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ORIGINAL ARTICLE

AMNIOTIC MEMBRANE GRAFT IN PTERYGIUM SURGERY

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ABSTRACT: Amniotic Membrane Transplantation is currently being used for a continuously widening spectrum of ophthalmic indications. It has gained widespread attention as an effective method of reconstruction of the ocular surface. Amniotic membrane has a unique combination of properties, including the facilitation of migration of epithelial cells, the reinforcement of basal cellular adhesion and the encouragement of epithelial differentiation. Its ability to modulate stromal scarring and its anti-inflammatory activity has led to its use in the treatment of ocular surface pathology as well as an adjunct to limbal stem cell grafts. Amniotic membrane transplantation has been used for reconstruction of the corneal surface in the setting of persistent epithelial defects, partial limbal stem cell deficiency, bullous keratopathy and corneoscleral ulcers. It has also been used in conjunction with limbal stem cell transplantation for total limbal stem cell deficiency. Amniotic membrane grafts have been effectively used as a conjunctival substitute for reconstruction of conjunctival defects following removal of pterygia, conjunctival lesions and symblephara. More recently, amniotic membrane has been used as a substrate for ex vivo cultivation of limbal, corneal and conjunctival epithelial cells. This article reviews the current literature on the applications of amniotic membrane transplantation and its outcome in pterygium surgery.

KEYWORDS: Amniotic Membrane Graft, Pterygium Surgery, Amniotic Membrane anatomy, Amniotic Membrane Characteristics.

INTRODUCTION: Pterygium surgery, even today remains one of the most controversial and challenging subject. Clinically, pterygium is characterized by a fleshy triangular portion of bulbar conjunctiva encroaching on to cornea. Also causing limbal stem cell deficiency and fibrovascular proliferation of subconjunctival tissue. The goals of surgery are to remove the pterygium completely without much tissue loss or scarring and prevent recurrences. Prevention of recurrences has always been the end point of treatment success of any modality.

Recurrence rate ranges from 5-90% in various studies. Recurrent pterygium is characterized by hyper-proliferation of subconjunctival fibrosis with more accelerated growth rate than primary pterygium. The resultant fibrosis can cause restriction of ocular movements and symblepharon formation. There are several techniques of surgeries like bare sclera amniotic membrane graft, conjunctival autograft, conjunctival sliding flap with or without adjuvants. One of the techniques with less recurrence is amniotic membrane grafting.[1]

Preserved Amniotic Membrane (AM) is currently being used for wide surface disorders. The basic tenets of Amniotic Membrane Transplantation (AMT) are to promote re-epithelialisation, to reconstruct the ocular surface and to provide symptomatic relief from surface aberrations. AMT is a useful technique for reconstruction of surface defects resulting from removal of surface disorders.

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AMT has effectively restored a stable corneal epithelium in eyes with peripheral epithelial dystrophies and corneal ulcers.[2] in the setting of acute ocular burns and Steven Johnson syndrome, AMT has satisfactorily reduced scarring and inflammation.[2] AMT alone may be an effective alternative for partial limbal stem cell deficiencies.

(a) Anatomy of Amniotic Membrane: The Human Amniotic Membrane (HAM) is the inner most layer of the placenta, composed of the outer chorion of maternal origin and the inner amnion of fetal origin. Histologically, the amniotic membrane is composed of the following layers. The HAM is devoid of any vasculature and is 0.02 to 0.5 mm in thickness.[2]

(i) Epithelial layer.
(ii) Thick basement membrane.
(iii) Avascular, hypo cellular stromal matrix.
    a) Compact layer.
    b) Fibroblast layer.
    c) Spongy layer.

(i) Epithelium: The amniotic membrane epithelium consists of a single layer of cuboidal cells with a single nucleus and a number of cytoplasmic vacuoles uniformly arranged on the basement membrane.[2] They are differentiated from the ocular surface epithelium by the presence of greater number of microvilli on the apical surface. Current cryopreservation techniques of AM storage tend to devitalize these cells by cell membrane disruption, leaving an intact basement membrane and stromal matrix.

(ii) Basement membrane (BM): Thick layer composed of network of reticular fibers. These are interdigitations of short, blunt processes from the basal region of epithelial cells with similar basement membrane processes. This is a tough layer and resistant to current cryopreservation techniques for AM storage. It is one of the thickest membranes found in human tissue and the support provided to the fetus during gestation stands testimony to the structural integrity of this remarkable tissue.

