

■ **Review article**

Role of amniotic membrane transplantation in ocular surface disorders

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Abstract

The advent of amniotic membrane (AM) and limbal stem cell grafts have transformed the treatment of diseases resulting in ocular surface failure. The current success may be attributed to cryopreservation of human AM, which retains its properties and renders the amniotic epithelial cells nonviable and thus nonimmunogenic. Its unique properties have prompted its application in a large number of ocular ailments. The present article reviews the properties of AM and its uses in ophthalmic practice.

Keywords: amniotic membrane transplantation, ocular surface disorders, chemical injury, dry eye

Introduction: Regenerative medicine is a new field based on the use of stem cells to generate biological substitutes and improve tissue functions. The three essential factors involved are: stem cells, which retain the capacity to renew themselves and may be able to restore damaged tissue with high proliferability and differentiability; the scaffolds that support them; and growth and differentiation factors. The advent of amniotic membrane (AM) and limbal stem cell grafts have transformed the treatment of diseases resulting in ocular surface failure.

History

In 1910 Davis was the first to report the use of fetal membranes as surgical material in skin transplantation (Davis 1910). Since then the use of amniotic membrane in surgery has been expanded. The earliest use in ophthalmology dates back when de Rotth (1940) used fetal membranes, both chorion and amnion, in treating conjunctival defects. Fresh membrane was used as a dressing. Sorsby & Symmons (1946) used dried and chemically processed human AM for chemical burns involving the eye. For almost 5 decades thereafter, it was unheard of until Kim & Tseng (1995) propelled it

into the limelight by using noncryopreserved human AM as a xenograft in rabbit eyes. The current success may be attributed to cryopreservation of human AM, which retains its properties and renders the amniotic epithelial cells nonviable and thus non-immunogenic. Its unique properties have prompted its application in a large number of ocular ailments.

Anatomy

The human AM is the innermost layer of the placenta and has a single layer of ectodermally derived columnar cells attached to a basement membrane with an underlying layer of mesenchyme. Histologically the amnion is a 0.02 mm to 0.5 mm five layered membrane. The epithelium consists of a single layer of cuboidal cells with a large number of microvilli on the apical surface. The basement membrane is a thin layer composed of a network of reticular fibers. Histochemically the basement membrane closely resembles that of the conjunctiva. The compact layer contributes to the tensile strength of the membrane. The fibroblast layer is the thickest layer of the AM made up of a loose fibroblast network. The outermost layer of the amnion is the spongy layer.

Functions

The structural integrity, transparency and elasticity of the amniotic basement membrane make it currently

Received on: 02.02.2010 Accepted on: 14.05.2010
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the most widely accepted tissue replacement for ocular surface reconstruction. The AM has several properties that render it extremely useful in ocular surgery:

1. Promotes epithelialization: AM acts like a basement membrane and facilitates the migration of epithelial cells (Lee & Tseng 1997). It reinforces adhesion of basal epithelial cells, promotes epithelial differentiation, prevents epithelial apoptosis, and improves corneal sensitivity and tear film stability; it also produces growth factors that promote epithelial cell growth. The AM can be used to promote non-goblet cell differentiation of the conjunctival epithelium.
2. Inhibits Fibrosis: Fibroblasts are normally responsible for the scarring associated with wound healing and are activated by transforming growth factor (TGF b). Amniotic membrane downregulates TGF-b and the receptor expression by fibroblasts and thus reduces fibrosis like in conjunctival and pterygial fibroblasts (Tseng et al 1999).
3. Antiinflammatory and antiangiogenic factors: The AM probably acts as a barrier against the tear film resulting in a reduced amount of inflammatory cells and hence the amount of inflammatory mediators (Chen et al 2000). Tissue inhibitors of metalloproteinase inhibitors (TIMPs) 1, 2, 3, and 4 interleukin (IL)-10; and IL-1 receptor antagonist anti-inflammatory factors along with endostatin (inhibit endothelial cell proliferation, angiogenesis and tumor growth) are present in human AM. In addition, the presence of proteinase inhibitors promote wound healing.
4. Antimicrobial and antiviral properties: AM seems to have antimicrobial properties that decrease the risk of postoperative infection (Talmi et al 1991). It also contains cystatin E, an analogue of cysteine proteinase inhibitors, which has complementary antiviral properties. Its antimicrobial and possible antiviral properties warrant further studies.
5. High hydraulic conductivity: The AM has a high hydraulic conductivity, thus facilitating its use in bleb repair following glaucoma-filtering surgery. The non-immunogenicity of the AM was thought as AM did not express HLA-A, -B, or -DR

