# DIAGNOSTIC AND SURGICAL TECHNIQUE

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# The Amniotic Membrane in Ophthalmology

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**Abstract.** The amniotic membrane is the innermost of the three layers forming the fetal membranes. It was first used in 1910 in skin transplantation. Thereafter it has been used in surgical procedures related to the genito-urinary tract, skin, brain, and head and neck, among others. The first documented ophthalmological application was in the 1940s when it was used in the treatment of ocular burns. Following initial reports, its use in ocular surgery abated until recently when it was re-discovered in the Soviet Union and South America. Its introduction to North America in the early 1990s heralded a massive surge in the ophthalmic applications of this membrane. The reintroduction of amniotic membrane in ophthalmic surgery holds great promise; however, although it has been shown to be a useful and viable alternative for some conditions, it is currently being used far in excess of its true useful potential. In many clinical situations it offers an alternative to existing management options without any distinct advantage over the others. Further studies will undoubtedly reveal the true potential of the membrane, its mechanism(s) of action, and the effective use of this tissue in ophthalmology. (**Surv Ophthalmol** 49:51–77, 2004. © 2004 Elsevier Inc. All rights reserved.)

**Key words.** amniotic membrane • glaucoma • limbus • ocular surface • oculoplastics

The amniotic membrane is the innermost of the three layers forming the fetal membranes. It is a translucent membrane composed of an inner layer of epithelial cells, planted on a basement membrane that in turn is connected to a thin connective tissue membrane by filamentous strands.[34] Amniotic membrane is derived from fetal ectoderm by cavitation within the fetal knot and is contiguous over the umbilical cord with the fetal skin. Outside the amnion is the chorion laevae comprising of connective tissue containing the fetal (chorioallantoic) vessels. The outermost layer of the fetal membranes, the decidua capsularis, is the only component of the fetal membranes of maternal origin and is composed of modified endometrium.

## Historical Perspective

In an extensive review, Trelford and Trelford-Sauder[186] recall that in early days, a child born with the fetal membranes intact (caul), was considered to be lucky. Early Scottish sailors born with unruptured membranes carried a dry remnant of the caul, "the sely how" to save them from drowning. With the healing qualities of the membranes becoming substantiated by scientific research, this folklore of bygone days is rapidly becoming established as a clinical reality.

Davies in 1910 was the first to advocate the therapeutic use of amniotic membranes in skin transplantation.[35] Following this, several reports of its

51

use as a biological bandage for dressing burns,[16,62,143,155,176,172,188] non-healing skin ulcers[15,165,179,204,205] and as an aid to physiological wound healing[32,45,61,134,158] appeared. In addition, this versatile material has been used in surgical reconstruction of the vagina,[21,37,55,127,186] repair of abdominal herniation,[56] closure of pericardium,[124] prevention of surgical adhesions,[7,146,187,211] and head and neck surgery.[214]

The use of amniotic membranes in ocular surgery was first suggested by de Rotth[36] and Sorsby[171,172] who examined its role in the management of ocular surface damage. It was first used, as a substitute for rabbit peritoneum, in the management of chemical burns of the eye. Very good results were reported with dried amniotic tissue "amnioplastin." Thereafter, for no evident reason, its use was abandoned or went unreported until recently, when renewed interest has developed in its potential role in the treatment of ocular surface disorders.

The early 1990s can be taken as a suitable starting point in tracing the modern history of the use of amniotic membranes in ophthalmic surgery. In May 1992, Juan F. Batlle and Francisco J. Perdomo (personal communication) presented a paper entitled "Placental membranes as a conjunctival substitute" to the annual congress of the Dominican Society of Ophthalmology held in Puerto Plata, Dominican Republic. In this presentation, Juan Batlle traced the use of "Soviet tissues" as allotransplants in conjunctival, tarsal, orbital, and tendon surgery.

He described how Dr. Horacio Serrano of Caracas, Venezuela, had visited Dr. Muldachev in Ufa of the former Soviet Union and witnessed the use of a "special tissue" in ocular transplantation with impressive results. The tissue was used and distributed within Europe by the Soviets. Dr. Serrano himself had started using the tissue in Venezuela in 1988 and his pupil, Dr. Carlino Gonzalez, supplied some of this "special tissue" to Dr. Batlle. Dr. Batlle, intrigued by the nature of the "mysterious tissue" but nevertheless impressed and motivated by the successful outcome following use of the material in ocular transplantation, set out to trace its history and scientifically determine its nature. Dr Batlle stated "The source of the Soviet tissues and the processes involved in the harvest, preservation, and transport remain in absolute scientific silence." After many trials and tests with different tissues obtained at autopsy, Batlle and Perdomo were able to determine that the tissue used by the Soviets for conjunctivoplasty contained human placental membranes (Fig. 1).

He and his colleague conducted a study in three parts. First, they performed histologic studies of the tissues obtained from the Soviet Union. Secondly,



*Fig. 1.* The 'Russian allotransplant' that was later proven to be human fetal membranes. Dispensed as a sheet spread on a carrier (*A*) or in a vial (*B*). (Courtesy of Juan Battle.)

they studied histologically human placental membranes preserved in ethyl alcohol (the preservative used by the Soviets), and thirdly, they conducted clinical trials using human placental membranes as a substitute for conjunctival tissues in several ocular surface disorders. They concluded that the placental membranes used were histologically similar to the "mysterious tissue" and clinically gave the same successful results. They were very impressed by the small amount of pain and discomfort experienced by the patients receiving the placental grafts. One day after surgery, the transplanted eyes are quiet with minimal edema and inflammation. They did not observe any rejection or sloughing of the grafted placental membranes and noted complete epithelialization with host epithelial cells within 72 hours of surgery. In June 1992 the work was presented at the Bascom Palmer Alumni Meeting in Miami, Florida, and later as a scientific poster (#25) at the American Academy of Ophthalmology, Annual Meeting in Chicago, Illinois, in November 1993.

From then on, Schaffer Tseng, from Bascom Palmer, Miami, together with innumerable colleagues further advanced and developed this concept of the use of amniotic membrane in ophthalmic surgery. They provided the sound clinical and scientific basis for its applications. By introducing the now well-accepted method of preservation and storage, by adding considerably to the knowledge of the key components of the membrane and developing some of the surgical techniques, Tseng and his team re-established a role for this membrane in ophthalmic surgery.

## Development and Anatomy

The amniotic membrane first appears at about days 7–8 following conception. It results from a separation from the inner cell mass of the germ disk at the

periphery of the ectodermal layer. These polygonal amniogenic cells produce a slit-like cavity, which becomes the amniotic cavity. Expansion of the amniotic membrane continues and presence of mitotic figures in the amniotic epithelium suggests that the majority of these new cells are derived from division of existing amniotic epithelial cells. The amniotic bubble fuses with the surrounding chorion at about the 12th week of gestation.

Amniotic mesenchyme is derived from the primary extraembryonic mesoderm. The primary mesoderm becomes compressed against the outer surface of the amniotic layer and forms a single layered mesothelium closely applied to the amniotic epithelium.

By the second month of gestation, the mesenchymal cells are separated from the epithelium by a layer of tissue containing loosely packed collagen fibrils with occasional interspersed separate cells, which are fibroblast in nature. The collagen content of the mesenchymal layer increases providing increased tensile strength. At term, the amnion consists of a layer of a single layer of amniotic cells firmly adherent to a mesenchymal layer six to eight cells thick, which is loosely adherent to the underlying chorion.[68,109,186]

The amnion varies in thickness from 0.02 mm to 0.5 mm in thickness.[19,34] It contains no blood vessels and has no direct blood supply.[19] Bourne described the amnion as consisting of five layers from within outward: (a) epithelium; (b) basement membrane; (c) compact layer; (d) fibroblast layer; and (e) spongy layer.[19] The epithelial layer consists of a single layer of amniotic membrane epithelium (Fig. 2A). These cells are polygonal in shape and vary from columnar over the placenta to cuboidal or flat away from the placenta. The basement membrane is a thin layer composed of reticular fibers. It is closely adherent to the amniotic epithelium from which multiple processes interdigitate into it. The compact layer is a dense layer almost totally devoid of cells and consists mainly of a complex reticular network. The fibroblastic layer is the thickest layer of the amnion and consists of fibroblasts embedded in a loose network of reticulum. The outermost spongy layer forms the interface between the amnion and chorion and consists of wavy bundles of reticulum bathed in mucin.

The amnion varies in histological appearance from conception to maturity and several different patterns are often noted even at term.[136] At term light microscopy reveals a single layer of epithelial cells which are columnar in appearance overlying the placenta but become more cuboidal and flatter over the extraplacental amnion. These cells overlie a basement membrane of partly amorphous partly microfibrillar substance.[201] The underlying mesenchymal layer consists of an extensive collagen matrix mixed in with fibroblastic-like cells.
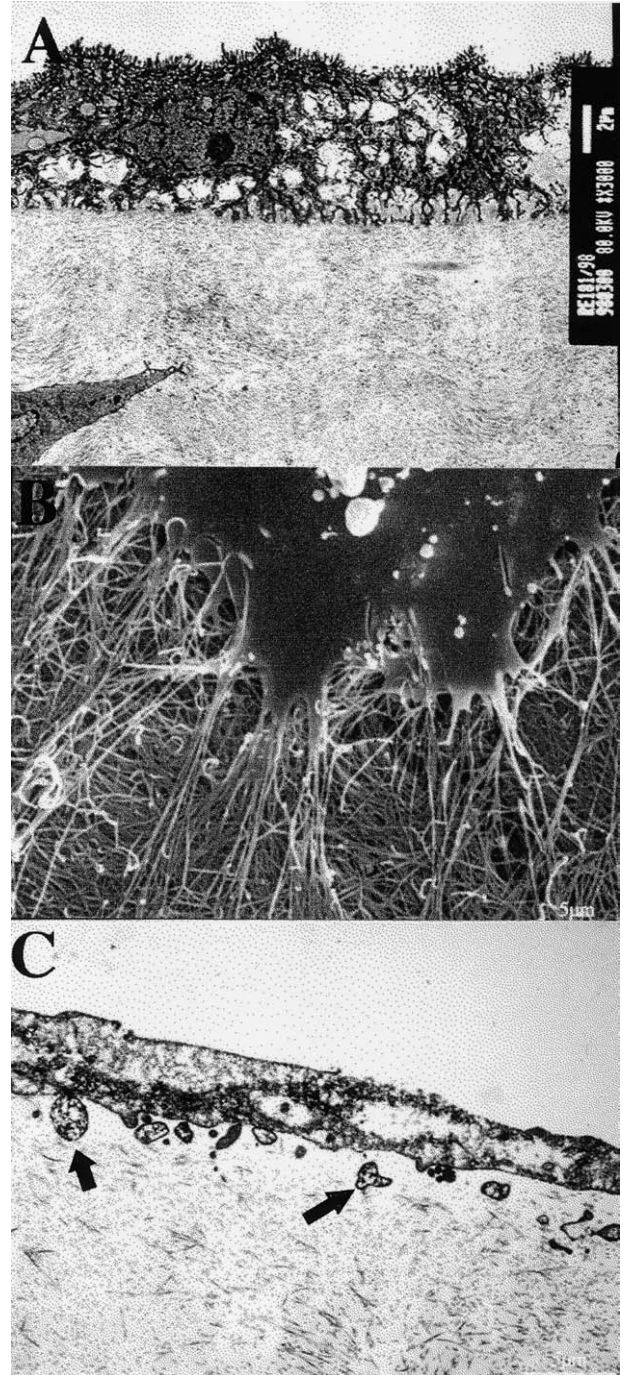


Fig. 2. A: Transmission electron micrograph of normal amniotic membrane showing a metabolically active epithelial cell with microvilli, resting on its basement membrane on top of the collagenous stroma (×3000).B: Scanning electron micrograph of the edge of a corneal epithelial cell with numerous filopodia cultured on amniotic membrane denuded of its epithelium and basement membrane. Note the coarse collagen meshwork of the amniotic stroma (×1200). C: Transmission electron micrograph of filopodia of the advancing edge of a corneal epithelial cell cultured on amniotic stroma. Processes are seen extending into the collagen matrix (arrows), in the absence of a basement membrane (×6000).

The apical surfaces of the amniotic epithelial cells are covered by microvilli,[34,136,201] the density of which varies during pregnancy. An amorphous material of unknown substance is seen on the surface of these microvilli at term.[136]

Microvilli are also numerous on the lateral aspect of these cells and may form intercellular canaliculi.[34,136,201] The cytoplasmic core of the microvilli has a fine fibrillar substructure, which probably has a supportive function. The amniotic cells are connected to each other laterally by numerous desmosomes, but there are no tight junctions[201] and the lateral intercellular space may provide an effective transcelluler pathway for macromolecule transportation. The amniotic cells have highly folded basal surfaces, which interdigitate intensely with the basal lamina[19] to which they are fixed by numerous hemidesmesomes[201] to compensate for the mechanical strain resulting from fetal movement.