This structural integrity, transparency and elasticity of the AM make it currently the most widely accepted tissue replacement for ocular surface reconstruction. Both collagen IV and VII, components of the corneal epithelial BM, are present in the basement membrane of the AM. In addition, collagens I, II, III and V are also present in the AM.[2] Basement membrane is known to promote epithelial cell migration, adhesion and differentiation. It also suppresses epithelial cell apoptosis. Histochemically, the basement membrane more closely resembles that of the conjunctiva It is an ideal substrate for supporting the growth of the epithelial progenitor cells by prolonging their life span and maintaining their clonigenicity.

This action explains why AMT facilitates epithelialization for persistent epithelial defects with stromal ulceration. In tissue cultures, AM supports epithelial cells grown from explant cultures and maintains their normal morphology and differentiation. The resultant cultured epithelium can be transplanted with the AM to reconstruct damaged corneas. The amniotic membrane can also be used to promote non-goblet cell differentiation of the conjunctival epithelium. These data support why goblet cell density is promoted following AMT in vivo.

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Amniotic epithelium produces basic fibroblast growth factor, hepatocyte growth factor and transforming growth factor β.[1] These growth factors may modulate proliferation and differentiation of stromal fibroblasts.

(iii) Stromal Matrix: Compact layer (5-20 μm) - thought to be strongest layer comprised of a complex network of reticular fibers. This layer is thought to contribute to the tensile strength of AM. Its acellular nature points to possible epithelial origin.[2]

Fibroblast layer- thickest layer of AM and is made of a loose fibroblast network embedded in a mass of reticulum.

Spongy layer- outermost layer of the amnion has wavy bundles of reticulin, made up of branching fibers with triangular shaped nodes at the junction. Scattered fibroblasts are present in this layer

(b) Characteristics: Certain characteristics of AM make it ideally suitable for its application in ocular surface reconstruction:

1) Easily obtainable.
2) Nearly unlimited availability.
3) AM does not express HLA-A, B or DR Ag and hence immunological rejection after its transplantation does not occur.
4) Believed to have antibacterial properties, reducing the risk of post-op infection.
5) Can be preserved at -80°C for several months, allowing sufficient time to plan surgery or consider a trial of other options.
6) Favours epithelial cell migration/growth promoting activity.
7) Reinforces adhesion of basal epithelial cells, diminishes their apoptosis and promotes their differentiation.
8) Has anti-adhesive properties.
9) Has anti-inflammatory effect not linked to its function as a basement membrane.
10) It is avascular and angiogenic.

(c) Mechanism of Action: Question arises whether corneal epithelium grows over or under the AMT. Both these possibilities exist.

When corneal epithelium grows beneath the HAM, the regenerating epithelium will cause the HAM to detach progressively from the ocular surface as healing continues; on the other hand, when regenerating epithelium grows over the HAM, this causes the HAM to be incorporated into host corneal stroma. In this instance HAM remnants have been detected as late as 13 months after transplantation.

The therapeutic effect of AM basically involves three key actions that work synergistically, namely promoting epithelialization, reducing inflammation and suppressing fibrosis. The AM stromal matrix, rich in fetal hyaluronic acid suppresses transforming growth factor β signaling, proliferation and myofibroblastic differentiation of normal corneal and limbal fibroblasts as well as normal conjunctival and pterygium fibroblasts. This action explains why AMT helps reduce scars during conjunctival surface reconstruction, prevents recurrent scarring after pterygium removal and reduces corneal haze following photorefractive keratotomy.

DOI: 10.14260/jemds/2014/3648

The AM stromal matrix also suppresses the expression of certain inflammatory cytokines that originate from the ocular surface epithelia, including interleukin 1α (IL - α), IL - 1β, IL – 8, interferon γ, tumor necrosis factor -α, β-fibroblast growth factor and platelet derived growth factor. The suppression of inflammation is the key element in prevention of conjunctival scarring, neovascularization and fibrosis.

The AM attracts and sequesters inflammatory cells infiltrating the ocular surface and contains various forms of protease inhibitors. This may explain some of the anti-inflammatory properties of the fetal tissue and how neovascularization is mitigated, actions important for preparing the stromal microenvironment to support subsequent limbal stem cell transplants.

