

Amniotic Membrane: A Treatment for Prevention of Blindness from Various Ocular Diseases

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Since 1910, human amniotic membrane has been used for many purposes, as reported by Davis et al. Both fresh and preserved human amniotic membranes have been widely used effectively as a biological dressing for acute burns, skin ulcers and abdominal wounds to relieve pain, promote epithelial healing and decrease infection rate of the wound. In 1940, deRoth first described the use of fetal membranes for ocular surface reconstruction. Clinical use of amniotic membrane for ocular surface reconstruction, as a new era was first introduced by Kim and Tseng in 1995. They showed that a corneal surface with total limbal deficiency can be reconstructed with a satisfactory result. Since then, the membrane has been used for many purposes in ocular surface reconstruction.¹

Why do we use amniotic membrane?

The reasons of using amniotic membrane are because amniotic membrane has property of promote epithelial healing, maintain corneal and limbal epithelial phenotype, anti-scarring effect, inhibit vascularization and decrease inflammatory process.¹⁻³ Besides, amniotic membrane is an nonimmunogenic and has a bacteriostatic substance.

Amniotic membrane (AM) is a thick basement membrane with abilities to facilitate epithelial healing, down-regulate inflammation and scar formation by down regulate TGF- β 1,2,3 signaling and TGF- β receptor II expression. It inhibits fibroblast proliferation and myofibroblast differentiation in the cornea and the limbus. It consists of collagen type IV, fibronectin and laminin-1 which are identical to the conjunctival basement membrane. It has been recognized that basement membrane facilitates migration of epithelial cells and reinforces adhesion of basal epithelial cells, causing rapid epithelialization. It expresses mRNAs for several growth factors to benefit epithelialization. It also promotes epithelial differentiation and prevents epithelial apoptosis.⁴

As AM matrix contains several antiangiogenic and antiinflammatory factors, it can inhibit a wide variety of pro-inflammatory cytokines including IL-1 α , IL-1 β , IL-2, IL-8, IL-10, IFN- γ , β FGF, and PDGF. It can also reduce PMNs in acute chemical injury, HSV-1 keratitis, inactivated macrophage¹ and preventing collagen degradation by inhibiting protease inhibitors.^{2,5}

Forms of amniotic membrane

Amniotic membrane has various forms for clinical applications: fresh and preserved amniotic membrane which is in lyophilized (dehydrated) and frozen preserve form, preserved in media and kept in -70 - 80 °C. One of the most concerns of using amniotic membrane is the avoidance of transmitted diseases. For this reason, before preparation, blood transmitted disease such as syphilis, hepatitis and AIDS were checked from donors in the same way as in other organ transplantations. At Siriraj Hospital, with the co-operation of the Bangkok Biomaterial Center, the Department of Ophthalmology and Department of Obstetric and Gynecology, amniotic membranes (Fig 1) were preserved and used for over 1,000 cases for ocular surface reconstruction from 1997 to 2006.

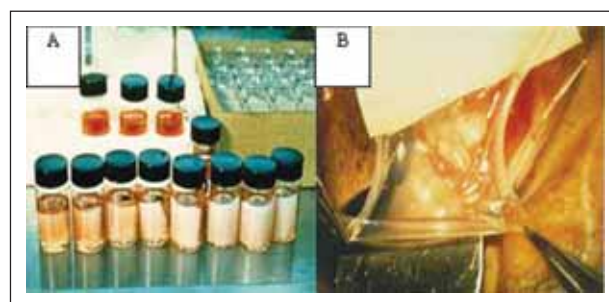


Fig 1. (A) shows preserved amniotic membrane attached with nitrocellulose paper kept in media storage, (B) demonstrate amniotic membrane removing from nitrocellulose paper before use.

Indications of amniotic membrane transplantation

Because of amniotic membrane transplantation (AMT) can give a rapid epithelialization, minimal fibrovascular, less scar formation and give a normal looking ocular surface. At present, it has been used under various indications and has been modified for more applications everyday. It can be used as either a permanent or temporary graft for both corneal and conjunctival reconstruction. It can be used for many purposes, for example : 1) to promote wound healing in persistent epithelial defect, corneal perforation, neurotrophic keratitis, acute burn and Stevens-Johnson syndrome; 2) to serve as adjunctive with limbal