Intracellularly, the nucleus is irregular and the nucleolus is large and homogenous. There is a well-developed cytoskeleton with filaments forming a network of bundles throughout the cytoplasm, which often come in close relation to the lateral membranes in the vicinity of the hemidesmesomes.[201] Both smooth and rough endoplastic reticulum are seen; ribosomes are scattered throughout the cytoplasm, and the Golgi apparatus is usually supranuclear in location. There are numerous lipid droplets and glycogen stores contained within the cytoplasm.

## Function and Molecular Organization

### PHYSIOLOGY

The general functional description of the amnion is an epithelial lining that contributes to the homeostasis of amniotic fluid. The role of the amnion in the homeostasis of amniotic fluid is still uncertain. Some authors have suggested the presence of intracellular vacuoles[19,201] and bleb formation[201] as indicators of secretory activity in amniotic fluid production and the presence of pinocytic vesicles as evidence of an absorptive function.[201]

The amniotic epithelium is highly metabolically active during pregnancy;[201,206] however, as it has no blood supply of its own, nutrition and oxygen are derived from the surrounding chorionic fluid, amniotic fluid, and fetal surface blood vessels. It is suggested that energy is derived primarily through an anaerobic glycolytic process due to this restricted oxygen supply.[13] Glucose transporter proteins 1 and 3 are found in the apical surface of the epithelial cells.[207]

### CYTOCHEMICAL MARKERS

Amniotic membrane epithelium expresses CA 125, which is believed to be a marker for tissues derived from fetal coelomic epithelium and is found on the periderm of the fetus and on the amniotic epithelium and is thus present on all tissues lining the amniotic membrane early in gestation.[126] Oxytocin receptors[14] are found on the amnion as well.

The cytoskeleton of amniotic membrane cells is extensive and actin, α-actin, spectrin, ezrin, cytokeratins, vimentin, and desmoplakin[12,128,208] have been found. These specialized arrangements of intracellular filaments indicate their role in cellular structural integrity and junctional permeability.[208]

Enzymes involved in prostaglandin synthesis, phospholipases, prostaglandin synthase, and cyclo-oxygenase have been found in amnion.[167,184] Prostaglandin production is modulated by human chorionic gonatrophin (hCG),[184,185] corticotrophin releasing hormone (CRH),[76] and glucacorticoids.[57]

### CYTOKINES

Interleukins 6 and 8 are the predominant cytokines associated with amnion cells.[78,145] Interleukin-6 and interleukin-8 are found in high concentrations in the amniotic fluid at term.[78] Keelan et al[78] found these cytokines in the media of cultured amnion cells in the same ratio (IL6:IL8 5:1) as found in amniotic fluid. Expression of these cytokines was increased in the presence of IL-1β, TNFα, and bacterial lipopolysaccharide. Expression of both these cytokines was downregulated by steroids (dexamethasone). IL-1α, IL-1β, and IL-1 receptor antagonist have all been demonstrated in amniotic fluid.[75] Interleukin-1β is produced by amniotic epithelial cells in vitro and is also thought to be involved in the regulation of prostaglandin production.[152] Prostaglandin dehydrogenase, a prostaglandin-inactivating enzyme, has also been demonstrated by Cheung et al.[27] Interleukin-4 has also been shown to suppress the activity of prostaglandin-H synthase-2 in amnion epithelial cells.[79]

Human amniotic interferon γ (IFN-AM) is antigenically unrelated to human IFN-α, -β or −γ. It has some biological activities similar to IFN-α and IFN-β and shows significant cross-species anti-viral activity unlike both IFN-α and IFN-β.[47] It has been suggested that IFN-AM is a novel sub-type 1 interferon.

Studies on human amniotic membrane preserved at −80°C for 1 month revealed the presence of EGF, TGF-α, KGF, HGF, bFGF, TGF-β1, and -β2 by RT-PCR for the mRNA and by ELISA for the protein products.[89] TGF-β3 and growth factor receptors KGFR and HGFR were also detected by RT-PCR. A higher level of various growth factors were found in amniotic membrane with epithelium than without epithelium indicating an epithelial origin for these

growth factors.[89] Significant levels of TGF-β1, -β2, -β3 and their type I and II receptors were also demonstrated in human term placenta. The simultaneous expression of ligands and their receptors support the hypothesis that TGF-β may play an important role in regulating growth, differentiation, and function of the human placenta.[157] Significant binding of EGF to amnion, suggesting presence of EGF receptors, has also been demonstrated.[142]

Endothelin-1 and leukotrienes, produced by amniotic epithelial cells, have also been demonstrated.[24,144] Carbonic anhydrase isoenzymes CA-1 and CA-2 are found in amniotic epithelial cells.[33,123] This enzyme, which is involved in bicarbonate/carbon dioxide metabolism, is thought to have a regulatory role in maintaining amniotic fluid pH. Degrading peptidase dipeptigylpeptidase IV is also present in amniotic epithelium.[60] Secretory leukocyte protease inhibitor is a protein found in various human fluids. It is a potent inhibitor of human leukocyte elastase and has recently been demonstrated in human amniotic fluid and in the amniotic membrane. Its concentrations can be upregulated by exposing amniotic cells to IL-1α, IL-1β, and TNF-α. It is hypothesized that this protein may contribute to immune mediated defense mechanisms during pregnancy.[213]

## MATRIX

Amniotic basal lamina contains large quantities of proteoglycans rich in heparin sulphate. The proteoglycans are thought to perform a barrier function to restrict permeability of the amnion. Amnion contains a large amount of collagen, hyaluronan, and predominantly smaller proteoglycans such a biglycan and decorin, with decorin being more prominent of the two and is located in close connection with the collagen fibrils.[111] The distribution of collagen, proteoglycans, and hyaluronan probably account for the biochemical properties of the membrane.[111]

Collagen types I, III, IV, V and VII,[6,80,120,212] laminin,[6] and fibronectin[102] have been identified in amniotic basement membrane and stromal amnion. Fukuda et al[50] demonstrated similarities between the laminin-1, laminin-5, fibronectin, and type VII collagen components of the basement membranes of conjunctiva, cornea, and amniotic membrane but identified the α-subchain components of collagen IV to be similar between amniotic membrane and conjunctiva but different between amniotic membrane and cornea. Much of laminin-5 contained within the basement membrane of the human amnion is covalently adducted with laminin 6 (α3β1γ1) and with another laminin isoform termed laminin-7 (α3β2γ1).[25] It is proposed that the association between laminin-5 and laminins-6 and -7 is

a mechanism used in amnion to allow stable association of laminin-5 with the basement membrane.[25] The hemidesmosome integrin α6β4 exhibits a distinct basal location in amniotic epithelium, whereas β-1 integrins (α3β1 and α5β1) are located basolaterally.[104] The basally located intgerins promotes cell basement membrane attachment and the basolaterally located integrins may be involved in cell-matrix interactions.[104]

## IMMUNOLOGY

Human amniotic cells do not express HLA-A,B,C, or DR antigens of β2-microglobulin on their surfaces.[2,73] However, radiobiological studies of in vitro cultured cells does suggest that small quantities of these substances are synthesized.[2] This was further verified by Houlihan et al,[71] who found expression of class 1b HLA antigen on full-term amnion but very limited expression of class 1a antigens. Sutton et al[180] have shown that the connective tissue underlying the amnion contains mononuclear phagocytes. Class II MHC antigens are acquired by an increasing number of placental macrophages from the second trimester onward, and by term they may be capable of antigen presentation. The immunologic response to transplantation is negligible: no human volunteers showed clinical signs of acute rejection following transplantation with human amniotic membrane and none of the volunteers tested produced antibodies against the HLA-antigens.[3] Kubo et al[94] used anti–human class I and class II antibodies and demonstrated strong class I expression in amniotic epithelium, mesenchymal cells, and fibroblasts in frozen amniotic membrane. They also found positive reaction against the antibody W6/32 that is known to capture the HLA-G molecule. Some fibroblasts were also positive for class II antigen. In a xenotransplantation model (human membrane in Lewis rats) they did not, however, detect significant immunological responses to membrane transplanted at the limbus or intracorneal.

Hsi et al[73] have identified a unique plasma membrane antigen. Marvin et al[108] studied an amnion epithelium-derived cell line (WISH cells) and demonstrated that amnion epithelial cells can be induced to express intercellular adhesion molecule-1 (ICAM-1) by the pro-inflammatory cytokines, tumor necrosis factor α (TNFα) and interleukin (IL)-1β. The expression of ICAM-1 was increased several fold compared to undetectable levels of expression in primary amnion cells. They postulated a role for amnion in inflammatory processes in addition to is accepted secretory role. ICAM-1 is a known ligand for lymphocytes facilitating adhesion and homing. The presence of ICAM-1 in amnion may implicate these cells in immune-mediated inflammation as well.

It is interesting that several cross-reactive proteins have been demonstrated between amnion and ocular surface tissues. Monoclonal antibodies raised against human amnion have been shown to react against human conjunctival epithelium (antibody GB4) and the central four-fifths of the corneal epithelium (antibody GB9). The peripheral epithelium near the limbus was not reactive.[72] Monoclonal antibodies raised against denuded amniotic basement membrane reacted against basal corneal epithelial cells and basal keratinocytes.[31] A mouse IgM monoclonal antibody raised against an extract of amniotic membrane reacted against the fetal periderm and fetal and adult cornea and conjunctiva.[158]

# Mechanism of Action

Several mechanisms have been proposed to explain the beneficial effects of amniotic membrane transplants in ocular surgery, primarily in ocular surface reconstruction. Some of the mechanisms of action of the membrane are inferred, from the composition of the membrane, rather than proven scientifically in relation to its application in ocular surgery. Not all the proposed mechanisms are applicable to both fresh and preserved membranes. Preserved membrane is considered to be inert tissue with no viable cells. The ability of preserved membranes to influence wound healing by changing the local milieu of growth factors and cytokines must be very limited or nonexistent.

## BIOLOGICAL BANDAGE

Bennett et al[15] used amniotic membrane as a temporary bandage over chronic leg ulcers prior to skin grafting and felt that this generated granulation tissue in the ulcer base, enhancing the success of grafting surgery. De Rotth[36] first used amnion as a replacement for lost conjunctival tissue, and Sorsby[171,172] first used amniotic membrane as a biological bandage in the treatment of caustic burns to the eye.

Hao,[67] who demonstrated the presence of mRNA for both antiangiogenic and anti-inflammatory factors in amniotic membrane, has suggested that amniotic membrane should be applied epithelial cell surface down in order to deliver a high concentration of these factors to the damaged ocular surface. This would be applicable more to fresh rather than preserved membranes.

Use of the membrane as a bandage to cover inflamed or exposed areas, due to injury or surgery, not only favorably influences the healing process but also has a dramatic favorable effect on the levels of pain and discomfort experienced by the patient. It is our clinical experience that when denuded areas

of the ocular surface, particularly the cornea, are covered by amniotic membrane, pain is significantly reduced. This appears to be a mechanical or physical effect because one study has shown that amniotic fluid application to the corneal surface of rabbits following excimer laser photokeratectomy actually enhanced corneal sensitivity and nerve regeneration.[97]

## SUBSTRATE (BASEMENT MEMBRANE) TRANSPLANT

Amniotic membrane has been used in vivo as a substrate for epithelial growth in the management of persistent epithelial defects following infection,[9,100] in neurotrophic corneas,[26,100] and chemical injuries[9,162] and for recurrent erosion syndrome and persistent epithelial defects associated with cicatricial conditions.[100] In addition, it has been transplanted onto corneas to provide a substrate for epithelial growth in cases of bullous keratopathy, resulting in significant reduction of bullae formation and improvement of patient comfort[135] and in combination with limbal stem cell transplantation to promote epithelialization.[162] It has been successfully used to replace stroma and encourage epithelial growth in cases of persistent ulceration associated with neurotropic corneas.[26,91] In this context, neurotrophic factors have been demonstrated in the amniotic membrane and amniotic fluid.[156,198] It is also shown to provide a good substrate for axonal regeneration when used as an amnion tube nerve conduit.[110,121,122] However, it has been unsuccessful in promoting re-epithelialization in cases of persistent epithelial defects with stromal thinning[9] or in patients with perforated corneas.[100] This may be explained by the necessity of restoring corneal biomechanical properties (or architectural structure) in these cases, which can only be achieved with the use of hard tissues, such as cornea, sclera, or others.