antigen since after transplantation it did not undergo rejection (Adinolfi et al 1982). Subsequent studies by several authors have shown class 1 antigen and co-manifestation of class 1a (HLA-A, -B, -C, -DR) and class 1b (HLA-G and HLA-E) antigens in amniotic epithelium and in mesenchymal cells and fibroblasts of human AM.

The technique of human AM processing and cryopreservation with Dulbecco Modified Eagle Medium and 50 % glycerol recommended by the FDA renders all the amniotic cells nonviable and hence its immunogenicity is of no consequence. In addition, the human AM appears to be an immune-privileged tissue despite the expression of class 1a and 1b antigens and contains immunoregulatory factors that include HLA-G and Fas ligand. Human AM also has the ability to suppress T lymphocytes.

Procurement and preservation

The current success may be attributed to cryopreservation of human AM, which retains its properties and renders the amniotic epithelial cells nonviable and thus nonimmunogenic (Mejia et al 2000).

Amniotic membrane is obtained from donors undergoing Caesarean section, who are negative for HIV, hepatitis and syphilis. Different protocols exist for the processing and storage. According to Kim et al (1995) the placenta is cleaned with balanced salt solution containing a cocktail of antibiotics (50 mg/ml penicillin, 50 µg/ml streptomycin, 100 mg/ml of neomycin as well as 2.5 mg/ml of amphotericin B) under sterile conditions. The amnion is separated from the chorion by blunt dissection. The separated membranes are cut in different sizes placed on nitrocellulose paper strips with the epithelial side up. Dulbecco Modified Eagles Medium/glycerol (1:1) is used for cryopreservation and the tissues are frozen at -80 degrees until further use. Amnion stored in 50-85 % glycerol is reliable and effective for over a year, with the added advantage of antibacterial properties. Lyophilized AMs were found to be impermeable to different strains of bacteria - Bacillus, Escherichia coli, Pseudomonas, Citrobacter, Flavimonas and Staphylococcus. Before use, the membrane is thawed by warming the

container to room temperature for 10 minutes. This technique of cryopreservation followed by thawing renders all the amniotic epithelial cells nonviable and the tissue nonimmunogenic.

Hyper-dry amnion

At present, in most cases, cryopreserved amniotic membrane tissue has been used clinically for membrane grafts. The immunogenicity of cryopreserved tissue is generally thought to be less than that of fresh tissue. The low-grade inflammatory responses were observed when viable amniotic epithelial cells were present (Akle et al 1981) suggesting that live amniotic membrane is immunogenic. To overcome these problems, a novel dried amniotic membrane (Hyper-dry amnion), using far-infrared rays and microwaves, in addition to α -irradiation for sterilization can be prepared. There is still a lack of appropriate indications or scientific evidence based on randomized comparative studies to prove that its use is better than other alternative therapies.

Uses in ophthalmology

Persistent epithelial defect and neurotrophic ulcer

The management of a persistent epithelial defect is aimed at treatment of the underlying disease process, control of inflammation, and protection of the surface. The AM has several properties that promote epithelialization and reduce inflammation. Multiple layers of AM restore stromal thickness in deep and perforated noninfectious ulcers and probably provides a substrate for collagens and growth factors for epithelial healing. (Fig 1, 2, 3 & 4). The limitations of AMT include continuous tissue destruction beneath the graft, the need for adequate stem cells at the limbus and normal keratocytes in the surrounding tissue, and intact sensory innervation for healing (Prabhasawat et al 2001).

AM has been used as an adjunct to FDA-approved fibrin glue for management of corneal perforations up to 2 mm. The AM provides better adhesion to the surrounding epithelium and prevents dislodgment of the glue.

