Indian Journal of Ophthalmology
Journal of All India Ophthalmological Society

OPHTHALMOLOGY PRACTICE
Year : 2002 | **Volume** : 50 | **Issue** : 3 | **Page** : 227–31

Preparation of amniotic membrane for ocular surface reconstruction

Hajib N Madhavan, K Priya, J Malathi, Patricia R Joseph
Vision Research Foundation, Sankara Nethralaya, 18 College Road, Chennai-600 006, India

Correspondence Address:
Hajib N Madhavan
Vision Research Foundation, Sankara Nethralaya, 18 College Road, Chennai-600 006
India

Abstract

We describe the preparation and preservation of human amniotic membrane required for transplantation in the management of ocular surface diseases. Informed consent is obtained and the donor is screened to exclude risk of transmissible infections such as human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, and *Treponema pallidum* infections. Ideally, the media and washing solutions needed for the preparation of amniotic membrane are prepared only a week to 10 days prior to use and not stored in the freezer weeks ahead. The AM obtained under sterile conditions after elective caesarian section is washed free of blood clots and chorion. With the epithelial surface up, amniotic membrane is spread uniformly without folds or tears on individually sterilized 0.22 µm nitrocellulose membranes of the required sizes. The prepared filter membrane with the adherent amniotic membrane is placed in the preservative medium and stored at -80°C. The membranes are released when the repeat serology for HIV after the window period has excluded virus infection in the donor. Depending on consumption they may be used up to 6 months after preparation, though many have recommended storage for an indefinite period. Since the amniotic membrane has only incomplete expression of HLA antigens and amniotic epithelial cells do not express them, it is not rejected after transplantation. The presence of several cytokines in the amniotic membrane promotes epithelialization with reduction of fibrosis during healing.

How to cite this article:
Madhavan HN, Priya K, Malathi J, Joseph PR. Preparation of amniotic membrane for ocular surface reconstruction.Indian J Ophthalmol 2002;50:227-31

How to cite this URL:
Madhavan HN, Priya K, Malathi J, Joseph PR. Preparation of amniotic membrane for ocular surface reconstruction. Indian J Ophthalmol [serial online] 2002 [cited 2016 Apr 15];50:227-31
Available from: http://www.ijo.in/text.asp?2002/50/3/227/14777

Full Text

Over the last several years there has been a growing demand for amniotic membrane (AM) transplantation to treat ocular surface diseases. We have been preparing AM during the last two years in our tertiary care ophthalmic hospital, for use by

ophthalmologists. This communication reviews the biological properties that form the basis of AM transplantation and attempts to provide practical guidelines and necessary laboratory set up for harvesting AM. In India AM preparation is entirely dependent on in-house preparation of media and solutions and is not available off-the-shelf as described in the Western literature.

Amniotic membrane is the innermost semi-transparent layer of the foetal membranes. It has an avascular stromal matrix, a thick collagen layer and an overlying basement membrane with a single layer of cuboidal epithelium.[1] Since 1910, AM has been used sporadically in clinical practice to encourage epithelialization in burns, as graft over skin ulcers, and in intra-abdominal and reconstructive surgery.[2] AM as a graft was first used for conjunctival reconstruction in 1940.[3] With standardization of the technique and further understanding of pathobiology, AM has been routinely used in ocular surface reconstructive surgery since 1995.[4] AM can be used either as a "substrate" to replace the damaged ocular tissue, as a "patch" (biological dressing), or a combination of both. Ocular surface diseases where amniotic membrane transplantation is applied include Stevens-Johnson syndrome, conjunctival cicatrisation/scar, symblepharon, conjunctivochalasis, conjunctival surface reconstruction, replacement of conjunctival autografts, pterygium surgery, trabeculectomy, chemical injury, limbal stem cell deficiency, persistent epithelial defects, corneal ulceration, and symptomatic keratoplasty.[5-12]

Biological properties of AM in relation to transplantation

The biological properties and the relationship of these characteristics are detailed in [Table:1]. Since only incomplete HLA antigens are found in AM, it is immunologically inert and not rejected by the recipient. Due to the availability of several biological factors in AM, epithelialization and healing with reduced fibrosis are facilitated when transplanted on the ocular surface. The presence of inflammation-inhibiting chemokines in AM reduces neovascularization and fibrosis during the healing process.