Several laminin isoforms, that are not characteristically present in the corneal basement membrane, are present in amniotic basement membrane.[25,95] Interestingly these laminin isoforms in amniotic membrane encourage rapid adhesion and enhanced spreading of corneal epithelial cells.[95] This may be an important reason why the amniotic membrane is effective as a substrate transplant.

In vitro amniotic membrane has been used to cultivate an epithelial sheet for direct transplantation onto the cornea. Koizumi et al[87] cultivated rabbit limbal and corneal epithelial cells on denuded human amniotic tissue. They demonstrated significantly improved growth of cells on membrane denuded of epithelial cells compared with intact

membrane. They subsequently successfully re-epithelialized injured rabbit corneas following transplantation of amniotic disks on which epithelial cells had been cultivated.[88] These cultured cells however failed to show demonstrable basement membrane deposition or basal hemidesmosome complexes.

Providing a suitable substratum for epithelial cell migration and stratification, as a substrate transplant, is perhaps the most important and effective mechanism of action of the tissue. The amniotic basement membrane usually survives the processing and storage procedures and affords a more suitable substratum for epithelial cell growth compared to the amniotic membrane stroma or matrix. The wider spaced collagen of the stroma retards epithelial spread, with cells tending to extend processes or podia into the pores of the collagen matrix (Fig. 2B and C). To effectively invoke this mechanism of action, the membrane, fresh or preserved, is placed on the ocular surface with the basement membrane side up. It is to be noted, however, that in the treatment of partial thickness burns, application of amniotic membrane to the affected skin encourages re-epithelialization to occur under rather than over the membrane.[186,140]

## PROMOTER OF EPITHELIALIZATION

In addition to providing a suitable substratum for epithelial cell migration, as described above, the membrane promotes epithelialization by other means as well. Amnion has long been used in the promotion of epithelialization following skin ulceration or burns.[11,179,205,206] Large epithelial defects created on rabbit corneas by excimer laser healed at a faster rate when they were covered by amniotic membrane compared to controls that were not.[209]

Recently, several authors have reported variable success in the use of amniotic membrane for re-epithelialization of ocular surface problems. Lee and Tseng[100] reported successful re-epithelialization of 10 out of 11 cases of persistent epithelial defect following amniotic membrane transplantation and postulated that amniotic basement membrane facilitates the migration of epithelial cells,[115,117] reinforces adhesion of basal epithelial cells,[81,170,182] promotes epithelial differentiation,[64,96,177] and is important in preventing apoptosis.[17,18] Azuara-Blanco et al[9] reported successful re-epithelialization in four of five patients with chronic epithelial defects but reported that three of these patients required further limbal stem cell transplants to restore ocular surface integrity or for visual rehabilitation. They also reported that amniotic membrane failed to prevent progressive stromal thinning or perforation in four out of four cases of persistent epithelial defect with

stromal thinning or recent perforation. Shimazaki et al[162] used amniotic membrane transplantation in combination with limbal stem cell transplantation to successfully restore corneal epithelium.

In addition to promotion of epithelialization there is evidence that amniotic basement membrane can prolong the life span of corneal and conjunctival progenitor cells grown in vitro[117,118] and maintain slow cycling limbal label retaining cells.[115] The morphology of epithelial cells grown on amniotic membrane is similar to that of normal epithelium[162] and the barrier function measured by fluorescien uptake was slightly higher than normal corneal epithelial cells. By impression cytology, Prabhasawat and Tseng[138] demonstrated increased epithelial cell and goblet cell density on amnion used to reconstruct conjunctival surfaces. Meller and Tseng[117] successfully cultured rabbit conjunctival epithelium on amniotic membrane and noted that amniotic membrane maintained the conjunctival non-goblet epithelial phenotype and allowed epithelial stratification and promoted cellular polarity when the cultivated sheet was lifted to an air interface. Kim and Tseng,[83] using a rabbit model of total stem deficiency, reported that new conjunctiva derived epithelium growing on the transplanted amniotic membrane expressed cornea-specific keratins, CK3 and CK12 in 40% of cells. Cho et al[28] cultivated rabbit conjunctival epithelium on human amniotic membrane and showed that transdifferentiation of conjunctival to corneal phenotype did not occur in vitro.

Koizumi et al[89] investigated the various growth factors present with in preserved amniotic membrane. They used reverse transcriptase–polymerase chain reaction (RT-PCR) to examine for expression of mRNA for eight growth factors (EGF, TGFα, KGF, HGF, bFGF, and TGFβ-1, -2, and -3) and two growth factor receptors (KGFR and HGFR). They found that all these growth factors were expressed by the amnion, in both epithelium and stromal regions. They proposed that these growth factors contributed a significant effect to epithelial regrowth. Shimazaki et al found that amnion produced transforming growth factor β (TGFβ) and basic fibroblast growth factor.[162]

## SUPPRESSER OF INFLAMMATION AND INHIBITOR OF SCARRING

Tseng et al[190] demonstrated that human corneal and limbal fibroblasts grown on the matrix surface of amniotic membranes had marked down-regulation of inflammatory mechanisms compared with controls. Specifically, they noted a marked down-regulation of the TGFβ-signalling system with reduced expression of TGFβ-1, β-2, and β-3 isoforms in addition to reduced expression of TGF-Receptor II. This

had the subsequent effect of preventing fibroblast activation into myofibroblasts as shown by reduced expression of α-smooth muscle actin, fibronectin-EDA, and integrin α5, which represent the biochemical markers of this morphological transformation. They maintain that this mechanism is primarily responsible for the anti-scarring properties of amniotic membrane. Similar results were found by Lee et al[99] when human conjunctival fibroblasts and pterygial fibroblasts were cultivated on the matrix side of amniotic membrane. In a recent study in rabbits, Choi and Tseng[30] demonstrated that corneal epithelial cells induce differentiation of keratocytes into myofibroblasts and this effect could be prevented by placing amniotic membrane as a "barrier" between the epithelial sheet and corneal stroma/keratocytes, both in vivo and in vitro.

On the other hand, Koizumi et al[89] found TGF β-1, β-2, and β-3 mRNA by RT-PCR and protein by ELISA in the amniotic epithelium and stroma. They also found high levels of EGF, KGF, HGF, bFGF, and concluded that these may play important roles in ocular surface wound healing.

In another study, Park and Tseng[131] performed transepithelial photorefractive keratectomy in rabbits and showed that subsequent keratocyte death was associated with polymorphonuclear cell infiltration. Amniotic membrane precluded polymorphonuclear cell infiltration and thereby reduced keratocyte death. Woo et al[209] showed reduced inflammation and corneal haze following excimer laser wounds in rabbit eyes. Choi et al[29] in a randomized prospective study of rabbit corneas undergoing excimer laser demonstrated a reduction of haze in the post operative period if amniotic membrane is applied to the wound, they hypothesized that this may be due to a reduction in inflammatory cell infiltration and loss of keratocytes in the early postoperative period. Hao et al[67] identified the presence of mRNA for cytokines IL-1RA (receptor antagonist) and IL-10 in both amniotic epithelial and mesenchymal cells. These cytokines are potent inhibitors of inflammation. Similar observations were made by Solomon et al.[169] They demonstrated that human corneal limbal epithelial cells cultured on human amniotic membrane stromal matrix showed a significant reduction in expression of IL-1α and IL-1β mRNA and protein, while expression of IL-1RA was upregulated.

Tissue inhibitors of metalloproteases (TIMPS) have been shown to be produced by both amniotic epithelial cells and mesenchymal cells.[67,153] Kim et al[84] compared inflammation in corneal wounds inflicted by an alkali-impregnated disk; they compared wounds covered with amnion against those not covered and found significantly less stromal thinning and faster epithelial healing in the amnion-covered groups. They noted that corneal opacities were least in the amnion-covered group with the epithelial cell side down. In addition infiltration with polymorphonuclear leukocytes and proteinase activity measured by zymology was much less in the amnion-covered groups.

Some of the anti-inflammatory and anti-scarring effects of amniotic membrane are believed to be due its effect on apoptosis. Fas (CD95), a cell-surface receptor that mediates apoptosis, has been demonstrated in amniotic epithelial cells.[154] Fas ligand-positive cells have been shown among the mesenchymal cells of preserved amniotic stroma.[94] Higher amounts of apoptosis was demonstrated in amniotic epithelial cells in the 23–30-week gestation membranes than in the 37–42-week gestation membranes.[154] In a rabbit PRK model, Wang et al[203] demonstrated reduced scarring (corneal haze), reduced inflammation, and reduced keratocyte apoptosis with use of amniotic membrane. They suggested that amniotic membrane matrix might reduce scarring by reducing keratocyte apoptosis. In another study where amniotic membrane was used on patients with persistent epithelial defects, Shimmura et al[164] showed trapping of inflammatory cells in the matrix of amniotic membrane. They also showed apoptosis of trapped inflammatory cells and suggested that this might explain some of the anti-inflammatory effects of amniotic membrane.

## INHIBITOR OF ANGIOGENESIS

Bennett et al[15] and Faulk et al[45] suggested an angiogenic effect for amniotic membrane because they noted that application of the membrane to an ulcer generated healthy granulation tissue prior to skin grafting. Hao et al,[67] using the reverse transcriptase polymerase chain reaction, demonstrated messages for several anti-angiogenic chemicals. Thrombospondin-1, a potent antiangiogenic chemical, was expressed by all amniotic epithelial cells and about 20% of mesenchymal cells. The powerful antiangiogenic and endothelial cell growth inhibitor endostatin, a component of basement membrane heparin sulphate proteoglycan is expressed by both amniotic epithelial and mesenchymal cells. In addition mRNA expression of all four tissue inhibitors of metalloproteases (TIMP-1, -2, -3 and −4) was demonstrated and these proteases are known to have a potent antiangiogenic effect. They suggest that these findings may explain the anti-angiogenic properties of amniotic membrane. In addition they suggest that the anti-inflammatory properties of amniotic membranes further dampen the stimulus to angiogenesis.

Kim et al[82] showed that rabbit corneas with limbal stem cell deficiency and total keratectomy were less

likely to become re-vascularized if covered with amniotic membrane. In their series they concluded that absence of vascularization in turn helped in the retention of amniotic membrane on the cornea promoting resurfacing with an epithelial phenotype similar to corneal epithelium.

As before, if the anti-angiogenic effects are due to chemical inhibitors, then it follows that fresh amniotic membrane should be more effective than preserved membrane. Clinically, retardation of vascularization is also observed with preserved membrane. This may result from a physical barrier effect of the membrane preventing diffusion of or "mopping up" inflammatory mediators and promoters of vascularization. When used in the management of limbal stem cell deficiency due to limbal ischemia, the rationale of this potential anti-angiogenic effect (promoting ischemia) may need to be balanced against its other potential beneficial effects.

## ANTI-MICROBIAL AGENT

Several studies have demonstrated that both amniotic membrane and amniotic fluid have antimicrobial properties in wound healing. Rao et al[143] demonstrated control of infection in patients with full-thickness burns treated with amnion, while Robson and Krizek[147] showed decreased bacterial counts in burns inoculated with *Pseudomonas* and covered with amnion compared with split skin graft or controls. Robson et al[148] showed amniotic membrane to be equal to isograft and superior to both allograft and xenograft at decreasing bacterial levels in full thickness rat skin defects. Antibacterial effects of both amnion and chorion have been demonstrated against a wide range of bacteria, including *Hemolytic streptococcus* group A, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.[85,86] Several antimicrobial factors have been demonstrated in amniotic fluid. Gudson[63] showed bactricidin and beta-lysin in 17 percent of amniotic fluid samples tested. Galask and Snyder[53] demonstrated lysozyme, transferrin, 7S immunoglobulin, and specific antibody activity in third trimester amniotic fluid samples. Hormones such as progesterone, which is known to be present in amniotic fluid, are also bacteriostatic for *staphylococci* and other Gram-positive organisms.[53,210] Hsu et al[74] demonstrated inducible nitric oxide synthase in human fetal amniotic membranes taken from patients with intrauterine infection but not in patients without infection. Similarly, Otsuki et al[129] demonstrated significantly elevated levels of lactoferrin and IL-6 in amniotic fluid of patients with chorioamnionitis compared to those without. Talmi et al[181] believe that there is no inherent antimicrobial substance contained within amnion but that its antimicrobial properties reside purely in its ability to adhere intimately to the underlying substrate. Kjaergaard et al[86] used group B *streptococcus* to demonstrate that amniotic membrane constituted an effective physical barrier against infection.