Indications of AMT in ocular surgery[2]:

- Conjunctival surface reconstruction.
- Pterygium surgery.
- Chemical burns.
- Cicatrizing conjunctivitis.
- Ocular surface squamous neoplasia (OSSN).
- Leaking blebs.
- Filtering surgery.
- Symblepharon release.
- Fornix formation.
- Socket reconstruction.
- Conjunctivochalasis.
- Entropion correction.
- Corneal surface reconstruction.
- PEDs.
- Non-healing stromal ulcers.
- Partial LSCD.
- Total LSCD.
- Bullous keratopathy.
- Band keratopathy.
- Scleral melt.
- Substrate for ex vivo expansion of limbal stem cells.
- Cicatrizing conjunctivitis.

MATERIALS AND METHODS:

(1) Amniotic Membrane Preparation: amniotic membrane is obtained under sterile conditions through elective cesarean section after a full-term pregnancy. There are several methods of preserving amniotic membrane like: [3]

- Heat-dried Amniotic Membrane.
- Air-dried Amniotic Membrane.
- Lyophilized (Freeze-dried) Amniotic Membrane.
- Preservation in Cold Glycerol.

DOI: 10.14260/jemds/2014/3648

- Cryopreserved Amniotic Membrane.
- Antibiotic Impregnated Amniotic Membrane (AIAM).

Among which most commonly used are air-dried Amniotic Membrane and antibiotic Impregnated Amniotic Membrane (AIAM).

(A) Air-dried Amniotic Membrane[3]: After separating and washing, the amniotic membrane is flattened under a lamellar flow hood and exposed to the air overnight to get dried. Packing and sterilization using gamma irradiation is then performed. Although high temperature is not applied in this method, some properties of the amnion are lost or altered remarkably due to dehydration. This type of prepared amniotic membrane is also often used for wound dressing

(B) Impregnated Amniotic Membrane (AIAM)[3]: After separation, the amniotic membrane is placed in an antibiotic solution composed of 5 types of wide-spectrum antibiotics and an antifungal agent overnight and then frozen at -80°C. The resultant amniotic membrane is suitable for management of infected wounds by providing an appropriate concentration of antibiotics to the wound surface.

(2) Surgical Procedure:

(A) Surgical Principles: Different techniques of use of Amniotic Membrane. a. Inlay Technique.[4]

Amniotic membrane may be applied by use of an 'inlay technique' or an 'overlay' ('patch') technique, or 'filling' or may be used as 'multilayered graft'. In the inlay method, AM is sized to fit just slightly larger than the size of the defect and sutured into place with the epithelial–basement side facing up. The AM thereby functions as a basement membrane over which new corneal or conjunctival epithelium can grow.

The basement membrane side can be identified by the fact that the AM is generally oriented on the nitrocellulose paper with the basement membrane side up and furthermore, the basement membrane side is less 'sticky' than the stromal side. Or blunt forceps can be used to draw up a 'vitreous like' strand from the stromal side but not from the basement membrane side. To suture AM to the cornea or sclera, 10-0 nylon or 10-0 polyglactin (Vicryl) is used. Care needs to be taken to flatten the AM over the underlying surface and to avoid trapping blood or fluid under the membrane.

Nylon sutures ultimately require removal. Recommend that suture knots be cut short, but not buried, to avoid detachment of the membrane at the time of suture removal.

b. Overlay Technique[4]: The second method of AM application consists of an overlay technique in which AM is placed over the entire cornea, limbus, and perilimbal area. When applied in this manner, the AM functions essentially as a biological contact lens.[5] Theoretically, orientation of the membrane is not as important as in the overlay method, although the AM generally is oriented with the basement membrane side facing down.

The patch is secured to the surrounding conjunctiva–episclera with interrupted 8-0 or 9-0 Vicryl sutures. An additional 10-0 Vicryl purse-string suture may be placed in the midperipheral cornea. If necessary, both techniques can be utilized in the same eye: that is, first an inlay graft is placed, followed by an overlay patch.

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c. Filling[4]: In the case of deep stromal ulceration, more than one layer of AM may be used to fill the ulcer cavity (that is, the AM is used as 'filling').[5] The orientation of the deeper layers probably is not important; however, the most superficial layer generally is oriented with the basement membrane side up, thus, allowing the growth of epithelium over it. Only the most superficial layer is sutured in place. Cutting AM into small pieces and filling the ulcer cavity with the pieces can also be done.[5]

d. Multilayered graft[6]: Indicated in ulcers of cornea and sclera, it is similar to fill in technique, but here multiple layers of AM are used, one over the other.