Preparation and preservation of AM in the laboratory

An elective caesarian delivery helps in the correct choice of a consenting donor and planned collection and processing of AM. Placenta collected after natural vaginal delivery may have structural defects associated with stretching of the membrane during labour and delivery, and may be contaminated by normal vaginal flora, Herpes, Chlamydia or other contaminant bacteria.[2]

For longterm tissue preservation, the method used must reliably disinfect the stored biological tissue. Antibiotic disinfection is currently the method of choice to preserve the tissue matrix. Safety against transmission of viruses is effected by donor selection and testing for serological markers of presently known transmissible viruses at the time of donation and again 3-4 months later.[2] The AM can be preserved in glycerol, by irradiation, cryopreservation or lyophilization techniques. Cryopreservation at -80°C is done with either glycerol or dimethyl sulfoxide (DMSO).[16]

The following steps may be followed to finally obtain quality AM for transplantation. The steps involved in the preparation of AM are shown in the Figure. The required transport medium, the washing solutions and preservative medium, properly checked for sterility should be made available at least a day before the collection and preparation of the membrane. The working solutions and media should have been prepared 7 - 10 days ahead with complete verification of pH and sterility. It is preferable to prepare these solutions when required and not stored in freezer weeks ahead. The preparation is described below.

The detailed medical history and clinical condition of the potential donor should exclude the risk of tissue-transmissible infections and unsuitability of the donors.

The consent of the donor is obtained for the donation (and subsequent use of AM). Donors are screened for human immunodeficiency virus (HIV) type 1 and 2, hepatitis B virus (HBV), hepatitis C virus (HCV) and Treponema pallidum infections. Competent laboratories should perform these tests and the records should be maintained for 11 years post-transplantation. Review of the records may become necessary if HIV or any slow virus infections develop in the recipient(s), assuming that these would develop not later than 10 years. Donor anonymity must be maintained.[16]

Consent for subsequent screening of blood to determine the HIV status after the "window" period of 3-4 months should also be obtained.

The AM should be obtained under sterile conditions after elective caesarian section.

The obstetrician must place the placenta in a sterile stainless steel 12-inch diameter basin, preferably covered with a sterile lid. During placement the clamp from the cord should not be removed, to avoid the AM getting covered with blood.

In the clean atmosphere of the operating room or the clean laminar flow workbench, the AM is dissected from the placenta in two large bits. As much of the chorion as possible should be peeled out before the bits are dropped into a sterile, wide mouthed 125 ml screw-capped reagent bottle containing 50 ml transport medium. The transport medium generally used is the commercially available Eagles' minimum essential medium (EMEM) supplemented with 3.3% L-glutamine and antibiotics (50 µg/ml gentamicin, 100 units/ml penicillin, 200 µg/ml ciprofloxacin and 1 mg/ml Amphotericin B). The media and the antibiotic preparations should be of recognized quality manufactured as per ISO 9002 standards as certified by a competent certifying authority. The membrane must be transported immediately to the laboratory.

In the laboratory, under the laminar flow hood, the AM is washed free of blood clots with EMEM containing antibiotics. Any leftover chorion attached to the AM and blood clots is gently peeled off the epithelial cell layer using round-ended forceps.

With the epithelial / basement layer surface up, the AM is spread uniformly without folds or tears on individually sterilised 0.22 µm nitrocellulose membranes of the required size (47 mm or 25 mm, commercially available - Millipore or Sartorius). The AM around the nitrocellulose membrane should be cut and allowed to adhere to the cellulose membrane.

If in doubt, a small piece of about 1 cm2 of the membrane may be examined on a microscopic slide under a phase contrast microscope to ascertain the epithelial side.

The filter membrane along with the adherent AM is fully placed carefully in the preservative medium in 50-ml wide mouthed screw-capped irradiated transparent plastic bottles. The preservative medium used is 1:1 (vol/vol) ratio of sterile glycerol (sterilized by autoclave) and EMEM with 3.3% L-glutamine, 25 µg/ml gentamicin, 50 units / ml penicillin, 100 µg/ml ciprofloxacin and 0.5 mg/ ml Amphotericin B.

The bottles are labeled with the appropriate size and date of preparation.

A random bottle from the batch is left over the work bench at room temperature for about an hour and about 5 ml of the same is inoculated into 100 ml of brain heart infusion medium and 100 ml of thioglycolate broth medium to check the bacterial and fungal sterility. These media are incubated for 21 days and if no growth of bacterium or fungus is observed, the batch should be considered as preserved free of cultivable microbial agents.

The AMs should be stored at -80°C to facilitate the devitalization of the epithelial cells.

Upon confirmation of the HIV-negative status of the donor by repeat serology done 3-4 months after the collection of AM, the membranes are released.