The clinical significance of the proposed antimicrobial effects of amniotic fluid have to be considered in the context of the knowledge that colonization of the amniotic fluid with bacteria from the upper vagina is common in women whose membranes have ruptured for over 6 hours.[58] Bacterial contamination of the amniotic fluid is a common finding in women with postpartum pelvic infections.[58] Walsh et al[202] studied the ability of amniotic fluid to inhibit growth of five common bacteria isolated from the vagina (*E. coli, Streptococcus* group B and group D, *Candida albicans*, and diptheroids). No inhibition of the growth of any organism was demonstrated.

## CARRIER FOR EX VIVO EXPANSION OF CORNEAL EPITHELIAL CELLS

The ex vivo expansion of corneal epithelial cells on fibrin sheets and subsequent transfer on the corneal surface in the management of stem cell deficiency was first described by Pellegrini et al.[133] In animal experiments He et al[69] demonstrated that both limbus derived human corneal epithelial cells and human amniotic cells could be cultured on the concave surface of collagen contact lenses. The populated lenses were then placed on the experimentally denuded corneal surface of rabbit corneas. Both corneal and amniotic epithelial cells showed polarisation and adhered firmly to the corneal stroma in 24 hours, with the formation of hemidesmosomes. The success rate was less than 50%, however, in this "xenograft" model. With the widespread use of the amniotic membrane as a substrate for epithelial cell migration and adhesion, it was only logical that it would be tested as a substrate to expand epithelial cells in vitro. Schwab reported success with 19 patients in whom the ocular surface had been reconstructed using in vitro cultured corneal epithelial cell transplants.[159] In addition to amniotic membrane, they tried other carriers such as corneal stroma, type 1 collagen, soft contact lenses, and collagen shields. Later, Schwab et al carried out a successful repair of the ocular surface of 10 patients using amniotic membrane devoid of amniotic epithelial cells (by trypsin digestion and mechanical scraping) as a substrate to expand corneal epithelial cells.[160] Koizumi et al surgically created total stem cell depletion in one eye of rabbits with ensuing conjunctivalization of the cornea.[88] They then took a small limbal biopsy from the contralateral eye and expanded the cells on acellular amniotic membrane. These sheets were used to successfully re-establish corneal epithelial

cover on the stem cell deficient eyes. Control eyes that were transplanted with amniotic membrane alone did not heal well. A very similar approach was adopted by Tsai et al[189] in treating patients with severe unilateral manifestations of stem cell deficiency. For transplantation, they used the populated amniotic membranes together with the limbal explant, and they reported success in all six patients. It has been experimentally shown that rabbit corneal epithelial cells expand better on amniotic membrane (human) that has been denuded of the amniotic epithelium than when the amniotic epithelium has been left in situ.[87] Clinically, however, this does not seem to be a major problem because in most surgical procedures in which amniotic membrane has been used, the amniotic epithelium is left in situ and rapid re-epithelialization of the membrane has occurred. We have demonstrated (unpublished observation) that human corneal epithelial cells migrate slowly on amniotic membrane that has been denuded of its basement membrane. The cells throw out numerous filopodia into the coarse collagen framework of the amniotic substantia propria (Fig. 2B and C). The cells migrate rapidly when limbal explants are placed on amniotic membrane (human) denuded of amniotic epithelial cells but with the basement membrane intact (using thermolysin to lift the amniotic epithelium off the basement membrane, Fig. 3A). The epithelial cells stratify (Fig. 3B) and form hemidesmosome attachments with amniotic basement membrane (Fig. 3C). Corneal epithelial cells grow relatively slowly when the amniotic epithelium is left behind and slowest when the membrane has been flipped over and the cells are grown on the stromal surface. In the latter situation, the morphology of the corneal epithelial cells also changes with the leading cells forming several filopodia, some of which extend through the spaces between the amniotic stromal collagen (Fig. 2B and C).

Conjunctival epithelial cells have also been successfully grown on amniotic membrane. Meller and Tseng[117] demonstrated that conjunctival cells expanded on amniotic membrane show a higher cell density and exhibit primarily a non-goblet epithelial phenotype. In an in vivo study on an experimental rabbit model of total stem cell deficiency, Kim and Tseng[83] reported that re-epithelialization over transplanted amniotic membrane was associated with a return of cornea phenotype (cytokeratin 3 and 12 positive cells) in approximately 40% of cells. In an in vitro study Cho et al[28] failed to demonstrate any transdifferentiation of conjunctival epithelial cells cultured on human amniotic membrane. It is therefore likely that in the in vivo experiment, some corneal phenotype of cells escaped the methods employed to create total stem cell deficiency.
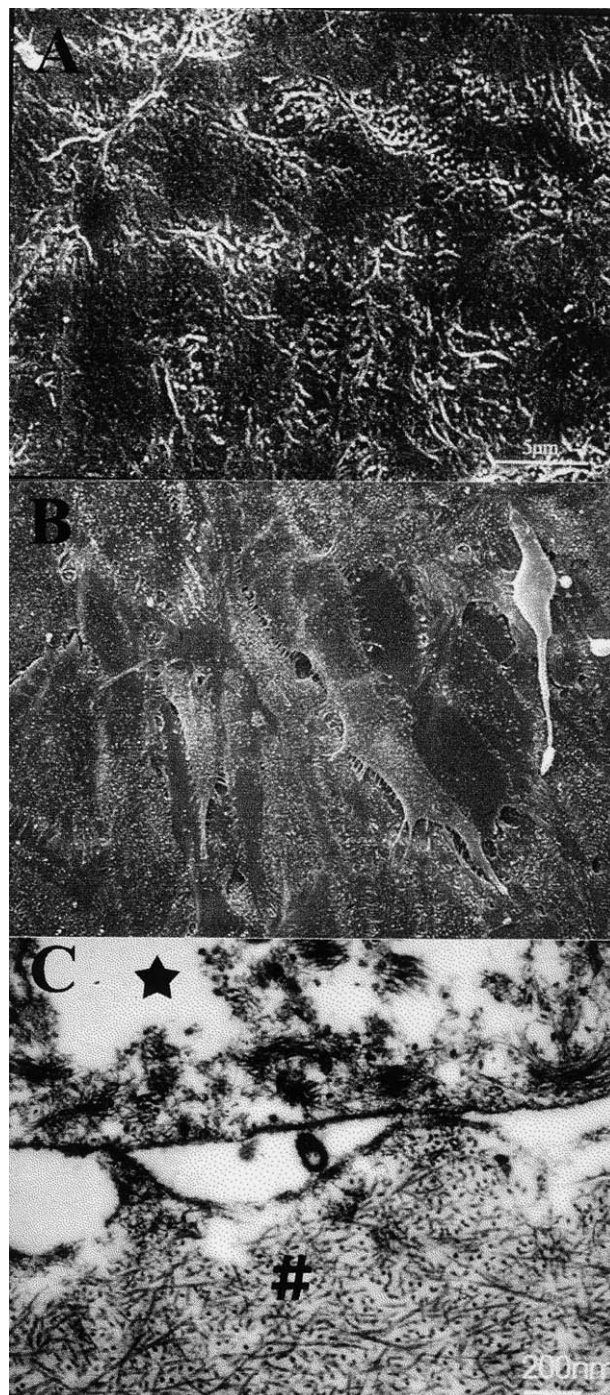


*Fig. 3.* *A*: Scanning electron micrograph of human amniotic basement membrane denuded of the amniotic epithelium with the enzyme thermolysin (×1200). *B*: Scanning electron micrograph of a sheet of corneal epithelial cells cultured on human amniotic membrane prepared as in A above (×250). *C*: Transmission electron micrograph showing hemidesmosomal attachments between a corneal epithelial cell (star) and amniotic basement membrane [#] (×30000).

## Procurement of Amniotic Membrane

Prospective donors are identified in the prenatal clinic with the help of the attending obstetricians and midwives. A social history that is compatible with a healthy mother (donor) is a good starting point. Individuals with a history of drug or alcohol abuse and multiple sexual partners are usually excluded. Informed consent is obtained from all donors and screening for communicable diseases, specifically syphilis, human immuno-deficiency virus (HIV), and hepatitis is carried out. These tests are mandatory and are carried out in the third trimester of pregnancy, as close to the date of caesarean section as possible. All the above tests, especially HIV, are repeated six months after delivery and the tissue used for surgery only if all tests, on both occasions, are negative or non-reactive.

Processing and preparation of the membrane is carried out under sterile conditions. An antibiotic cocktail to cover Gram-negative and Gram-positive bacteria and fungi is used in washing and storage solutions. Two different protocols are in vogue. One popularized by Tsuboto's group[9,41,162] wherein the membrane is cut into pieces measuring 10 cm × 10 cm and rinsed sequentially for five minutes in each of 0.5M dimethyl sulfoxide (DMSO) (4%w/v in 0.01M phosphate buffered saline PBS), 1.0M DMSO (8%w/v in 0.01M PBS), and 1.5M DMSO (12% w/v in 0.01M PBS). The second method was popularized by Tseng and co-workers[82,83,100] and consists of storing the pieces of membrane in 50% glycerol in Dulbeco's modified Eagle Medium (DMEM, Gibco) or TC-199. The pieces of membrane are usually spread epithelial side up, on nitrocellulose paper before storage in medium. The tissue is stored frozen at −80°C and released for use only after the second serological screening test, carried out six months after delivery is normal. Tissue has been stored and used for up to 2 years post-delivery. In the U.S., one company (Bio-Tissue Inc, Miami, Florida) supplies most of the tissue used. Frozen pieces of membrane are transported to the user site in dry ice. The tissue is thawed and rinsed in buffered normal saline immediately before use.

Maral et al[105] preserved human amniotic membrane in 85% glycerol solution at 4°C for over a year and showed that its performance was as good as fresh amnion in the treatment of partial thickness skin burns and reducing bacterial levels in infected burn wounds (in rats). Glycerol has antiviral and antibacterial properties that are dependent on concentration, time, and temperature.[105,106,199,200] Cameron et al[23] have demonstrated that storage of HIV-1–infected cadaver skin in 85% glycerol at 4°C results in complete inactivation of the virus after 5 days. These considerations would give glycerol preservation a distinct advantage over other methods but it must be noted that the current protocols using glycerol for the preservation of amniotic membrane for ocular use utilise a 50% glycerol in tissue culture medium.

Other methods of preserving and storing amniotic membrane for ocular and other uses have been described. These include lyophilization,[22,175] air drying,[107,143] glutaraldelhyde and polytetrafluoroethylene treatment,[124] and irradiation.[107,143,197] Different antibiotic cocktails, 0.5% silver nitrate[65] and 0.025% sodium hypochlorite solution,[147] have been used to render the membrane sterile. Recently, Adds et al[1] examined fresh membranes obtained by elective caesarean and normal vaginal deliveries. They found bacterial contamination in all membranes but they recovered a greater number of different species from membranes obtained by vaginal deliveries. The risk of infection is therefore real and adequate sterilization procedures must be employed, not only during the preparation and storage of the membrane but also periodically, during clinical use of the membrane, to monitor contamination.

Several workers have used fresh membrane for clinical use.[11,54,112,130] Whereas there may be some theoretical advantages of fresh membranes over preserved membranes, the risk of HIV infection despite sero-negativity, due to the window period between infection and sero-conversion, is real.[166] Due the risk of infection with HIV and hepatitis C, tissue transplantation laws in different countries require different protocols for preservation, testing, and storage.[46] In the UK, for any tissue that can be stored, the donor needs to be tested at the time of harvesting and 6 months thereafter.

Most methods employed in the preservation of the membrane affect it in some manner. Kruse et al[90] demonstrated that cryopreservation significantly impaired the viability and proliferative capacity of amniotic membrane and its cells. They concluded that amniotic membrane grafts function primarily as a matrix and not by virtue of transplanted functional cells. Kubo et al[94] have shown that after 2 months of freezing, at least 50% of amniotic cells are viable and capable of proliferation. After 18 months of cryopreservation, they were not able to demonstrate a significant amount of cell survival. Fujisato et al[48] cross-linked amniotic membrane with chemical means (glutaraldehyde) and with gamma-ray and electron beam. They showed that radiation cross-linked membranes degraded rapidly in vitro compared to chemically cross-linked membranes. The weight of the evidence available however, supports the notion that viability of the tissue components of the

amniotic membrane is not essential for its biological effectiveness.

