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## ORIGINAL ARTICLE / RESEARCH

# Amniotic Membrane in Trabeculectomy: An Experimental Study

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### ABSTRACT

**Background:** To assess the potential use of amniotic membrane graft (AMG) for augmenting trabeculectomy.

**Methods:** Sixteen eyes of 8 rabbits were randomly divided into a study group that underwent trabeculectomy with AMG, and a control group that underwent trabeculectomy alone.

**Result:** The mean duration of blebs was greater in the study group as compared to the controls ( $p=0.009$ ). On day 1, the IOP was low in both groups ( $p<0.001$ ). At 1 week, the study group IOP remained low but reached preoperative levels at 2 weeks and 1 month. In the control group the IOP reached preoperative levels at 1 week, 2 weeks and 1 month. Histologically, the early functioning bleb showed filtering spaces. There was subconjunctival fibrosis at 1 week in the controls and at 2 weeks in the study group.

**Conclusion:** AMG had a short term augmenting effect on trabeculectomy in this animal model.

**Key words:** Amniotic membrane, Rabbits, Trabeculectomy, Experimental study.

### Introduction

Amniotic membrane grafting (AMG) is being evaluated as an adjuvant in trabeculectomy in view of its potential to overcome subconjunctival fibrosis, which is the primary cause of failure of glaucoma fistulizing surgery [1]. Trabeculectomy is a routine surgery in glaucoma patients in which a piece of trabecular meshwork in the angle of anterior chamber is removed and aqueous humour

from the eye is made to drain into the subconjunctival space. This is especially true of clinical management of refractory glaucoma, where the success rate with alternative modalities such as antifibrotic agents, drainage implants, and controlled cyclodestructive procedures has been modest. In addition, some complications that have come to be identified with these procedures have led to a rethink on their widespread use

The present study aims to address the role of AMG in augmenting trabeculectomy in the rabbit eye. This animal model was chosen for its very rapid wound healing response following trabeculectomy, which closely approximates the

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human eye with refractory glaucoma in respect of a high risk of failure of trabeculectomy [2] .

## Materials and Methods

This experimental study in rabbit eyes was conducted at the Ocular Pharmacology Department in collaboration with the Glaucoma services of our Centre between March 2000 and June 2002. Certification was obtained from the Ethics committee of All India Institute of Medical Sciences, Delhi, India. The study was so designed as to comply with the Helsinki Declaration of 1975, and with the guidelines for the ethical treatment of animals issued by the Government of India.

Sixteen eyes of 8 pigmented rabbits were randomly divided into two groups. One eye of each rabbit was randomly assigned to the study group, where trabeculectomy with AMG was performed, and the other eye to the control group, where trabeculectomy alone was performed.

All the rabbits were anaesthetized with intravenous 20 mg/ml sodium pentobarbital at a dose of 35-40 mg/kg. The anesthesia level was monitored by the blink reflex and the toe withdrawal reflex. A baseline IOP was measured with the Schiotz tonometer in both eyes. The mean of 3 readings were taken. Both eyes were operated in the same sitting, with the right eye being operated first. Using a superior approach, an 8-0 polyglactin traction suture was passed through the corneal stroma. The conjunctiva and Tenon's capsule were dissected as a single layer to form a limbus based flap. A 3×3 mm partial thickness scleral flap was raised and dissected towards the limbus. Next an amniotic membrane strip of 2×6 mm was sutured to the scleral bed with continuous 8-0 polyglactin (Vicryl) suture, keeping the mesenchymal surface in contact with the sclera. Utmost care was taken to prevent obstruction of the proposed sclerotomy site with the anterior edge of the graft. A full thickness internal sclerotomy of 1×3 mm was made and a peripheral iridotomy performed. The scleral flap was sutured over the graft at its corners with 10-0 monofilament nylon (MFN), and the conjunctiva closed with 8-0 polyglactin. The anterior chamber was formed with balanced salt solution. The contralateral eye underwent conventional trabeculectomy without AMG, with a similar technique. In both groups, IOP reduction and bleb formation were confirmed intraoperatively. All the surgeries were performed by the same surgeon (SK).

The procedure of human amniotic membrane procurement, preparation and storage has been described previously[3]. Briefly, placenta was obtained from cases of elective caesarean section. HIV, Hepatitis B and C, and syphilis were ruled out with appropriate tests. Erle's balanced salt solution was used to clear the placenta of clots under the lamellar flow hood. The chorion and the amnion were separated by blunt dissection. The amnion was then placed on a strip of nitrocellulose paper with the epithelial basement surface up. It was then cut into strips of 3×4 cm<sup>2</sup> and stored at -80°C in a sterile vial containing Dulbecco's modified Eagle medium and glycerol in the ratio of 1:1.