The membranes may be used for up to 6 months after preparation. The use of AM within a period of 6 months is suggested because of the following reasons. The cellular viability is found to be reduced by more than 50% in two months.[15] Damage of AM cells due to cryopreservation results in a decrease in AM associated levels of growth factors. [16,18,21] As a policy, only cryopreserved AM at -80°C less than 3 months is used clinically in Tokyo Medical University. [15]

The membrane is thawed by keeping the bottle either at 4°C for 30 minutes or at room temperature for 10 minutes.

The prepared membrane should be handled properly avoiding microbial contamination during transportation to the operating room in ice. The colour of the storage medium after thawing should be light pink. A change towards yellow should be taken as suggestive of microbial contamination; any such membrane is discarded.

When the AM is detached from the cellulose acetate membrane in the bottle, and the surgeon is unsure of the epithelial

side of AM, it is identified by its stickiness to the tip of the cotton swab.

The above protocol has been found to be extremely safe, and AM so harvested is expected to have a long shelf life. The chemicals and drugs needed for AM preparation are listed in [Table:2].

Amniotic membrane, with its unique properties on inflammation, healing and promoting epithelialization has been a very useful biological material for surgical management of certain ocular surface diseases. Its preparation in a laboratory requires expertise in preparation of media and solutions and good laboratory practice of sterilisation and preservation of biological materials.

References

1 Blanco AA, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. *Br J Ophthalmol* 1999;83:399-402.

2 Adds PJ, Hunt C, Hartley S. Bacterial contamination of amniotic membrane. *Br J Ophthalmol* 2001;85:228-30.

3 DeRoth A. Plastic repair of conjunctival defects with fetal membrane. *Arch Ophthalmol* 1940;23:522-25.

4 Kim JCI, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea* 1995;14:473-84.

5 Tseng SCG, Prabhasawat P, Zee SH. Amniotic membrane transplantation for conjunctival reconstruction. *Am J Ophthalmol* 1997;124:765-74.

6 Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pteryguimexcision. *Ophthalmology* 1997;104:974-85.

7 Tseng SCG, Tsubota K. Amniotic membrane transplantation for ocular surface reconstruction. In: Holland EJ, Marris M J, editors. *Ocular Surface Diseases: Medical and Surgical Management*. New York: springer-Verlag; 2002. p226-31.

8 Zee SH, Tseng SCG. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997;123:303-12.

9 Tseng SCG, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal autografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol* 1998;116:431-41.

10 Sridhar MS, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation in acute chemical and thermal injury. *Am J Ophthalmol* 2000;130:134-37.

11 Meller D, Pires RTF, Mack RJS, Figuiredo F, Heiligenhaus A, Park WC, *et al*. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology* 2000;107:980-90.

12 Sridhar MS, Sangwan VA, Bansal AK, Rao GN. Amniotic membrane transplantation in the management of shield ulcers of vernal keratoconjunctivitis. *Ophthalmology* 2001;108:1218-22.

13 Akle CA, Adinolfi M, Welsh KI, Leibowitz S, McColl L. Immunogenicity of human amniotic membrane epithelial cells after transplantation into volunteers. *Lancet* 1981;2:1003-5.

14 Terada S, Matsuura K, Enosawa S, Miki M, Hoshiki A, Suzuki S, Sakuragawa N. Inducing proliferation of human amniotic epithelial (HAE) cells for cell therapy. *Cell Transplant* 2000;9:701-4.

15 Kubo M, Sonada Y, Muramatsu R, Usui M. Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Inves Ophthal Vis Sci* 2001;42:1539-46.

16 Dua HS, Blanco AA. Amniotic membrane transplantation. *Br J Ophthalmol* 1999;83:748-52.

17 Solomon A, Rosenblatt M, Monray D, Ji Z, Pflugfelder SC, Tseng SCG. Suppression of interleukin 1a and interleukin 1b in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. *Br J Ophthalmol* 2001;85:444-49.

18 Koizumi N, Inatoni T, Sotozono C, Fullwood N, Quantock A, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res* 2000;20:173-77.

19 Hui-Kang D, See LC, Zian SB, Tsai RJF. Amniotic membrane graft for primary pterygium: Comparison with conjunctival autograft and topical Mitomycin C treatment. *Br J Ophthalmol* 2000;84:973-78.

20 Joussen AM, Kruse FE, Sim B, Baumann J, You L. Morphology of the amniotic membrane at transplantation on the ocular surface. *96th DOG Annual Meeting*, 1998. p 481.

21 Sippel KC, Ma JJK, Foster CS. Amniotic membrane surgery. *Curr Opin Ophthalmol* 2001;12:269-81.