## Indications of Amniotic Membrane Use in Ophthalmic Surgery

### OCULAR SURFACE

In the vast majority of cases, the amniotic membrane is used as a substrate to provide a suitable substratum or basement membrane for corneal or conjunctival epithelial cells to grow on. In such situations it is secured in place with the epithelial (basement membrane) side up and the matrix side down in close apposition to the corneal or episcleral stroma. In other instances, particularly in the presence of acute inflammation, the membrane may be used primarily to protect against the deleterious effects of inflammation. It is then secured in place with the epithelial side down and the matrix side towards the palpebral aperture. It is believed that the matrix traps inflammatory cells and induces apoptosis (see above) thereby reducing or downregulating the inflammatory response. Two membranes, one with epithelial side up and the other, superimposed on it with the epithelial side down may be used together.

### Ocular Surface Reconstruction in Stem Cell Deficiency (Figs. 4 and 5)

Cicatrizing diseases of the ocular surface associated with acute or chronic stem cell loss constitute a major indication for amniotic membrane transplantation. Chemical or thermal burns, Stevens–Johnson syndrome (SJS), and cicatricial pemphigoid are the important conditions in this group. Amniotic membrane transplantation is usually combined with some form of stem cell transplantation, which may be performed simultaneously or subsequently, after the surface has been adequately prepared by amniotic membrane transplantation.

Initial experiments in a rabbit model of stem cell deficiency, created by n-heptanol application and limbal keratectomy, showed that application of amniotic membrane without associated stem cell transplantation reduced vascular in-growth and preserved corneal transparency. It was also associated with a return of cornea-like epithelial phenotype.[82,83] First clinical applications of the membrane in patients with cicatricial pemphigoid, Stevens–Johnson syndrome, and chemical and thermal burns were reported by Tsubota and colleagues.[162,194] Tsubota et al[194] reported very good results in all seven treated eyes with cicatricial pemphigoid and two of four eyes with Stevens–Johnson syndrome. The amniotic membrane used was frozen at −80°C for two weeks before use. Subsequently Shimazaki et al[162] reported a similar success using preserved membrane (frozen in

Fig. 4. A: Amniotic membrane used as a graft to cover the corneal surface in a patient who had an allo limbal transplant for total stem cell deficiency. The amniotic graft is held in place with 10 O nylon sutures. Arrows indicate the limbal allotransplant placed 360° around the cornea. B: Amniotic membrane used as a patch in a patient who had an allo limbal transplant and corneal graft for stem cell deficiency secondary to ocular cicatricial pemphigoid. The membrane is being removed after two weeks.

dimethyl sulfoxide) in five patients with chemical burns and two with thermal burns of the ocular surface. Tseng et al[191] used the membrane in the treatment of 31 eyes of 26 patients with partial and total stem cell deficiency. They extended its application to include patients with a primary diagnosis of toxic epidermal necrolysis, pseudopemphigoid, contact lens–induced keratopathy, aniridia, atopy, and iatrogenic stem cell disease. The majority of their patients, however, had chemical burns or Stevens–Johnson syndrome. They concluded that patients with partial stem cell deficiency could be successfully treated with amniotic membrane transplantation alone, thereby avoiding limbal transplantation. Patients with total stem cell deficiency required both amniotic membrane and limbal transplantation. This series was extended to a total of 47 eyes of 42 patients and published in the German literature with the same conclusions.[116] The same group of investigators[4] further reported their consolidated experience with the use of amniotic membrane, without limbal transplantation, in the treatment of partial stem cell deficiency. They reported success in all 17 eyes of 15 patients thus studied. Amniotic membrane was applied to the corneal surface after debridement of the abnormal epithelium or superficial fibrovascular tissue. All patients had a stable corneal epithelial surface and visual acuity improved in 93% of the eyes. Whereas there is no doubt that amniotic membrane provides a suitable surface for rapid and stable re-epithelialization to occur, as this study has shown, it is still unclear whether amniotic membrane transplantation is superior to simple debridement alone. Such controls were not included in this study or in the previous similar study.[191] Dua et al[42] and Dua[38] have shown that simple debridement, without

Fig. 6. A: Preoperative picture of a neurotrophic corneal ulcer with stromal melting in a patient with a history of recurrent herpes simplex keratitis. B: The area of stromal melt is filled in with a piece of membrane cut to size. C: A second piece of membrane is placed over the first to cover the area denuded of epithelium. D: The membrane sutured in place with interrupted sutures. The patient made a satisfactory recovery with the membrane becoming epithelialized with corneal epithelium.
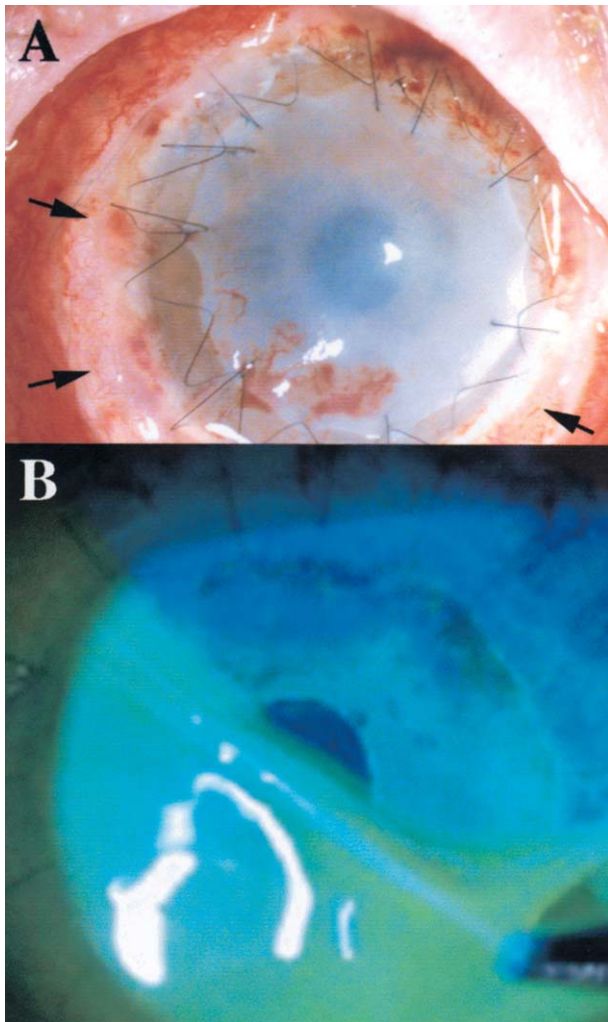
amniotic membrane transplantation, is very effective in corneal re-epithelialization in cases with superficial conjunctivalization associated with partial stem cell deficiency. The use of this technique in the prevention of conjunctivalization in partial stem cell deficiency or in the management of stem cell deficiency
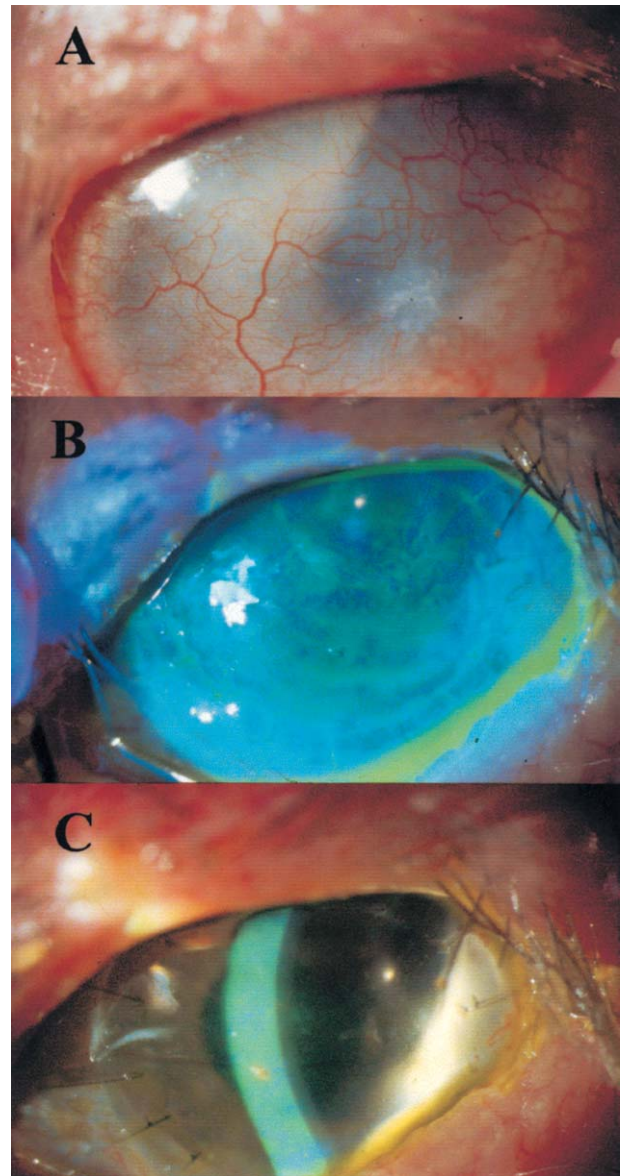




*Fig. 5.* *A*: Clinical picture of a patient with stem cell deficiency due to Stevens–Johnson syndrome. The cornea is scarred and heavily vascularized. *B*: Postoperative fluorescein-stained picture 12 months after conjunctival allo limbal transplant and amniotic membrane transplant. There is no epithelial defect as indicated by the lack of fluorescein stain on the corneal surface. *C*: Clinical picture of the same eye that then underwent a corneal transplant. The graft is clear with a smooth surface 6 months after the transplant.
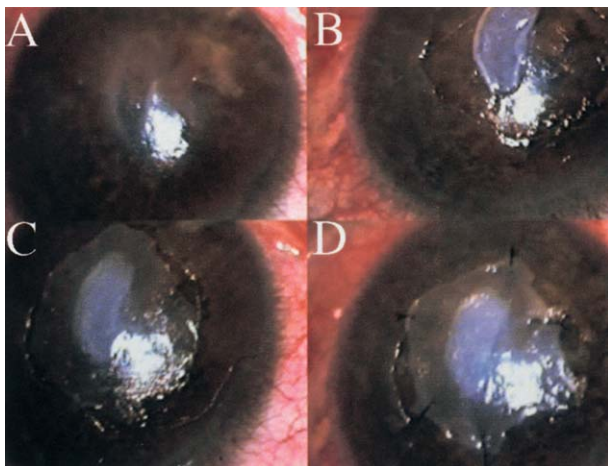
(with or without limbal transplantation) is referred to as Sequential (Sector) Conjunctival Epitheliectomy (SSCE).

Gomes et al[59] reported the results of ocular surface reconstruction with AMT in 11 eyes from 10 patients with cicatricial keratoconjunctivitis (4 with SJS, 6 chemical injuries, 1 trauma) with a mean follow up of 5.22 months. They reported failure in only two

patients with Stevens–Johnson syndrome. Five eyes underwent amniotic membrane transplantation with living related allogeneic HLA matched limbal and conjunctival transplantation. Similar successes have been reported by several other workers.[70,93,119,139,196]

The role of amniotic membrane transplantation in ocular surface reconstruction in patients with stem cell deficiency can best be described as an adjunct to limbal transplantation, especially in patients with total stem cell deficiency.[39] Besides auto- or allolimbal transplantation, several other procedures such as tarsorrhaphy, botulinum toxin–induced ptosis, tenoplasty, lamellar or full thickness corneal grafts, fine needle diathermy or laser occlusion of deep vessels, cataract extraction with or without implants, and drainage or valve procedures for control of intraocular pressure are also required for successful outcomes. One of the major factors affecting successful outcome, whether or not amniotic membrane is used, is the preoperative tear function. Most of the patients with cicatrizing diseases who undergo ocular surface reconstruction have at least moderate dry eye. The use of non-preserved artificial tears and autologous serum drops as advocated by Tsubota et al[194,195] goes some way in addressing this problem. Several workers routinely use autologous serum drops, at least in the initial postoperative period. However, in marked dry eye states with keratinization, specially in immune-mediated diseases as SJS, reconstructive procedures are significantly less successful.[163,193]

Recent reports on the use of amniotic membrane in acute chemical and thermal burns indicate that it helps in the restoration of the corneal surface with preservation of good visual acuity, reducing limbal stromal infiltration, and restoring the conjunctival surface by limiting symblepharon formation.[114,173] However, Joseph et al[77] have reported poor outcomes. The two patients reported by Sridhar et al[173] had around half the limbus surviving. The series of 11 patients reported by Meller et al[114] included cases from seven different centers. The extent of limbal involvement, which is a key to the success or otherwise of corneal resurfacing, was different even among the grade IV burns (Roper-Hall[150]). These variables probably account for the differences between the successful and poor outcomes reported, and they highlight the limitations of the present Roper-Hall[150] system of classification of ocular surface burns, particularly in grade IV, where anything from no surviving limbus up to 50% surviving limbus is grouped together. Another drawback of this classification is that it does not allow for the extent of conjunctival involvement at all. The controversy over the reported success or failure of amniotic membrane transplantation in the management of acute chemical burns reflects the differences in the severity of the burn treated. As

grade IV burns included anything from 50% to 0% surviving limbus, a good prognosis, with amniotic membrane transplantation, is to be expected in burns closer to the former category compared to those closer to latter (total limbal involvement) category. In the context of our present understanding of the concept of stem cells of the ocular surface and the surgical approaches derived thereof, the recent analogue scale of classification (clock hours of limbal involvement/percentage of conjunctival involvement) proposed by Dua et al[40,43] is proving to be more useful.

