



## Amniotic Membrane Transplantation in Ocular Surface Disorders – A Clinical Comparison of Fresh Versus Cryopreserved Membrane

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### ABSTRACT

**PURPOSE:** The purpose of this study is to compare fresh and cryopreserved amniotic membrane transplantation in various ocular surface disorders in terms of efficacy, cost effectiveness as well as simplicity of its use.

**MATERIAL AND METHODS:** This study was a randomized, prospective, interventional and comparative clinical trial conducted at RIO, Allahabad which included 30 eyes with various corneal pathologies and divided in two groups of 15 eyes in each group (group I with fresh and group II with cryopreserved membrane).

**RESULTS:** The overall success rate of 65.5% was observed with cryopreserved membrane showing a significant higher success rate of 86.5% as opposed to 40% in hypothermic grafts. The best results were seen in corneal ulcer (90%) and the worst in Steven Johnson syndrome (16.67%)

**CONCLUSION:** A cryopreserved graft was seen to be superior to hypothermically stored graft.

**KEYWORDS :** Amniotic membrane transplantation (AMT), ocular surface disorders, cryopreserved amniotic graft.

**INTRODUCTION:** Ocular surface disorders comprise wide spectrum of diseases ranging from corneal ulcers, degenerations and dystrophies to chemical burns. Conventional treatment of such disorders is aimed at eliminating the underlying disease mechanism as well as control of inflammation and protection of the corneal surface. However some of these diseases fail to respond to treatment and progress to stromal ulceration and perforation. In such scenarios Amniotic Membrane Transplantation (AMT) is currently being used and has gained widespread attention as an effective method of reconstruction of the ocular surface. It can be used as an adjunct to limbal stem cell graft with the potential to modulate stromal scarring. In this study, patients with refractory corneal ulcers, symptomatic Bullous Keratopathy (BKP), severe alkali burns and Steven Johnson syndrome (SJS) with recurrent epithelial defect were studied for their response to fresh as well as with cryopreserved amniotic membrane transplantation

In 1940, *De Roeth* was the first to describe the use of live placental membranes including both amnion and chorion for the repair of conjunctival defects. The low success rate was probably related to the allograft rejection due to inclusion of highly immunogenic chorionic membrane. After this documented study the use of Amniotic Membrane (AM) disappeared from medical literature for several decades. Later in 1995 *Kim and Tseng* reintroduced the use of AM for ophthalmic uses in rabbits.

Amniotic membrane is the innermost layer of placenta which functions to protect the fetus from unwanted insults during embryological development. It has been recognised that intrauterine fetal surgeries performed in third trimester via the skin of the fetus does not bear any scarring after birth. This phenomenon of 'scarless wound healing' remains to be elucidated. Thus, the tough semitransparent nature of the amniotic membrane has valuable qualities as a graft for ocular surface reconstructions. It promotes epithelization and acts as a scaffold for the migration of epithelial cells over it. It is an immunopre-privileged tissue and does not express HLA-A, B or DR antigens. Hence no immunological reactions occur. The antimicrobial properties reduce the risk of post operative infections. It produces several growth factors that stimulate epithelization and the avascular stroma inhibits vasogenesis. It prevents apoptosis of epithelial cells and fibroblast activities giving it an anti-cicatricial property.

### AIMS & OBJECTIVES:

1 To assess the efficacy of amniotic membrane transplantation in patients with non healing corneal ulcers, severe alkali burns, symptomatic Bullous Keratopathy as well as patients with SJS with recurrent epithelial erosions.

2 To compare between and evaluate

(a) Fresh (hypothermically stored) AM stored in ringer lactate solution at -4°C and used within 72 hours

(c) Cryopreserved AM stored in 50% glycerol at -80°C and used within 30 days

- Comparison was done in terms of
- Efficacy in results
- Cost effectivity
- Simplicity of use

**MATERIAL AND METHODS:** The study was conducted at Regional Institute of Ophthalmology in M.D. Eye hospital and MLN medical college, Allahabad. This study was a randomized, prospective, interventional and comparative clinical trial. A total number of 30 eyes of 27 patients with various corneal pathologies were taken for study and divided in two groups of 15 eyes in each group.

Group I: AM used in 12-72 hours after hypothermic storage (-4°C) in ringer lactate with broad spectrum antibiotics

Group II: AM used within 1 month of storage after cryopreservation in 50% glycerol at -80°C.

**Inclusion criteria:** Patients included in the study were grouped into four categories-

- Non healing corneal ulcers refractory to conventional methods of treatment, including perforations
- Severe cases of alkali burns
- Symptomatic Bullous Keratopathy
- Steven Johnson Syndrome with recurrent epithelial erosions

**Exclusion criteria:** Patients with acute infective fungal keratitis, acute bacterial keratitis with positive culture reports, patients with endophthalmitis and patients not fit for surgery were excluded.

**Procurement and membrane processing:** Maternal blood was screened for HIV, HbsAg, hepatitis C and syphilis. The membrane was processed by following strict aseptic techniques and stored accordingly.

### Surgical technique:

(a) Inlay or graft technique: Amniotic membrane in tailored size was secured in a single layer with its basement membrane and epithelial side up to allow migration of the surrounding epithelial cells on the membrane, thus acting as a scaffold.

(b) Overlay or patch technique: When more than one layer of the amniotic membrane is used akin to a biological contact lens in order to protect the healing surface defect beneath, it is referred to as a patch. When used as patch the membrane is secured with its epithelial side up.

Post operatively broad-spectrum topical antibiotics were used for two weeks initially, until the epithelium heals. Topical steroids were used for eight weeks in tapering doses to reduce surface inflammation. Artificial tear substitutes and cycloplegics were prescribed. Patients were frequently followed up for 4 months in terms of symptomatic re-

lief, visual acuity, corneal tissue healing, vascularisation, corneal opacity, state of graft and complications.