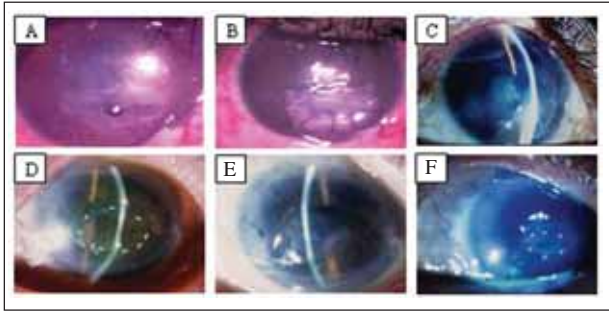


Fig 2. Demonstrate a patient with a history of recurrent HSV keratitis developed a corneal perforation (A). A multi-layer AMT was performed (B). Four months post-operative, a membrane completely dissolved revealing a fully restored stromal thickness with no epithelial defect (C). Pre-operative appearance of patient who had persistent epithelial defect and thinning of the corneal stroma 5 mm at the central cornea (arrow) from herpes (D). Post-operatively epithelial defect was healed completely and stromal bed thickening at 3 weeks (E) Post-op without fluorescein staining (F). Amniotic membrane partially dissolved but still attached at the lesion (arrow)

transplantation *in vivo* or *ex-vivo* cell culture; 3) to be used as a graft in pterygium, conjunctival tumor, conjunctivochalasis, symblepharon, repair socket, lid correction, leaking filtering bleb and covering scleral graft, 4) adjunctive with treatment of corneal infection; 5) to reduce irritation in bullous keratopathy or band keratopathy; and, 6) used in refractive surgery.

Main indications for innovation for sight

1. Corneal reconstruction

*a. Persistent epithelial defect (PED) and ulceration*⁵ The membrane possesses several properties, as mentioned above, to serve all basic principles for managing PED. It acts as a biological bandage lens to prevent mechanical trauma from lids, prevent exposure and reduce surface dryness. Amniotic membrane has been proved by several investigators⁵ that it can promote ocular surface healing and increase corneal thickness. So, it can be successfully used in PED and corneal perforation. (Fig 2)

b. Limbal stem cell deficiency

Corneal limbal stem cell deficiency can cause many problems for cornea⁶ such as vascularization, scarring, PED, ulceration, melting and perforation. Conventional corneal transplantation cannot give good results to these patients. AMT, with or without limbal transplantation, has been successfully used in patients with diffuse or partial limbal stem cell deficiency including Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid, chemical and thermal burn (Fig 3). Not only amniotic membrane



Fig 3. Demonstrate of using AMT combine with limbal transplantation in Stevens-Johnson syndrome. (A) pre-op manifestation shows limbal cell deficiency at superior half with symblepharon. (B) post-operatively lysis symblepharon with amniotic membrane transplantation. After 4 mo of AMT, allo-limbal transplantation was performed, (C) was the 8 mo follow-up post limbal transplantation.

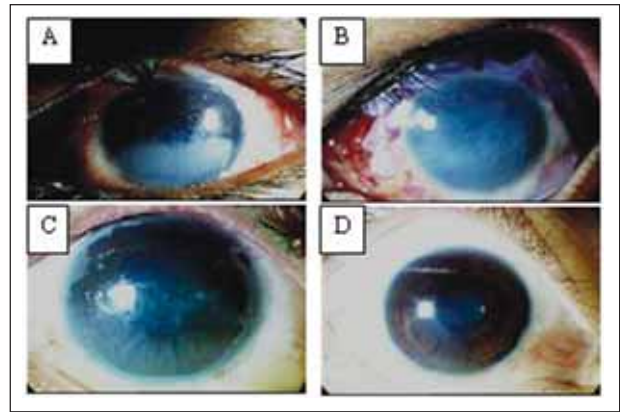


Fig 4. Shows grade 4 ocular burn with large area of limbal ischemia and corneal haze (A). (B) illustrates AMP over the whole cornea and ischemic area. (C) demonstrates ocular burn grade 3 pre-operative. (D) shows the same eye with clear cornea and non-inflamed conjunctiva seven months later.

and limbal stem cell transplantation can be used directly for these patients, autologous and allo-logous limbal-corneal epithelium can be cultured on amniotic membrane (*ex vivo* corneal epithelial cell culture) to expand the number of stem cell and transplant back to the patients for ocular surface reconstruction.^{1,3,6}

Recently, because high graft rejection rate of allogous limbal-corneal epithelium transplantation in bilateral limbal deficiency especially Stevens-Johnson syndrome (SJS), cultivated autologous oral mucosal epithelial transplantation with AMT was introduced to use in these groups of patients. It can treat persistent epithelial defect, to reduce vascularization and improve corneal clarity.⁷

c. Acute SJS, chemical and thermal burn

Acute SJS and burn is one of the most difficult ocular emergencies encountered by ophthalmologists. Severe inflammation in SJS and chemical toxicity, thermal effect and the inflammatory process in burn and can produce extensive damage to the ocular surface. Several reports^{1,8} have addressed a new adjunctive treatment of acute SJS and ocular burn by using amniotic membrane patching with a favorable outcomes. The rationale of AMT application is mainly its ability to promote epithelial healing and reduce inflammation as mentioned above. It promotes reepithelialization underneath amniotic membrane by acting as a biological bandage lens which prevents mechanical trauma from the lids, prevent corneal exposure and reduce surface dryness. (Fig 4) For these reasons, amniotic membrane patches can prevent further damage from acute SJS and burn. However, in severe burn as grade VI, the result of AMT is still not impressive.