Shield ulcers

Since the AM enhances epithelialization, and has antiscarring and antiinflammatory properties, it was

used in 7 eyes of 4 patients with grade II and III shield ulcers unresponsive to conventional treatment (Sridhar et al 2001). It helps in early healing.

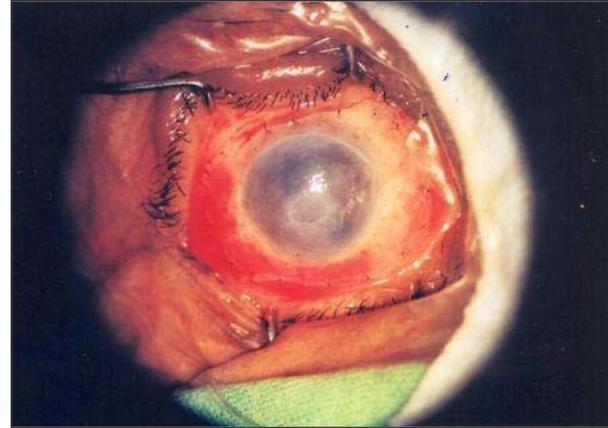


Fig 1: Preoperative photograph showing persistent epithelial defect



Fig 2: Postoperative photograph showing healed epithelial defect after AMT

Fig 3: Preoperative photograph showing persistent epithelial defect after deep anterior lamellar keratoplasty (DALK).



Fig 4: Photograph showing healed epithelial defect after AMT

Infectious keratitis

AMT has been used effectively in microbial keratitis of bacterial, parasitic, and fungal etiology, although the duration of therapy prior to AMT and the time to resolution following AMT are not mentioned (Kim et al 2001). Monitoring the disease process beneath the membrane may be difficult (Fig 5, 6 & 7).

Bullous keratopathy

AMT has been used for alleviating pain in eyes with symptomatic bullous keratopathy with poor visual potential. Relief of pain ranged from 88 % to 90 % over a period of 4 weeks to 45 months (Mejia et al 2002). The exact mechanism of action of AM in reduction of pain is unknown. This property has been observed in patients with severe skin burns. Several of the properties described earlier promote ocular surface epithelial healing and cell adhesion.

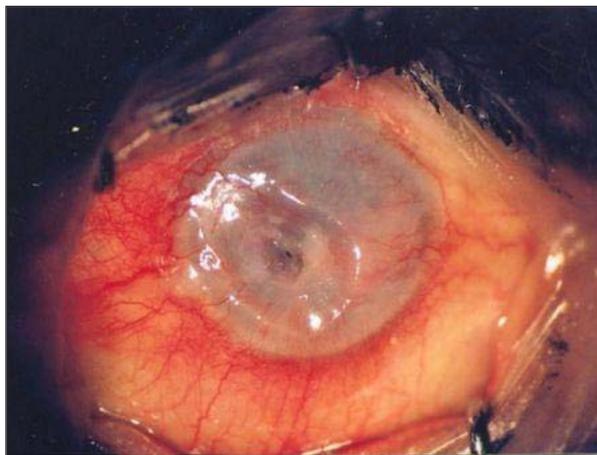


Fig 5: Preoperative photograph showing corneal fistula



Fig 6: Postoperative photograph showing healed corneal fistula after AMT

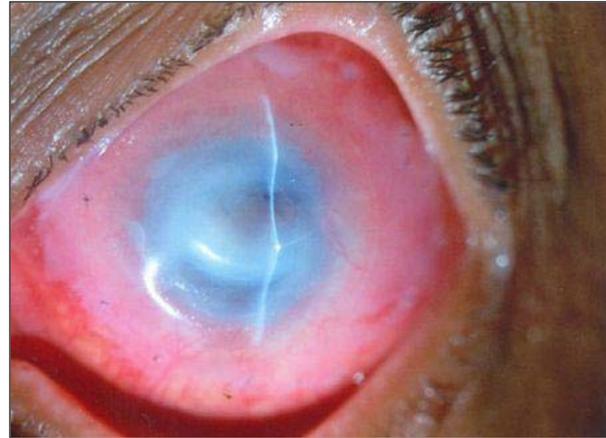


Fig 7: Perforated corneal ulcer with AMT

Band keratopathy

Patients with band keratopathy experience ocular pain due to corneal epithelial breakdown and ocular surface instability. In a series of 16 eyes with band keratopathy, superficial keratectomy with or without ethylene diamine tetra-acetic acid (EDTA) chelation followed by AMT resulted in an improved ocular surface and a pain-free postoperative course in 15 (93.75 %) eyes (Anderson et al 2001).