(B) Pterygium Excision[7]:

- Under subconjunctival anesthesia with 4% lignocaine (Xylocaine) the head of the pterygium should be first separated at the limbus and dissected towards the central cornea with a pair of spring scissors.
- After excising the head and most of the body, Tenon and subconjunctival fibrovascular tissue should be separated from the overlying conjunctiva, undermined and excised extensively upward and downward towards the fornices and medially towards but not reaching the caruncle; caution was taken not to damage the medial rectus.
- Cautery should be gently applied to bleeding vessels.
- The conjunctiva above and below the pterygium should be trimmed to create a rectangular area of bare sclera of approximately 5x7 to 6x8 mm.
- Residual fibrovascular tissue over the cornea should be detached toothed forceps or by gentle scraping with a #15 surgical blade.

(C) Amniotic Membrane Transplantation[7]:

- The area of bare scleral should be covered with amniotic membrane, which should be oriented with the basement membrane side up.
- The amniotic membrane should be sutured through the episcleral tissue to the edge of the conjunctiva along the bare sclera border with seven to eight interrupted 8-0 Vicryl sutures and the eye should be patched.

Complications[8]:

- Intractable corneal perforation.
- Graft necrosis.
- Active infection.
- Recurrence.
- Graft slippage.

Main complication common to all is recurrent disease which is more difficult to control. It is believed that surgical trauma and postoperative inflammation activate subconjunctival fibroblast and vascular proliferation, and deposition of extracellular matrix proteins, all of which contribute to recurrence of the lesion.[9]

Amniotic membrane contamination remains a potential risk which cannot be overlooked.[7]

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CONCLUSION: Ocular surface reconstruction techniques have advanced considerably during the last years, moving away from bare sclera techniques, through free conjunctival autograft, oral and nasal mucosal grafts, and the more potent and physiological weapon- the limbal autograft. However there are cases that cannot be solved with the mentioned techniques and their prognosis is dismal. It is in these complicated cases where the Amniotic Membrane Transplantation has proven to be helpful.

According to Ma Hui-Kang David, et al.[10] (2000) performed amniotic membrane graft for primary pterygia in 80 eyes of 71 patients. This was compared with 56 eyes of 50 patients receiving conjunctival autografts and 54 eyes of 46 patients receiving topical mitomycin C, where they showed amniotic membrane graft as an effective and preferred grafting procedure for primary pterygium.

As conventional methods in the management of ocular surface disorders have a limited success, AMT remains one of the most challenging entities facing the clinician today. AMT has now become a powerful surgical tool in the armamentarium of ophthalmology.

Insult to ocular surface had led to delayed epithelialisation of ocular surface, persistent inflammation and progressive tissue melting. Healing may occur with neo-vascularisation and conjunctivalisation. Conjunctival involvement too may lead to scarring, symblepharon formation and tear film deficiency. Until Kim and Tseng[12] showed that amniotic membrane transplantation facilitated corneal surface reconstruction.

Rapid healing and reduction of ocular surface inflammation following AMT can be explained by the following mechanisms of action.

Amniotic membrane provides a new basement membrane, which is an important substrate for supporting adhesion and growth of epithelial progenitor cells, including the stem cells.[13,14,15]

Amniotic membrane exerts an anti-inflammatory effect.

Amniotic membrane stromal matrix has a direct anti-scarring effect as evidenced by its suppression of TGF-β signaling and myofibroblast differentiation.[16]

Combination of the above three actions may help to re-establish a micro-environmental niche that is conducive for the growth of epithelial progenitor cells.

Amniotic membrane may promote nerve regeneration by maintaining nerve growth factor (NGF) signaling.

Thus AMT, having following advantage namely easy availability, relative ease of surgery and devoid of risk of allograft rejection, very useful technique which not only supplements other treatment modalities but also supplant them.

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Fig. 1: Commercially available air dried amniotic membrane

Fig. 2: Air dried Amniotic membrane

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Fig. 3(a to f)–Surgical Procedure of Pterygium Excision and amniotic membrane graft transplantation.