d. Infectious keratitis

Infectious keratitis is a major problem in corneal diseases. So far, it is the most common cause of corneal blindness in Thailand. There are many reports of amniotic membrane used to promote wound healing after eradicate the infectious organism, and also to inhibit infection in active stage of both bacterial and fungal keratitis.⁹ There may also be benefit from amniotic membrane in reducing collagenolysis, promoting wound healing and preventing melting. Besides, it may prolong anti-microbial agent effect due to absorbing anti-microbial medications in itself.

e. Refractive surgery.

Because amniotic membrane can inhibit keratocyte proliferation and inflammatory cell, previous reports³ showed the efficacy of amniotic membrane patch in reducing corneal

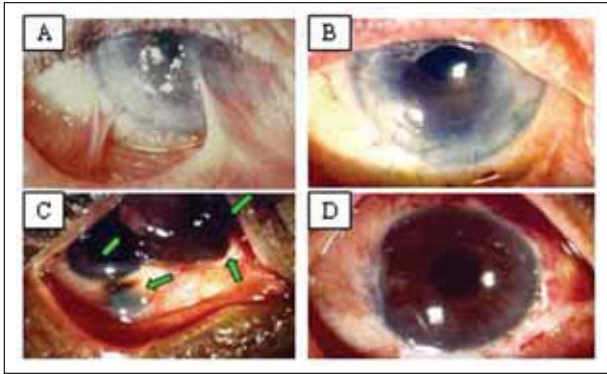


Fig 5. Illustrate multiple recurrent pterygium caused vision decrease to Fc 1 ft (A). Post lysis symblepharon with AMT combine with MMC and sector limbal transplantation 2 months (B). Demonstrate a large conjunctival malignant melanoma (arrow) which need a graft to cover 360 (C), Post tumor excision with AMT at 1 mo follow-up (D).

haze after photorefractive keratectomy (PRK) in animal and human studies. Recently, Lee et al. reported the use of amniotic membrane as a pressure patch in the treatment of epithelial ingrowth under a damaged laser in situ keratomileusis flap.

2. Conjunctival reconstruction

AMT for conjunctival purpose is not directly to improve. However, in severe cases such as multiple recurrent pterygium or advance cases of pterygium, conjunctival tumor or symblepharon can also be harmful to vision (Fig 5). In these cases, amniotic membrane helps to serve the need of a large graft without destroying their own conjunctiva or mucosa. Also, as in corneal reconstruction, amniotic membrane can be used as a substrate to culture conjunctival epithelial stem cell.

3. Scleral disease.

In cases of large scleral graft or lacking of conjunctiva, to cover scleral graft, amniotic membrane can be used to cover the graft. (Fig 6). This procedure helps to protect the globe perforation.

4. Glaucoma problem.

Bleb leaks after filtration surgery can harmful the whole globe from hypotony, shallow anterior chamber, choroidal detachment and endophthalmitis. There were reports

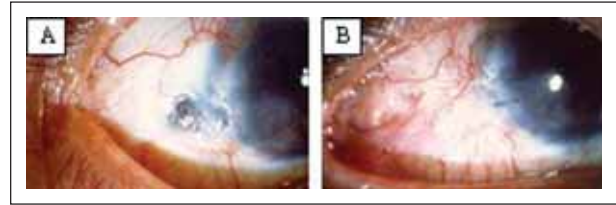


Fig 6. Demonstrate a patient of scleral thinning post pterygium excision with MMC (A). (B) Illustrate the clinical manifestation 2 months after scleral graft covered with AMT and conjunctival flap.

of amniotic membrane used for revision of leaking blebs. However, the result does not seem to be highly effective.

Recently, fibrin glue was introduced to fix amniotic membrane instead of suturing technique in many operations such as pterygium excision, persistent epithelial defect, and lysis symblepharon. This technique reduces scar reaction and shortens the surgical time.

In summary, amniotic membrane appears to serve as an important resource to ophthalmologists to manage the severe ocular surface diseases that are failed by conventional therapy. Indication and applications of amniotic membrane is rising every year and the efficacy is attractive. It is an innovative procedure to improve and protect the sight.

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