Photorefractive keratectomy and phototherapeutic keratectomy

AMT following excimer laser photorefractive keratectomy in rabbit eyes demonstrated no effect on epithelial healing, reduced influx of inflammatory cells, decreased keratocyte apoptosis, decreased keratocyte proliferation, reduced late subepithelial fibroblast hyperplasia, and more regular architecture of corneal lamellae, thus resulting in reduced corneal haze (Choi et al 1998).

There is only 1 report of AMT in human eyes for reducing subepithelial fibrosis following severe corneal haze and regression after phototherapeutic keratectomy and laser-assisted epithelial keratomileusis (Lee et al 2003). In all 3 eyes, visual acuity improved to 20/40 from less than 20/100, with minimal haze after epithelial debridement, phototherapeutic keratectomy, and AMT.

Chemical injuries

In the acute stage, severe ocular surface inflammation and epithelial breakdown may progress to tissue melting. The aim of treatment is to reduce inflammation, promote epithelialization, and prevent

tissue necrosis, thereby avoiding the scarring sequelae and debilitating visual loss that ensues in the chronic stages. In mild to moderate chemical injuries, AMT restores corneal and conjunctival surfaces. It prevents symblepharon formation in severe burns (Meller et al 2000) (Fig 8,9,10 &11). In severe cases, owing to extensive ocular surface inflammation with deep stromal ischemia and near-total destruction of the limbal stem cells, the AM may at best reduce inflammation, prevent further stem cell damage, and prevent symblepharon formation in the acute stages.

Conjunctival surface reconstruction

Conjunctival autograft and mucous membrane grafts have been used in ocular surface reconstruction despite the poor cosmesis, risk of infection, limited availability, and scarring at the donor sites (Neuhaus et al 1982). It was used successfully for ocular surface reconstruction in OCP and Steven Johnson Syndrome (SJS) patients and has been used as a “substrate” for conjunctival defects after the excision of cicatricial tissue, excision



Fig 8: Preoperative photograph showing symblepharon



Fig 9: Postoperative photograph after symblepharon release with AMT



Fig 10: Preoperative photograph showing symblepharon



Fig 11: Postoperative photograph after symblepharon release with DALK and AMT

of dysplasia and tumors, acute toxic epidermal necrolysis, conjunctivochalasis, ocular cicatricial pemphigoid and Stevens-Johnson syndrome.

The ability of AM to reduce scar and reduce inflammation and promote epithelialization described earlier are beneficial in ocular surface reconstruction. The other benefits of AM in ocular surface reconstruction include improved cosmesis, ability to monitor local recurrence of tumor beneath the transplanted AM when used following excision of ocular surface squamous neoplasia or malignant melanoma as compared with the thicker buccal mucous membrane grafts, reservation of eyelashes due to AMT on the lid margin in toxic epidermal necrolysis (TEN), and entropion reversal due to release of cicatricial tissue (John T et al 2002).

The factors responsible for failure are dry eye (Calonge M 2001), previous treatment with mitomycin-C or beta irradiation resulting in unhealthy conjunctiva surrounding the AM, uncontrolled systemic pathology resulting in ocular surface inflammation necessitating the use of immunosuppression, and total destruction of conjunctival epithelial stem cells.

Partial limbal stem cell deficiency

In unilateral cases with partial LSCD, limbal stem cell transplantation is not always required. The patient may be kept under close observation, subjected to repeated mechanical debridement also known as sequential sector conjunctival epitheliectomy (SSCE) or AMT. The AM was effective in reducing symptoms, restoring the ocular surface stability, and improving vision in a majority of patients with limbal stem cell deficiency ranging from 90 to 330 degrees who were followed up for 12-34 months (Dua HS 1998).

