Review Article :

AMNIOTIC MEMBRANE IN OPHTHALMOLOGY

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Introduction :

In 1910 Davis was the first to report the use of fetal membranes as surgical material in skin transplantation later on De Rotth in 1940 used amniotic membrane for the first time after pterygium excision. Certain characteristics make the amniotic membrane ideally suited to its application in ocular surface reconstruction.

· It is very easily available and unlimited.

· The tissue can be preserved for several months.

· Amniotic membrane does not express so immunological reaction is negligible after its transplantation.

· It has antimicrobial properties.

· Antifibroblastic activity.[2,3 28-30]

· Cell migration/growth promoting activity.[4,5 31-33]

Purpose of this paper is to review the characteristics of amniotic membrane that make it potentially useful to treat ocular surface disorder and to discuss the current indications, the surgical technique, and the outcome of AMT. Mechanism of Action

· **Promoter of epithelialisation:**

By serving as a "transplanted basement membrane", it acts as a new healthy substrate suitable for proper epithelialisation.

· The amniotic membrane produces various growth factors such as *basic fibroblast growth factor*, *hepatocyte growth factor*, and *transforming growth factor â*, that can stimulate epithelialisation.[4,63141]

· Amniotic membrane also inhibits protease activity.[3,7 3 042]

· It has also been shown that in some instances the amniotic membrane, rather than providing a substrate, acts as a "bandage contact lens" allowing epithelialisation to occur under its cover.[8]

Inhibitor of fibrosis

It has been shown that amniotic membrane induces a downregulation of transforming growth factor â signalling, responsible for fibroblastic activation in wound healing.[2]

The amniotic membrane may also function as an anatomical barrier.

Indications of Amniotic Membrane Transplantation (AMT) in ophthalmology:

1. Ocular Surface Disorders.
2. Limbal Stem cell deficiency.
3. Glaucoma Surgery.
4. Miscellaneous.

1. **OCULAR SURFACE DISORDERS :**

· PERSISTENT EPITHELIAL DEFECTS :

Amniotic membrane transplantation has been successfully used in patients with persistent epithelial defects unresponsive to medical treatment.[8,9 4344]

The frequency of success in two recent series was 10 of 11 cases[9] and four of five cases[8]respectively.

The use of more than one layer may be effective in covering ulcers with substantial stromal depth.

PTERYGIUM SURGERY :

Amniotic membrane has been used as an alternative to conjunctival autograft during the removal of pterygia.

The recurrence rate of pterygium after AMT (10.9% for primary pterygia) was lower than the bare sclera technique (45%), but higher than autologous conjunctival graft (2.6%).[10,45]

AMT has been successfully used in the treatment of recurrent pterygium associated with severe symblepharon and diplopia.[4 31] Similarly, AMT has been used successfully in 13 of 16 eyes in the reconstruction of conjunctival defects created during surgical removal of large conjunctival lesions.[5 33]

CORNEAL STEM CELL DEFICIENCY :

Corneal stem cell deficiency is associated with conjunctivalisation of the cornea and can be complicated with persistent epithelial defects, vascularisation, scarring, calcification, ulceration, melting, and perforation of the cornea.

Lamellar or penetrating keratoplasty provides only a temporary replacement.

In cases with diffuse corneal stem cell deficiency, limbal transplantation (allo or auto) is now considered essential for corneal surface reconstruction.[1146]AMT combined with limbal transplantation has been successfully used in patients with diffuse limbal stem cell deficiency.

GLAUCOMA SURGERIES :

Fujishima *et al* [12.58] recently used amniotic membrane in guarded filtration procedures supplemented with mitomycin C to inhibit scarring and promote filtration.

Budenz*et al* [13.60] recently reported favourable use of human AMT for revision of leaking blebs after glaucoma surgery in five patients.

Surgical principles

PREPARATION OF AMNIOTIC MEMBRANE

Amniotic membrane is obtained under sterile conditions after elective caesarean delivery.Mandatory serological tests performed to exclude:

· Hepatitis A,B,C

· HIV

· HTLV

· Syphilis

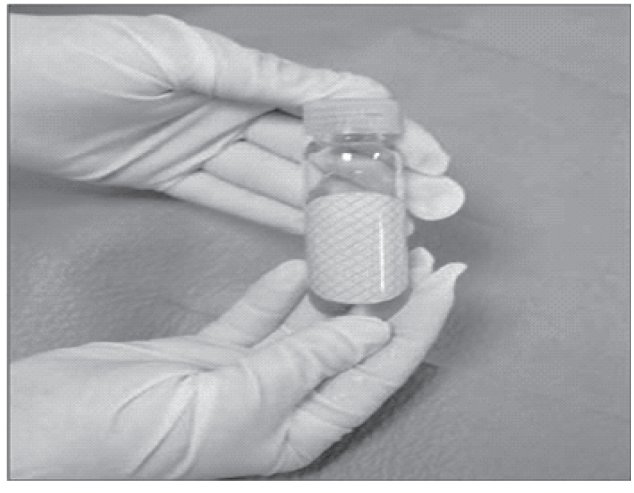
(A) PROCEDURE – 1.

1. Under lamellar flow hood, the placenta is first washed free of blood clots with balanced saline solution containing 50 ìg/ml of streptomycin, 100 ìg/ml of neomycin, and 2.5 ìg/ml of amphotericin B.

2. The inner amniotic membrane is separated from the rest of the chorion by blunt dissection. The membrane is then flattened onto a nitrocellulose paper, with the epithelium/ basement membrane surface up.

3. The membrane with the paper is cut into 4 × 4 cm pieces and placed in a sterile vial containing Dulbecco's modified Eagle's medium and glycerol at a ratio of 1:1 (vol/vol).