Almost all the above reports have the common limitation of a short follow-up period of less than 1 year. This is to be expected of a technique which is relative new and recent in its application. Tsubota et al[193] presented the first long term follow up results of ocular surface reconstruction. They evaluated 70 limbal transplantations from cadaveric eyes into 43 eyes of 39 patients with severe ocular surface disorders and stem cell deficiency (25 SJS or ocular cicatricial pemphigoid [OCP] and 14 chemical or thermal injury). The mean follow up time was 1,163 days after surgery. The authors used amniotic membrane as a replacement substrate when underlying stromal tissue had been destroyed, but they did not mention in how many eyes this was used. They found that 51% of the eyes had corneal epithelialization (41% for SJS or OCP and 71% for ocular chemical or thermal burns). Twenty-eight percent of the patients with SJS or OCP and 50% of the patients with ocular burns had clear corneas. Visual acuity improved by two or more lines in 60% of all cases.

From all the above studies (and experience of various surgeons presented at scientific meetings) the emerging consensus is that amniotic membrane transplantation alone is very likely to succeed in the presence of partial limbal stem cell deficiency. However, in patients with total limbal stem cell deficiency, an associated limbal stem cell transplantation procedure is also required.

## Transplantation for Persistent Epithelial Defects (Including Neurotrophic Ulcers and Corneal Perforations) (Fig. 6)

Among the first papers reporting the clinical use of amniotic membrane transplantation in the present surge of interest, is the report and Lee and Tseng.[100] They performed amniotic membrane transplantation in 11 eyes of 11 consecutive patients with corneal ulcers of different causes (3 after microbial infection, 4 neurotrophic keratopathy, 1 recurrent erosion caused by bullous keratopathy, 2 cicatricial keratoconjunctivitis, and 1 keratoconjunctivitis sicca with corneal perforation). The corneal epithelial defects

had persisted for a mean of 17.5 ± 13.9 weeks. Amniotic membrane grafts corresponding to the area of defect were sutured and covered with a bandage contact lens. Ten patients healed in 3.9 ± 2.3 weeks without recurrence for 9.0 ± 5.9 months. One patient failed to heal because of pre-existing corneal perforation due to severe rheumatoid arthritis. Other reports of similar successes in persistent epithelial defects including neurotrophic ulcers soon followed.[61,91–93] Azuara-Blanco et al[9] reported the use of amniotic membrane in cases of epithelial defect with or without severe stromal thinning and noted that it was only effective to promote corneal healing in patients with persistent epithelial defect without severe stromal thinning.

In 1999, Kruse et al[91] reported the use of multiple layers of amniotic membrane for reconstruction of deep corneal ulcers in 11 patients (6 with herpetic keratitis and 5 with other forms of neurotrophic keratitis). Depending on the depth and the configuration of the ulcer, two or more pieces of membrane were stacked one above the other to fill the cavity of the ulcer. This membrane was then secured in place with sutures and covered with a bandage contact lens for 4 weeks. At the end of 1 year of follow-up, the defects had remained healed and stable in 9 of the 11 patients. It also appeared to help to increase stromal thickness to some extent. A modification of this technique was employed by Hanada et al[66] in 11 patients with deep corneal and scleral ulcers. They used the membrane as a space filler (amniotic membrane filling), and covered the defect with a membrane to act as substrate for epithelium to grow on (amniotic membrane graft) and covered this with a larger patch (amniotic membrane patch) in lieu of a bandage contact lens. They reported success in eight patients with complete re-epithelialization in 16.5 ± 8 days. Chen et al[26] used up to three layers of amniotic membrane with an additional patch in some cases, in 16 eyes with neurotrophic ulcers. The neurotrophic state was secondary to keratoplasty, diabetes, radiation, herpes simplex keratitis and neuro-surgery. All but four patients showed healing in an average of 16.6 days. Rosenthal et al[151] used fluid-ventilated gas-permeable scleral contact lenses to treat persistent corneal epithelial defects in 14 eyes. They had six failures of which one healed after multiple amniotic membrane grafts.

In a recent study, Letko et al[101] tried inlay (as a graft) and overlay (as a patch) techniques to treat persistent corneal defects with amniotic membrane. In their patients other methods, such as bandage contact lens and tarsorrhaphy, had previously failed. They observed that their success rate was not as high as reported in the other studies and that there was no difference between the overlay and inlay techniques with respect to incidence of recurrence of defect or time to healing.

Rakowska et al[141] used the membrane in 18 eyes with persistent epithelial defects of which nine had perforated corneal ulcers. They reported "prompt" healing in seven of the nine eyes with perforation and observed that the transplanted amniotic membrane dissolved quickly if the bed was vascularized. Su and Lin[178] reported a case of combined use of amniotic membrane and tissue adhesive in treating corneal perforation. They placed a 1.5-mm piece of amniotic membrane in the anterior chamber (through the perforation) directly under the perforation and applied cyanoacrylate adhesive over the perforation. A bandage contact lens was used. Three weeks later the glue dislodged but the perforation was sealed. Duchesne et al[44] used human fibrin glue and amniotic membrane to seal 2-mm corneal perforations in three patients with satisfactory results. The membrane was applied on top of the fibrin plugging the perforation and in turn covered with a extended-wear bandage lens. Total re-epithelialization occurred in an average of 15 days. Long term, some scarring and thinning was seen at the site of perforation but in no case did the perforation recur.

Amniotic membrane has also been successfully used in the treatment of shield ulcers of vernal keratoconjunctivitis.[174] Mechanical debridement of the ulcer was combine with amniotic membrane transplantation in 7 eyes of 4 patients. All healed within 2 weeks.

## Conjunctival Reconstruction (Fig. 7)

The conjunctiva is an integral part of the ocular surface and conjunctival involvement invariably occurs together with corneal involvement in the cicatrizing conditions described above. Most procedures employing use of amniotic membrane in reconstruction of the ocular surface following burns or cicatrizing diseases address both corneal and conjunctival involvement.[59,162,191–194] Honavar et al[70] used "restoration of adequate bulbar surface free of symblepharon and good fornix depth" as their main outcome measure in 10 patients with SJS treated with amniotic membrane transplantation. They reported success in all patients. Meller et al[114,116] used amniotic membrane transplantation in acute chemical burns and reported that it restored the conjunctival surface without debilitating symblepharon. Others have reported similar successes in the management of symblepharon.[9,52]

Amniotic membrane has also been used to reconstitute the conjunctival surface following resection of conjunctival tumors or other lesions. It has been used in conjunction with beta irradiation and mitomycin
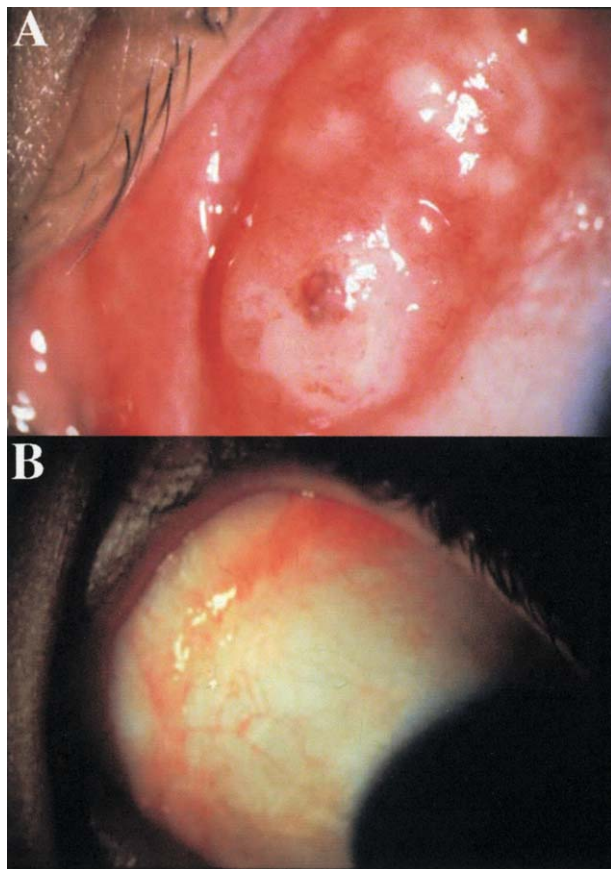
Fig. 7.   *A*: Preoperative picture of a conjunctival granuloma in a 10-year-old boy with Perinaud syndrome. *B*: Two weeks postoperative picture following excision of the granuloma and use of an amniotic membrane graft to the conjunctiva. The membrane is fully epithelialized with conjunctival epithelium.
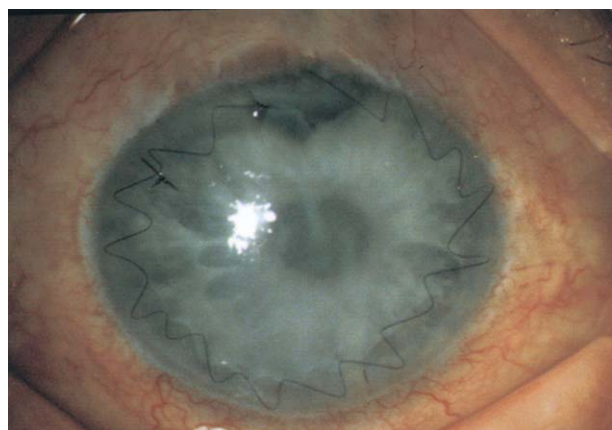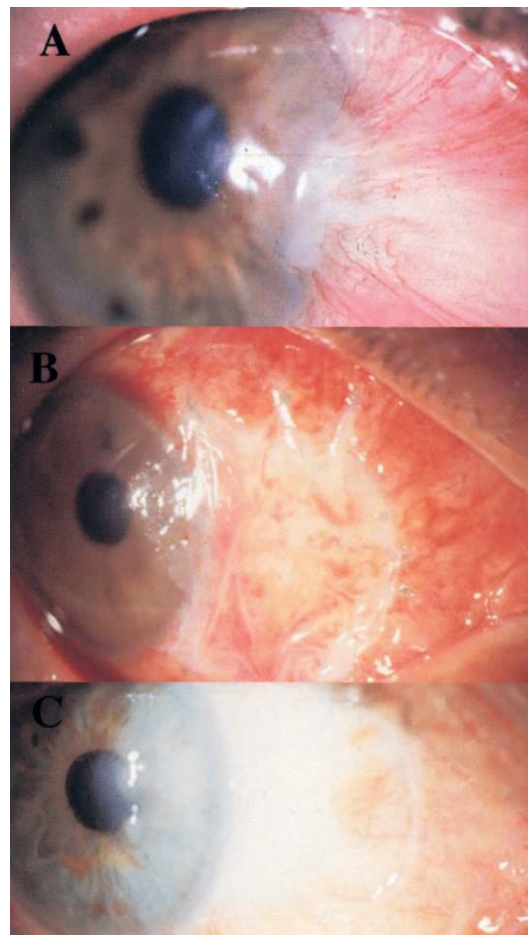




Fig. 9.   Symptomatic pseudophakic bullous keratopathy and secondary glaucoma in a blind eye, one week post amniotic membrane transplant. The membrane is epithelialized with corneal epithelium. The running suture was removed after this picture. The patients remains asymptomatic 6 months later.
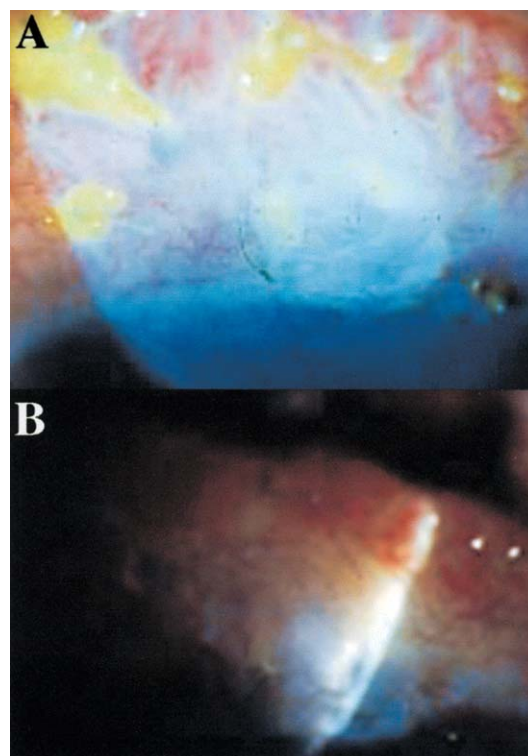
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C.[132,197] Paridaens et al[132] used amniotic membrane to cover large areas denuded after wide excision of conjunctival melanomas and primary acquired melanosis. They reported that the cosmetic result of a thin membrane was far superior to the bulk of thicker mucus membrane grafts. The transparency of the membrane allowed them to monitor for local recurrence.