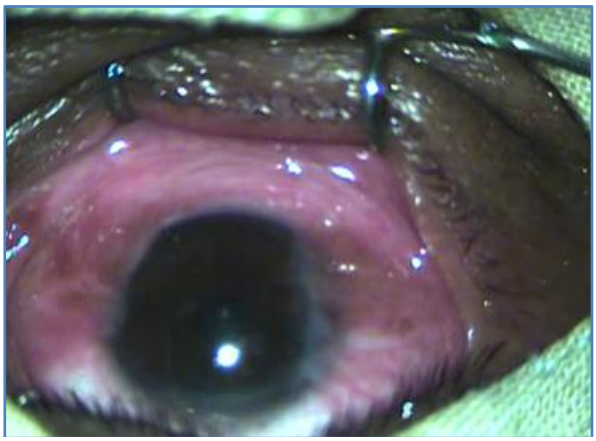

Fig. 3(a) – Double headed Pterygium

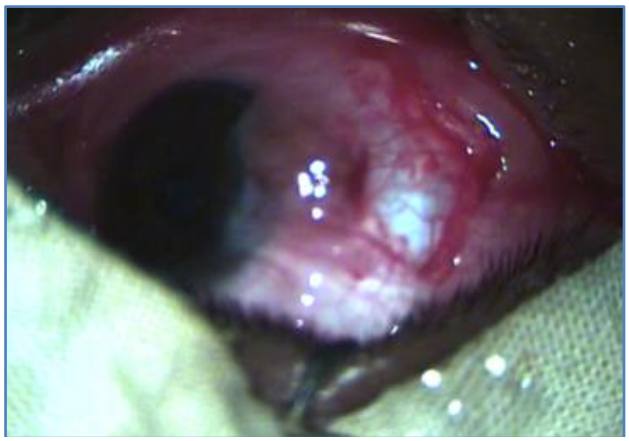

Fig. 3(b) – Dissection of Pterygium at neck

Fig. 3(c) – Dissection of Pterygium towards the central cornea with spring scissors

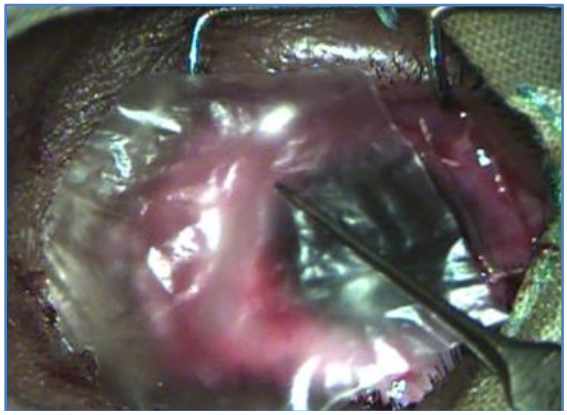

Fig. 3(d) – Orientation of Amniotic Membrane

Fig. 3(e) – Measuring and cutting Amniotic Membrane little more than size of the conjuctival defect.

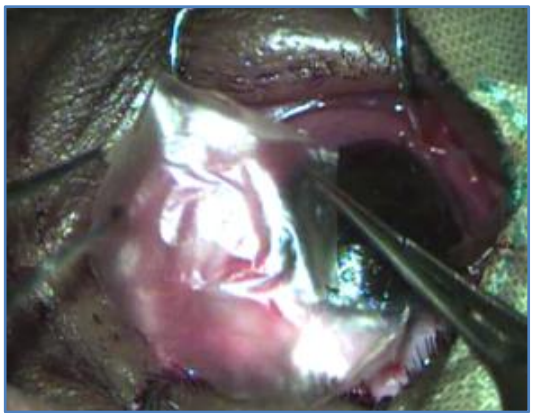

Fig. 3(e)

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Fig. 3(f) – Application of amniotic Membrane Graft Covering conjuctival defect and peripheral part of cornea.

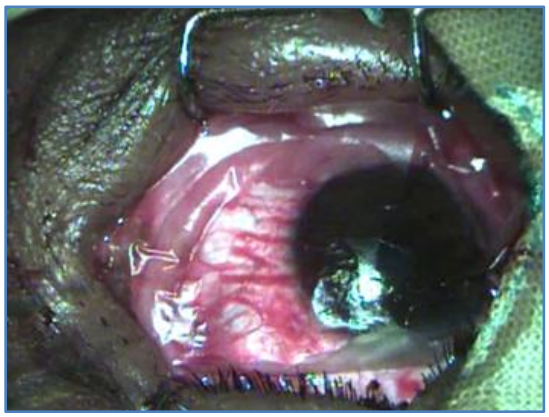

Fig. 3(f)

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