4. The vials are frozen at "80°C. The membrane is defrosted immediately before use by warming the container to room temperature for 10 minutes.[5]



· Preserved amniotic membrane over nitrocellulose paper

(B)PROCEDURE – 2.

1. After washing with physiological saline or 0.01 M phosphate buffered saline (PBS) containing 100 mg of dibekacinsulphate.

2. The amniotic membrane with the chorion is separated from other uterus tissue by blunt dissection.

3. The membrane is then cut into pieces measuring 5 × 5 cm and rinsed three times in 0.01 M PBS.

4. Each piece is rinsed in 0.5 M DMSO(dimethyl sulphoxide) dissolved in PBS, then in 1.0 M and 1.5 M DMSO in PBS, for 5 minutes each.

5. The membrane is placed in a plastic container and preserved at "80°C until use.

6. Just before use, the container is warmed to room temrerature and the membrane is rinsed three times in saline, then once in saline containing 100 mg of dibekacinsulphate.

7. At the time of surgery the amniotic membrane is separated bluntly from the underlying chorion with forceps.-[53]

MICROBIOLOGICAL SAFETY :

· Transmission of infectious agents is one of the risks associated with transplantation of human organs and tissues. Safety criteria applied to organ transplantation should be applied even more strictly to tissue transplantation such as amniotic membrane.[15-64]

· Infection may also occur as results of procurement procedures and tissue processing. There is therefore a need for microbiological quality control of tissue procurement and any associated banking as well as for prevention of transmission of infection from the donor.[15]

· Serum samples from all donors must be tested for anti-HIV-1 and 2, hepatitis B surface antigen (HBsAg), and anti-hepatitis C virus (anti-HCV). Testing for syphilis is also required. Donors infected with HIV-1 or 2, HCV, HBV, or syphilis should be excluded.

SURGICAL TECHNIQUE

· The membrane is always sutured to the ocular surface with its epithelial side up and the mesenchymal surface in contact with the eye, to facilitate adherence of the membrane to the ocular surface.

· For this reason it is important to be able to distinguish its two surfaces. This is easiest when the membrane is fresh, but when dealing with membranes that have been thawed after storage at "70°C it becomes difficult.

For example,

· Mounting the membrane on nitrocellulose paper, the right way up, so that the correct side can be determined when the membrane is thawed.

· Others will use a suture, with the knot as the marker or indelible marker pen, to mark one side of the membrane.

· After spreading the membrane on the ocular surface we apply the tips of a blunt fine forceps to one surface of membrane and pinch lightly with the forceps and lift. A fine strand of "vitreous-like" substance can usually be drawn up from the mesenchymal but not the epithelial (basement membrane) side of the amniotic membrane.

· The amniotic membrane is spread on to surface of the eye and cut to appropriate size and shape, keeping the final piece slightly larger than the size of the defect to be covered. It is usually sutured to the cornea with 10-0 Nylon sutures and to the conjunctiva with 9-0 Vicryl sutures.

· After surgery topical steroids and antibiotics are used. Sutures can be removed at 3 weeks.

· The membrane stains with fluorescein

stain and like the cornea, attracts ciprofloxacin deposits, if the drug is used topically, postoperatively.[8]

· In cases of persistent epithelial defects, the base of the ulcer and loose epithelium adjacent to the edge of the ulcer are debrided before applying the membrane.

· The amniotic membrane is trimmed and fitted to cover the epithelial defect and sutured to the edge of the defect (Fig 1).[9]

· If the epithelial defect is large, a 360 degreeperitomy is done and the membrane sutured to cover the cornea from limbus to limbus (Fig 2).

· In pterygium or symblepharon surgery, the membrane is applied to cover areas of conjunctival defects after removal of fibrotic tissue (Fig 3).[3]

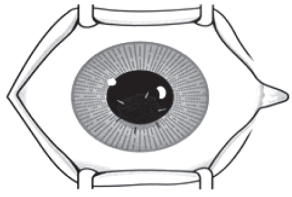


Fig.1 The diagram illustrates the amniotic membrane sutured to the cornea and covering a paracentral corneal epithelial defect.

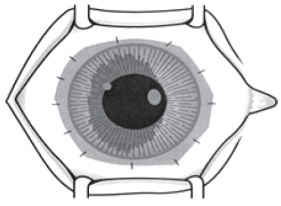


Fig.2 The amniotic membrane is sutured to perilimbalepisclera and to the edge of the conjunctiva (after peritomy) covering the whole corneal surface.

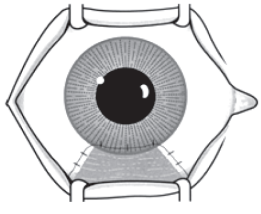


Fig.3 The amniotic membrane can be used to cover a conjunctival defect after releasing adhesions during symblepharon surgery, and in a similar manner (nasally or temporally) after excision of pterygium.

The following problems that can occur after amniotic membrane transplantation.

· The amniotic membrane may disintegrate before epithelialisation, in some cases within 2 weeks after transplantation.

· Necrosis of the amniotic membrane may be related to collagenases present on the ocular surface.

· The amniotic membrane will not remain attached to the ocular surface if the mesenchymal surface is not facing the host.

Summary

· Amniotic membrane has unique properties that can be helpful to treat different ocular surface diseases.

· AMT is useful in promoting normal epithelialisation of cornea and conjunctiva.

· It is also effective in preventing excessive fibrosis during ocular surface reconstruction.

· Because of the potential risk of infection strict safety criteria must be applied.

· . Controlled clinical trials will be needed to establish the role of AMT in ocular surface reconstruction.

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