Meller et al[113] used amniotic membrane to cover large defects created after conjunctival resection for conjunctivochalasis in 47 eyes of 40 patients. They reported improvement in most symptoms including episodic epiphora, in these patients. Focal inflammation, scarring and suture granulomas were observed complications.

## Pterygium Surgery (Fig. 8)

Prabhasawat et al[137] first reported the used of amniotic membrane in pterygium surgery and recommended that it can be used as an alternative first choice in the management of primary pterygium where the recurrence rate was inherently low. When compared to conjunctival autografts they noted, however, that the recurrence rate was significantly higher with amniotic membrane grafts. In a larger study of 80 eyes of 71 patients, Ma et al[103] compared amniotic membrane grafts with conjunctival autografts and topical mitomycin in patients with primary pterygia. They found no differences in recurrence rates in the three groups and suggested that amniotic membrane should be the preferred procedure. Shimazaki et al[161] used amniotic membrane in four patients with recurrent pterygium with symblepharon. They combined this with autologous limbal transplantation and found that a satisfactory outcome was achieved in these patients who had previously undergone multiple procedures for pterygium. Gabric[53] also reported success with amniotic membrane in recurrent pterygia. Solomon[168] used amniotic membrane grafts together with an intraoperative injection of a depot steroid, for primary and recurrent pterygia and observed a recurrence rate of 3.0% (33 cases) for primary and 9.5% (21 cases) for

◀

*Fig. 10.* *A*: Leaking trabeculectomy (with mitomycin C) bleb. *B*: Successfully repaired with an amniotic membrane graft 4 months postoperative.

recurrent pterygia. They proposed it as a useful alternative to conjunctival autograft in pterygium surgery.

## Transplantation for Symptomatic Bullous Keratopathy (Fig. 9)

Amniotic membrane transplantation has also been used effectively for the treatment of symptomatic bullous keratopathy. The technique was described by Pires et al.[135] The bullous epithelium was debrided and the exposed stroma covered with an amniotic membrane graft sutured in place and in turn covered with a bandage contact lens. During the follow-up period of 33.8 weeks after amniotic membrane transplantation, 90% of the 50 patients with intolerable pain preoperatively became pain-free postoperatively. Among the five eyes with residual pain, three received repeated amniotic membrane transplantation, one required a conjunctival flap, and one had reduced pain. We (Dua and Gomes) have used a 9-mm trephine to punch out disks of amniotic membrane and to mark the area of epithelium to be debrided. The edge of the epithelium is rolled outward and then rolled on to the edge of the membrane after securing it in place. The technique was applied in 16 patients and rapid epithelialization of the membrane was noted, usually within 7 days. Postoperative pain scores, both in terms of severity and frequency, were significantly less than preoperative scores. Long-term follow-up results are awaited (unpublished observations).

## Miscellaneous Indications

Amniotic membrane has been used in the management of band keratopathy,[5] repair of a large scleral perforation in a patient of Marfan syndrome,[149] and in an animal model of keratoprosthesis.[98] Anderson et al[5] used amniotic membrane in 16 eyes after surgically removing the calcific deposits in band keratopathy. Ethlene-diamine-tetra acetic acid was used in some patients. They reported reduced pain and a stable epithelium. This did not prevent recurrence of the calcium deposition.

Its potential role in animal models of excimer laser refractive surgery has been assessed and initial reports suggest significant reduction in corneal haze and stromal infiltration. The rate of re-epithelialization appears to be unaffected.[29,131,203,209] We (Dua) used amniotic membrane after photorefractive keratectomy in one patient who was referred to us with marked haze following excimer laser surgery for correction of 6 diopters of myopia. In addition to haze he had a residual 2 diopters of myopia. Repeat PRK was carried out after mechanical debridement of the epithelium and a 9 mm amniotic membrane graft was sutured to the corneal surface immediately after

laser ablation. The patient had minimal pain post laser and re-epithelialization occurred rapidly (5 days). The haze was reduced by 50% but not enough to improve his quality of vision.

## GLAUCOMA SURGERY (FIG. 10)

Many of the features of amniotic membrane make it an attractive tissue for use in glaucoma surgery. It has been used in filtering surgery, as an adjunct to reduce scarring, for repair of leaking blebs, and as a cover for valve implants and exposed pericardium patch.

Fujishima et al[49] incorporated a layer of amnion under the scleral flap to prevent adhesion between the trabeculectomy flap and the underlying scleral bed. They carried out surgery on 13 patients with previously failed mitomycin C trabeculectomies (5 patients), following penetrating keratoplasties (PK) (7 patients), and pseudophakia (1 patient), the mean preoperative IOP was 42.7 mm Hg. All patients had limbal based conjunctival flaps with 0.4 mg/ml of mitomycin C applied for 2 minutes, in addition they had a 2 × 7-mm flap of amniotic membrane sutured epithelial side up between the scleral flap and sclera. They reported limited success from this technique with two patients requiring similar repeat surgery and two requiring laser trabeculoplasty. At 24 months follow-up, all patients had an IOP controlled at <20 mm Hg (mean = 13.8 mm Hg), 2 patients requiring topical medications and 2 requiring topical and oral medications. They concede that these results may be due in some part at least to the use of mitomycin C in the procedure.

Budenz et al[20] suggested that amniotic membrane could be used as an alternative to conjunctiva for treatment of leaking trabeculectomy blebs. They performed a prospective randomized controlled trial comparing bleb revision by amniotic patch transplant or conjunctival advancement. In the amnion-treated group, the old drainage bleb was completely excised and the surrounding healthy conjunctiva undermined for 2–3 mm, the superior cornea was debrided of epithelium, and the amniotic membrane placed over the bare area; its edges were sutured under the undermined conjunctiva and to the debrided cornea anteriorly. They found that nearly 50% (7/15) of the amniotic membrane repairs failed after a mean follow-up of 19 months compared with no failures in the conjunctival-advancement group. These failures were due primarily to bleb leaks. They concluded that amniotic membrane does not provide a suitable alternative to conjunctiva for repair of leaking trabeculectomy blebs. Barton et al[10] used human amniotic membrane in glaucoma-filtration surgery in rabbits and compared it to conjunctival flaps.

*Fig. 11.* Diagram illustrating the different ways in which the amniotic membrane may be used in ocular surface reconstruction. *A*: As a small patch or graft on the cornea, for example, in a persistent epithelial defect. *B*: As a subtotal patch or graft over the cornea, for example, in bullous keratopathy. *C*: As a total corneal patch or graft, for example, in surface reconstruction with or without limbal transplantation. *D*: For release of symblepharon, usually as a graft. *E*: For total ocular surface cover, for example, after acute chemical or thermal burns. The membrane is held in place by fornix retaining sutures tied over bolsters (*F*).

They found that amniotic membrane could be successfully used in place of conjunctiva for constructing filtering blebs. The membrane was associated with longer bleb survival. However, a late xenograft reaction with granuloma formation occurred resulting in disintegration of the membrane.
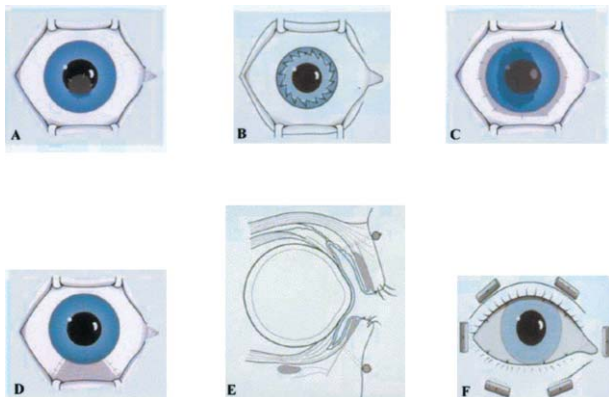
We (Gomes) have used amniotic membrane transplantation in 4 eyes of 4 patients presenting posttrabeculectomy with mitomycin C complications (leaking blebs, 3 eyes; and inadvertent filtering bleb, 1 eye). Complete resolution was achieved in all cases. In one case, it was necessary to add cyanoacrylate tissue adhesive and place a therapeutic contact lens. We (Dua) have also used it in 2 patients to cover the exposed pericardial patch, which was in turn used to cover the tube of an Ahmed valve implant. On both occasions the membrane failed to become epithelized, and underwent necrosis. It was subsequently replaced with an autologous conjunctival patch with good results.

In addition to its use in glaucoma surgery, amniotic membrane has been used to restore ocular surface integrity following partial stem cell deficiency caused by repeated 5-flourouracil injections following glaucoma surgery.[134]

## OCULOPLASTIC PROCEDURES

There are anecdotal reports appearing as posters or presentations at meetings on the use of amniotic membrane in oculoplastic procedures. The membrane has been used for lid reconstruction, punctal

*Fig. 12.* *A*: Microphotograph of a frozen amniotic membrane showing a layer of (nonviable) tall amniotic epithelial cells that overlie the amniotic basement membrane (arrow). Immediately below the basement membrane is the substantia propria (SP) of the membrane followed by the spongy layer (SPON). The spongy layer has a mucin-like consistency (toulidine blue stained). *B*: Photograph illustrating a strand of spongy layer that is being lifted off with a forceps. This allows identification of the undersurface of the membrane for proper orientation.

*Fig. 13.* Exposed "duragard" patch (procine pericardial patch) overlying the tube of an Ahmed valve implant. The exposed patch was covered by amniotic membrane which sloughed off without epithelization in less than 2 weeks.

occlusion, or as a cover for orbital prostheses at the time of insertion or later to cover an area exposed due to extrusion of the implant. A recent report has evaluated the use of the membrane in entropion
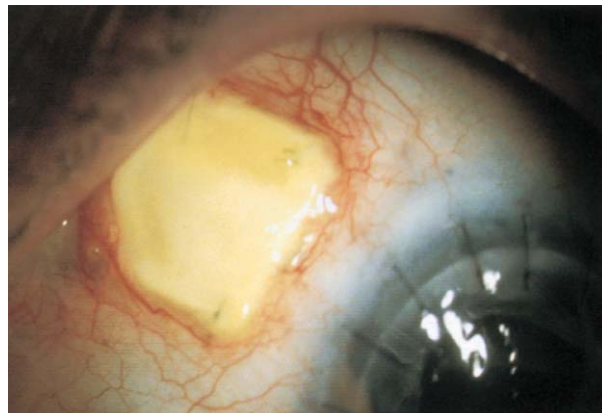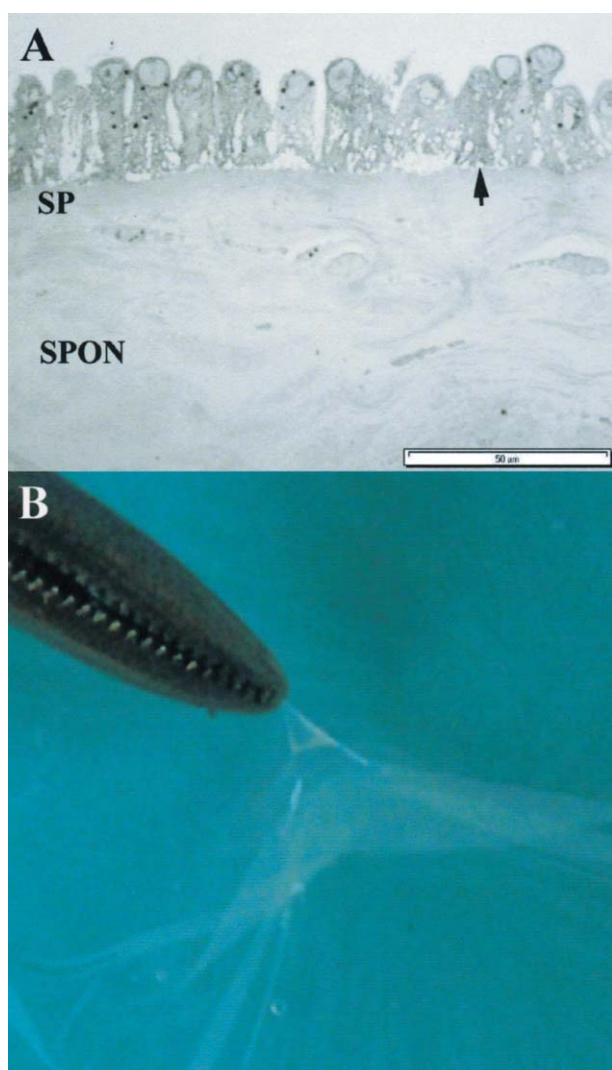
surgery. The membrane was used in a lid-split procedure for correction of cicatricial entropion. It was used to cover the bare tarsus plate up to the lid margin and was found to help the bare tarsal plate to re-epithelialize rapidly.[183] Use of amniotic membrane
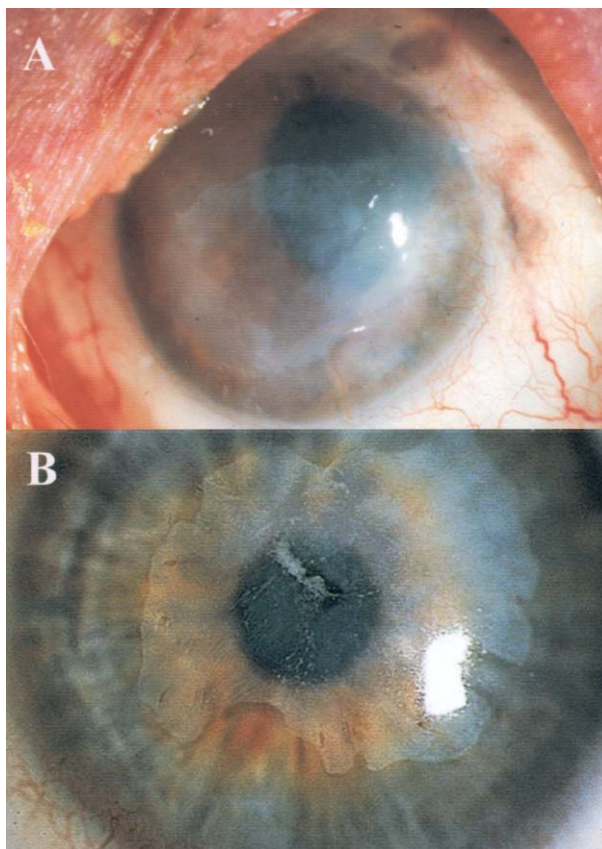