Pterygium: (Fig 12 & 13) The ability of AM to suppress normal conjunctival and pterygium body fibroblasts among its other properties (vide supra) has prompted its use in management of pterygium. Following a modification of the surgical technique, recurrences of 3 % and 9.5 % for primary and recurrent pterygia respectively were reported (Solomon et al 2001). Results were comparable to those following conjunctival autograft (Ma DH et al 2000), and much less than that for bare sclera technique (10.7 % versus 38.7 %) (Tekin NF et al 2001). AMT has been used in combination with conjunctival or limbal autograft, and intraoperative mitomycin C. AMT has been recommended as the first line of management for primary pterygium, especially for double-headed pterygium to cover a large conjunctival defect or to preserve superior bulbar conjunctiva for future glaucoma surgery (Ti & Tseng 2002).

Cultured stem cells

The AM has been used for culturing conjunctival epithelial cells to promote a predominantly conjunctival nongoblet epithelial phenotype with expression of microvilli, intercellular junction, and increased density of desmosomes and hemidesmosomes. The AM preserved conjunctival epithelial progenitor cells for goblet and nongoblet

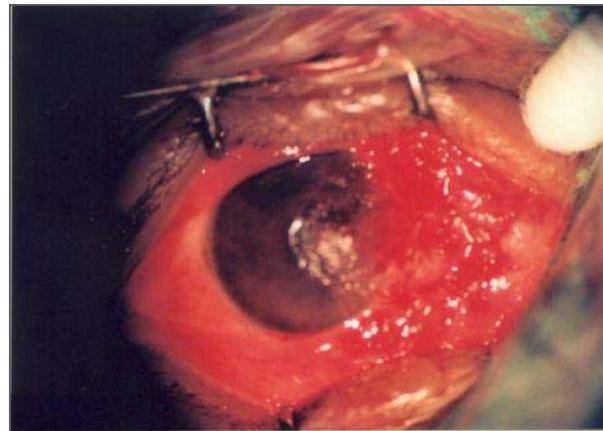


Fig 12: Preoperative photograph of pterygium



Fig 13: Postoperative photograph after pterygium excision with AMT

cell differentiation. Goblet cell differentiation requires a more stringent environment and may depend on fibroblasts, although further studies are needed to explore this aspect (Meller et al 2002). Regrafting of Cultured Corneal Epithelium using AM was done and successful surface reconstruction for over a year was achieved with cultivated allolimbal stem cells on AM in 3 eyes with failed cultivated limbal stem cell transplants due to epithelial rejection (Nakamura T et al 2003).

Cultured limbal and conjunctival epithelium was used for bilateral ocular surface disorders. The ocular surface remained stable with improvement in vision at 1-year follow-up (Sangwan VS et al 2003). AMT was used for filtering blebs as an adjunct to glaucoma-filtering surgery as a substitute for antifibrotic therapy (Barton K et al 2001). The AM was placed beneath the scleral flap to inhibit scarring

(Budenz DL et al 2000). Cicatricial entropion may be due to chronic blepharoconjunctivitis, trachoma, burns, chemical injuries, trauma, and systemic mucocutaneous disorders like Stevens-Johnson syndrome and ocular cicatricial pemphigoid. It promoted rapid epithelialization of the bared tarsus within 2 weeks but did not prevent subsequent lid margin keratinization or tarsal shrinkage.

Surgical technique

AM may be used either as a graft (inlay) or a patch (overlay) or in multiple layers. If preserved at -80°C , it should be thawed to room temperature before use. The membrane is usually sutured with the epithelial side up and the mesenchymal side down to facilitate adherence to the ocular surface (Dua & Azuara-Blanco, 1999). It is thus important to identify the 2 surfaces of the membrane. While placing the membrane on nitrocellulose paper, the basement membrane side is allowed to face up or a suture may be tied with the knot indicating the basement membrane. Blunt forceps or a surgical sponge may be used to identify the mesenchymal side since the forceps draw a vitreous-like strand from this side and tends to adhere to a surgical sponge. The loose epithelium around the epithelial defect and slough within the base of the corneal lesion is removed. The AM is spread onto the surface ensuring that no blood or fluid is trapped underneath it and sutured to the cornea with 10-0 monofilament nylon sutures and to the conjunctiva with 8-0 or 9-0 Vicryl sutures.