## RESULTS AND DISCUSSION:

Out of the 30 eyes with different ocular surface disorders 21 were males(70%) and 9 were females(30%). Most of the patients were in the age group of 31-40 years(40%).

With complete dissolution stem cells located at the limbus migrates over the membrane which gets incorporated into the ocular tissue. Of total 30 cases, 20 eyes(67.7%) showed complete dissolution of membrane, of which, group II achieved better dissolution (93.3%) than group I patients(44.8 %).

Membrane transparency is the sign of graft survival. In our study, a total number of 27 cases achieved transparency by 1<sup>st</sup> week(90%). 3 cases which did not achieve graft transparency were from group I. This could be attributed to the fact that cryopreservation significantly impairs the viability and proliferative capacity of the membrane. Absence of immunological reactions may also help in achievement of early transparency of the cryopreserved membrane. The time taken to achieve transparency also varied in both groups, group II showing transparency on the first post-operative day in 93.3% cases while group I over the period of 7 days.

Ocular stability was confirmed on the basis of active epithelization by clinical examination with a slit lamp and fluroscein staining over the period of 21 days. 25 out of 30 cases achieved total ocular stability within 3 weeks(83.3%). Ocular stability had a better success rate in group II(93.3%) than group I patients(73.3%). 4 patients of group I and 1 patient from group II did not achieve epithelization. This case of group II was a 65 year male patient of corneal ulcer of more than 15 days duration with a deep stromal abscess.

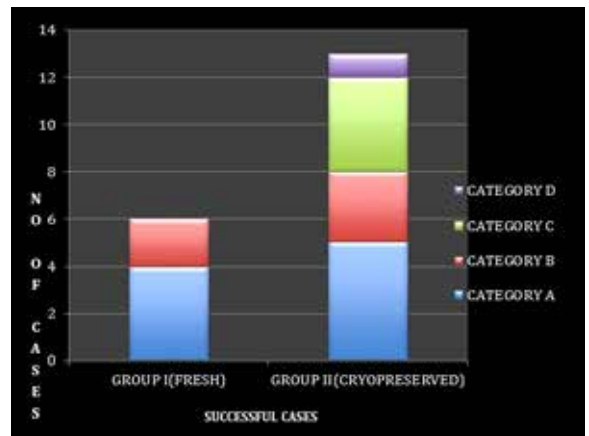
Improvement in visual acuity of more than two lines on Snellen's chart was observed in 10 cases(33.4%). Most of these cases were in category A(4/10 cases)(40%) and category C(4/7 cases)(57.1%). In patients of SJS (category D) only 1 of 6 cases(16.7%) showed visual improvement. 18 out of 30 cases did not experience any change in visual acuity(60%) while 2 cases showed deterioration.

26 out of 29 cases(89.4%) achieved symptomatic relief with pain, watering and photophobia by 15<sup>th</sup> post-operative day. 1 patient was symptomless. Recurrence of symptoms were more in group I (75%) than group II patients(25%).

Complications observed were necrosis with premature shedding of the membrane, host versus graft inflammatory reaction, detachment of membrane from the ocular surface and recurrence. 10 patients showed necrosis or rolling up of membrane with detachment. All these patients belonged to group I with most of the cases in the corneal ulcer category(3/10 cases)(30%). This might be due to infective and high inflammatory state of the eye in such cases. None of the cryopreserved membranes showed this complication. 3 cases(20%) showing inflammatory reaction at the host graft junction belonged to group I. Thus inert behavior of the cryopreserved graft membrane may again be attributed to the lack of viable epithelium thereby lacking immunogenic/inflammatory response. 13 out of 30 cases showed detachment of the graft membrane from the ocular surface, of which 10 cases(76.9%) were from group I as opposed to 3 cases(23.1%) from group II. The cause of detachment in our study was as varied as from loosening of sutures to rubbing of eyes by the patients. Recurrence of epithelial defect was observed in 8 of 25 cases. Out of these 6 cases were from group I(75%) and 2 cases were from group II(25%).

Overall rate of success was determined by stable ocular surface with complete epithelization, no recurrence during follow up for 24 weeks, absence of symptoms and maintenance of anatomical integrity of the eye. A success rate of 65.5% was achieved overall with highest success in category A in corneal ulcers of various depths including perforations(90%). Least successful category was D(SJS) with success rate of 16.67%. This may be related to extremely low tear function in this group leading to low survival probability of the graft. We had also observed higher overall success rate in patients with cryopreserved membranes(group II)(86.5%) as opposed to hypothermically stored membranes(group I)(40%) (figure 1). This is again explained by the non

immunogenicity of the cryopreserved graft.



**FIGURE 1: Bar graph showing number of successful cases in each category of patients in fresh as well as cryopreserved groups.**

A better surgical outcome was seen in case of inlay or graft technique(16/24 cases)(67%) as compared to overlay or patch technique(3/5 cases)(60%).

**CONCLUSION:** A cryopreserved graft was seen to be superior to hypothermically stored graft in terms of better outcome, less time taken to induce a stable ocular surface and almost minimal complications. A cryopreserved graft is also more

feasible and cost effective as one single placenta can provide for 20-30 grafts and can be used for a longer period. Ringer stored hypothermic membranes meanwhile can be kept only for 72 hours minimizing the amount which can be used. We found that AMT can give swift and effective results in non healing corneal ulcers, symptomatic bullous keratopathy and acute chemical burns and can be considered as an effective treatment of these conditions. As for diseases like Steven Johnson Syndrome, a careful preoperative tear function should be evaluated for better results.

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