*Fig. 14.* *A*: Subepithelial remnants 12 months after amnniotic memrbrane graft for bullous keratopathy. *B*: Five months after PRK where it was used to reduce haze that occurred after the first ablation.

in punctal occlusion is a modification of the punctal patch technique described by Murube et al.[125] Instead of using a patch of conjunctival mucosa, a patch of amniotic membrane is applied over the denuded punctal orifice.

# Surgical Principles

## CLINICAL APPLICATIONS

The suture material used in conjunction with the amniotic membrane is usually 10-O nylon, 8- to 10-O vicryl or prolene. The sutures may be interrupted, running, or mattress in type. Mattress sutures are generally placed tangential to the limbus, tacking the membrane to the episclera or superficial sclera.

## PATCH OR GRAFT

When the amniotic membrane is used to cover an area of the ocular surface and is eventually removed or falls off, it is referred to as a "patch." When it is used with the expectation that it will become epithelialized and incorporated into the host tissue, it is referred to as a "graft." When used as a patch it is expected that epithelialization will occur beneath the membrane, with the membrane acting as a bandage. When used as a graft, epithelialization is expected to occur on the membrane (substrate transplant).

## PARTIAL OR SUBTOTAL CORNEAL COVER

The membrane may be used to afford partial cover to the cornea when a small non-healing area is covered by a membrane of appropriate size and held in place with a few sutures. It is usually trimmed manually to a size and shape to fit the defect. This is facilitated by first suturing one edge of the membrane to one edge of the defect and then trimming and suturing it along the outline of the defect, a small length at a time. The membrane has a certain degree of elasticity and will stretch. Subtotal corneal cover is usually required in bullous keratopathy or when it is used as a graft in association with auto or allo- limbal transplant. A 9-mm or 10-mm disk of the membrane is trephined and sutured, epithelial side up, on to the denuded corneal surface with a single running or interrupted sutures. In the above situations, the knots may not be buried but the sutured membrane is covered with a bandage contact lens (Fig. 11).

## COMPLETE CORNEAL COVER

In large corneal epithelial defects or in association with limbal transplant operations, it may be necessary to suture the membrane 360° around the limbus to

peritomized conjunctiva. It may act either as a patch or a graft depending on the state of the underlying corneal stroma once the fibrovascular membrane has been removed (Fig. 11).

## BULBAR AND FORNICIAL OR PALPEBRAL COVER

In lid surgery and conjunctival surgery, especially after release of symblepharon or excision of pterygium, the membrane may be used as a patch or graft to cover areas of denuded sclera or episclera. In such situations it is usually applied as a graft. In fornix reconstruction, fornix-deepening sutures may need to be placed and tied on the skin surface over bolsters (Fig. 11).

## TOTAL OCULAR SURFACE COVER

In severe ocular surface burns when extensive areas of the corneal and conjunctival epithelium have been destroyed, the membrane can be used to cover the entire ocular surface. A large patch of membrane is placed over the lids and with a blunt instrument such as a squint hook, the membrane is tucked into the fornices so that a double layer is formed, one covering the palpebral surface and one covering the bulbar surface and cornea. Fornix-deepening sutures are placed and tied on the skin over bolsters, superiorly, inferiorly, medially, and temporally. Excess membrane is then trimmed at the lid margin and the edge tacked to the lid margins (Fig. 11).

In any of the above clinical applications, ex vivo bioengineered membrane populated with limbus derived epithelial cells may be used.

## ORIENTATION OF MEMBRANE

### Epithelial Side Up

The epithelial side of the membrane, with or without the amniotic epithelium, is smooth compared to the stromal (chrionic) side, which demonstrates the rougher spongy layer (Fig. 12A). When required as a substrate for migrating cells, that is, when used as a graft, the membrane has to be sewn in place with the basement membrane or epithelial side up. In clinical practice it is common to use the membrane with the amniotic epithelium attached. When the membrane is supplied, spread on a filter paper, the epithelial side is usually up, with the stromal side applied to the surface of the paper. It is then easy for the surgeon to decide on the orientation of the membrane. When this is not the case or if the need arises to decide on the orientation of the membrane after it has been removed from the paper, a simple technique is helpful: The surface of the membrane is gently pinched with a blunt forceps and lifted. If a thin "vitreous-like" strand is seen extending from the

membrane to the tip of the forceps (Fig. 12B), it indicates the stromal (chorionic) side. This may need to be performed at a few points for confirmation. Conversely, if such a strand cannot be elicited, the membrane should be flipped over and the maneuver repeated on the other side.

### Epithelial Side Down

When used as a biological bandage, primarily to contain the inflammatory reaction while epithelialization is occurring beneath the membrane, it is sutured with the epithelial side against the ocular surface. The stromal side of the membrane traps inflammatory cells and induces apoptosis reducing inflammation.

### Combined Approach

Two membranes can be used, one epithelial side up and the other down. The inner membrane applied to the ocular surface is sutured with the epithelial side up, to act as a graft. The other, usually larger membrane is sutured on top of the first, with the stromal side up. The second membrane acts as a protective bandage for the first membrane and the cells growing on it. One technical adaptation that we (Dua) employ is to overlap the edges of the second membrane with the recessed and peritomized conjunctiva. The conjunctival edge is then tacked on to the membrane. This ensures that any centripetally migrating epithelium from the conjunctiva will grow on the second membrane and not on the corneal/first membrane surface (as can happen when the second membrane is not used). When only one membrane is used as a graft, the advancing conjunctival epithelium has to be sequentially scraped away until the corneal or amniotic graft surface is covered by limbus derived epithelial cells.[82] Avila et al[8] have also described a technique, in rabbits, using two amniotic membranes to sandwich limbal tissue in the management of experimental stem cell deficiency.

### *Multiple Layers*

Multiple layers of amniotic membrane, stacked one on top of the other, can be used to fill in an area of corneal melt or thinning. The final layer is slightly larger than the others, is placed epithelial side up, and sutured to the corneal surface.

The surgical techniques employed in glaucoma surgery and oculoplastic surgery are alluded to under the Indictions of Amntiotic Membrane Use in Ophthalmic Surgery section of this review. These techniques are still to be established but the above principles still apply.

## Fate of Membrane

When used as a patch, the membrane usually falls off, often earlier than desired, or may be eventually removed. When used as a graft, it becomes incorporated into the substratum of the host epithelium and persists for a long time. Fibrillar remnants have occasionally been demonstrated on histology. It may be completely "absorbed" leaving no clinically visible trace (personal observations). At times, a few remnants of an otherwise disintegrated membrane may be visible as wavy white lines or superficial 'scar' tissue. Disintegration of the membrane is often visible as large holes or lacunae developing in the membrane under the covering epithelium (personal observations). When used in the presence of acute inflammation, as after acute chemical burns, it can undergo necrosis and slough or cut through sutures and fall off.

## Complications

Despite the widespread use of amniotic membrane in ocular surgery, very few complications have been reported. Most of the reported complications, such as suture granuloma or persistent inflammation, are not specific to the membrane. Gabler and Lohmann[51] are the only ones to report hypopyon after repeated amniotic membrane transplantation in a case of neurotrophic ulceration who developed a hypopyon 2 days after the second and again after the third amniotic membrane transplant. On both occasions it responded to topical steroids. The authors attribute this to an immune reaction and suggest that use of membrane from different donors (when required repeatedly) may help minimize the risk.

Accumulation of blood (hemotoma formation) under the membrane can occur in the immediate postoperative period or during suture removal a week or so after transplant (personal experience). The blood usually absorbs but if excessive, may have to be drained. A granulomatous reaction, unrelated to sutures, was observed by one of us (Gomes) a few days after ocular surface reconstruction combined with living-related conjunctival limbal allograft in a Stevens–Johnson syndrome patient.

Failure to achieve the intended effect with amniotic membrane is perhaps the single most significant drawback (Fig. 13). The incidence of this should decrease as experience with the membrane increases and enhances our understanding of appropriate patient selection. In our experience one important drawback is the loss of membrane, either by degradation or by cheese wiring of the sutures, in the immediate post operative period. Another less significant undesirable effect is the residual subepithelial membrane that persists in some cases (Fig. 14A and B).

It is our impression that this is more likely to happen if the membrane used is from the relatively thicker portion of the amnion, near the umbilical cord. When this occurs in the visual axis, it can be annoying to the surgeon and the patient.

One must not lose sight of the potential danger of spread of virus and bacteria. As membrane from a single donor can be used in several patients, the risk of single donor to multiple recipients is real. Adequate donor screening to cover the window period, proper handling, processing, and storage, and frequent microbiological tests on used and stored membranes should minimize this risk.

## Conclusion

The reintroduction of amniotic membrane in ophthalmic surgery brings with it great promise. It has been shown to provide a viable alternative or option in many clinically challenging situations. Nevertheless, it is not the panacea that some reports may lead us to believe. Early successes with the membrane have prompted clinicians to explore its possible role in every conceivable condition pertaining to the ocular surface. Disappointments and failures with its applications are therefore inevitable. In some the clinical outcome can be obtained just as easily without the membrane, in others despite the membrane, while in a definite few it will find its niche and prove to be the option of choice. Our understanding of the best method of preservation and its mechanism(s) of action are far from complete. In this regard it will continue to stimulate the clinician and the scientist for the foreseeable future.

## Method of Literature Search

The authors undertook a Medline search (1966–2002) of articles using the key words *amniotic membrane transplantation* (260 citations), *amniotic membrane AND ocular surface* (59 citations), *amniotic membrane AND limbus* (20 citations), *amniotic membrane AND glaucoma* (6 citations). The search was restricted to publications in English and other-language publications with English abstracts. The first set of key words yielded all articles covered by the other searches. A search using the key words *amniotic membrane* yielded over 4,800 citations. All full publications related to ophthalmology were considered. Data published as abstracts only (without a full report) were by and large not covered in this manuscript. Although all ophthalmology-related articles were considered, when several publications dealt with the same issue and expressed the same opinion, only

the first and other significant contributors were referenced. Several key papers dealing with the development, anatomy, biochemistry, immunology, and microbiology of the amniotic membrane, published outside the ophthalmic literature, especially in the obstetric and gynecology literature, were considered and are referenced.

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