Inlay technique: The AM is trimmed a little larger than the size of the defect, anchored in place with the basement membrane side facing up. It thus functions as a basement membrane on which the corneal epithelium can grow

Overlay technique: (Fig14). The AM is spread over the whole cornea and the perilimbal area and anchored with 10-0 monofilament sutures at the limbus and occasionally the mid-peripheral cornea with a running 10-0 polygalactin suture. It functions as a bandage contact lens and also acts as a barrier to protect the cornea from inflammatory cells and proteins in the tear film (Letko E et al 2001). The inlay and overlay techniques may be combined, ie, first an inlay graft followed by an overlay patch.

Multilayered technique: (Fig 15). In cases of deep ulceration, multiple pieces of AM may be used to fill up the defect. The orientation of these pieces does not matter. The most superficial piece is placed with the basement membrane side up and sutured as an inlay graft enabling corneal epithelium to grow over it (Kruse FE et al 1999).

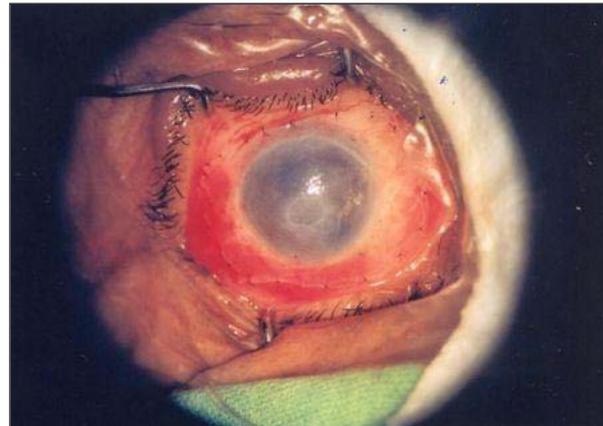


Fig 14: Overlay technique of AMT

Postoperative care

A large hydrophilic bandage contact lens may be placed after surgery. Topical steroids and antibiotics are used until epithelialization is complete and inflammation subsides. The translucent membrane enables observation of the healing epithelial defect beneath it. In the presence of excessive inflammation, it disintegrates faster and may have to be repeated several times (Petersen et al 2001).

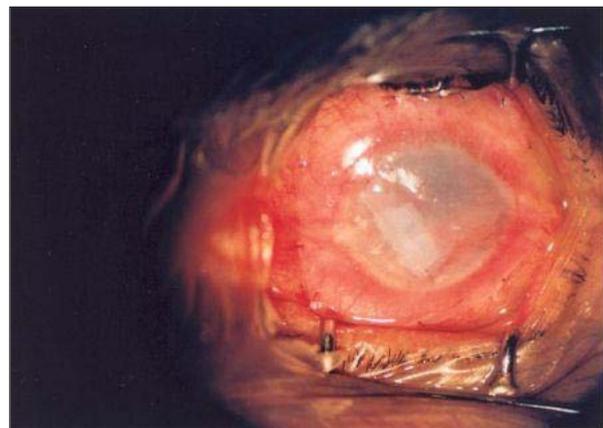


Fig 15: Multilayered filling technique of AMT

Conclusion

AM has been used for a variety of conjunctival and corneal disorders with varying success rates. Due to its ability to promote epithelialization and reduce inflammation and scarring, it appears to have a beneficial effect on persistent epithelial defects, neurotrophic ulcers, shield ulcers, chemical injury, pterygium excision, and conjunctival surface reconstruction. Its role in bullous keratopathy, band keratopathy, glaucoma-filtering surgery, and entropion has to be further evaluated. The AM devoid of epithelial cells provides an excellent substrate for culturing limbal stem cells and conjunctival epithelial cells. This may be attributed to the production of several growth factors that promote epithelial growth and its ability to preserve and maintain the existing progenitor cells. Thus, patients with limbal stem cell deficiency, either partial or total, may benefit from transplantation of AM alone or limbal stem cells cultured on AM. The results of long-term clinical studies in this area are awaited. Hyper-dry-amnion and cell sheets will also be attractive materials in the field of tissue engineering.

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Source of support: nil. Conflict of interest: none