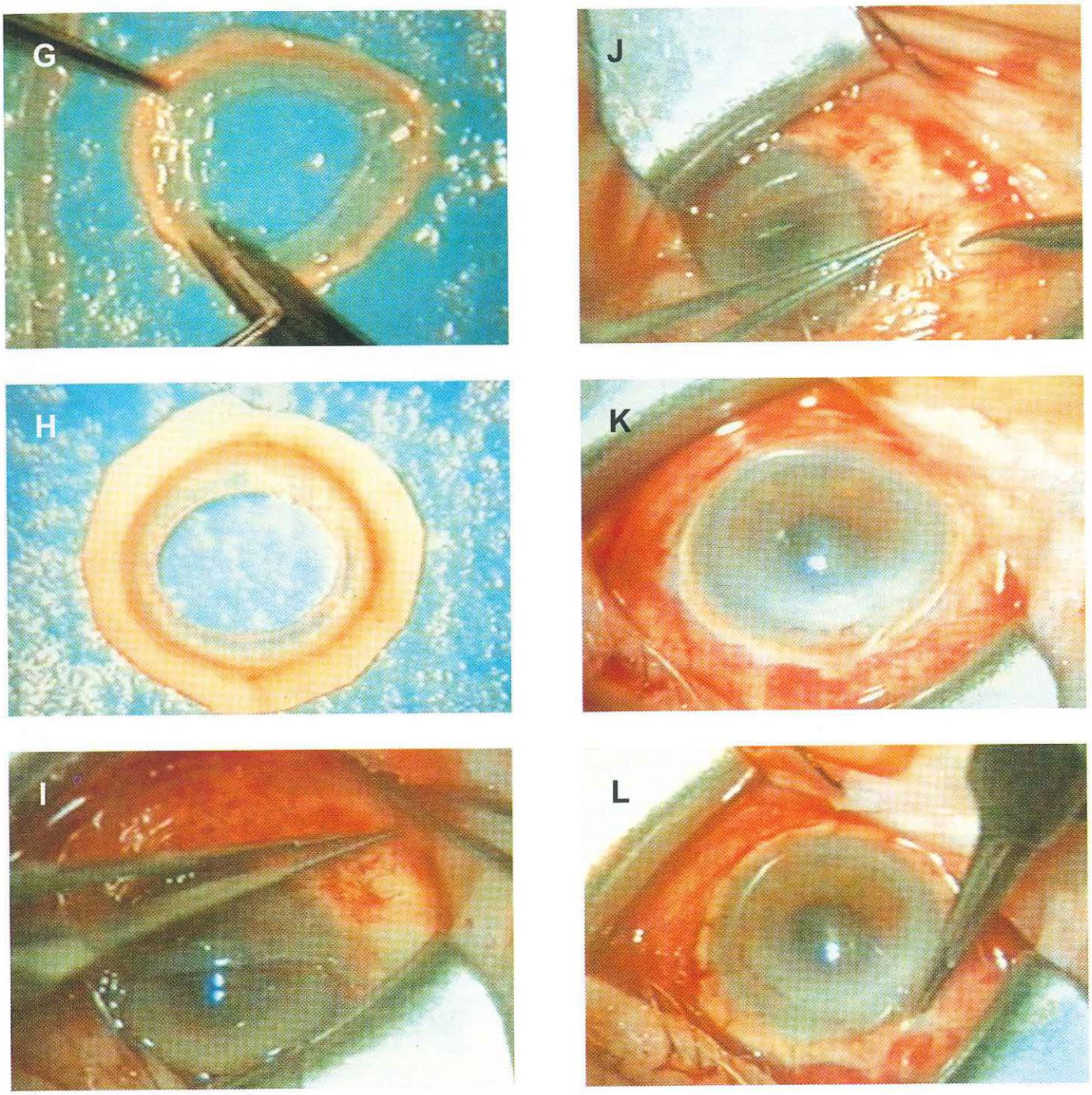


Figure 2. Key steps of the surgical procedures for amniotic membrane transplantation and limbal allograft.

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