Post operatively only topical dexamethasone 0.1%, ciprofloxacin 0.3% and tropicamide 1% were used. Diclofenac was given intramuscularly for pain relief. IOP was measured under anesthesia on day 1, week 1, and at 1 month after surgery. During follow up, daily slit lamp evaluation (Hand held slit lamp) was done for the nature of the bleb, and its size measured with calipers from the limbus.

For the pathological study, the rabbits were sacrificed with a high dose of sodium pentobarbital. The eyes were enucleated and 1.5 mm diameter trephine biopsies of conjunctiva, tenons capsule, and AMG were taken, as applicable, from three sites: away from the bleb area, adjacent to the bleb area, and over the bleb area. Each biopsy specimen were divided into two halves and transported in 10% formaldehyde, one half for light microscopy and the other half for electron microscopy. For light microscopy the paraffin sections were cut, and Hematoxylin and Eosin sections were examined for fibroblast and epithelial cell outgrowth. Massons Trichrome stain was used to confirm the diagnosis.

The primary outcome measures were related to the formation of bleb, and the parameters studied were intraocular pressure (IOP), bleb duration, and histopathological appearance of the bleb area. Anterior chamber depth was not measured.

**Bleb duration:** The criteria to determine bleb failure were -

1. Bleb size of 3 mm or less as measured with a caliper from the limbus
2. Reduction from cystic to minimal elevation
3. Progression of bleb vascularity as determined by serial slit lamp examination

**IOP reduction:** The criterion for successful IOP reduction was -

1. A fall in IOP by  $\geq 3$  mm as compared to the preoperative IOP, or,
2. A fall by  $\geq 3$  mm Hg as compared to the control group.
3. A reduction of IOP by  $< 3$  mm Hg was taken as failure of surgery.

### Statistical analysis

All data were managed on Microsoft Excel spreadsheet, and statistical analysis performed on STATA 8.0 (Intercooled version). The Descriptive statistics for all parameters were analyzed for mean and standard deviation and changes following surgery within each group were assessed using paired 't' test. Analysis of variance was performed to compare the difference in preoperative and post-operative IOP.  $P < 0.05$  was considered as statistically significant.

### Results

Two rabbits were sacrificed as soon as the bleb started failing at 1 week in the control group. There was a statistically significant fall in IOP in both groups on day 1 as compared to the preoperative IOP ( $p < 0.001$ , for both groups). There was a significant increase in IOP in the control group ( $p < 0.001$ ) at 1 week, which was maintained at 2 weeks ( $p < 0.001$ ) and at 1 month ( $p = 0.001$ ) as compared to Day 1, but these IOP values were not significantly different from the preoperative IOP ( $p = 0.300$ ,  $p = 0.327$ ,  $p = 0.46$ , respectively). In the study group, the IOP at 1 week also was significantly lower than the preoperative IOP ( $p = 0.000$ ), but at 2 weeks ( $p = 0.27$ ) and at 1 month it was comparable to the preoperative IOP ( $p = 0.97$ ).

Paired samples t-test for equality of means was done to compare the IOP between the two groups of eyes at different follow up intervals. It was seen that on day 1, the IOP in the two groups was comparable ( $p = 0.38$ ), but at 1 week the IOP in the control group was significantly higher ( $p = 0.001$ ). The IOP in the two groups were statistically comparable to each other at 2 weeks and at 1 month. [ Table/Fig 5 ]

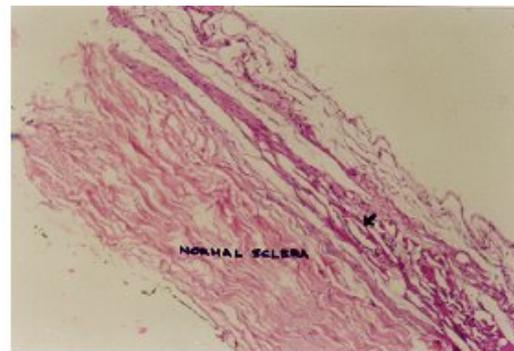
The mean duration of blebs was  $12.59 \pm 1.32$  days ( 8-15 days ) in the study eyes, and  $7.52 \pm 1.27$  day (7-12 days ) in the control group. This difference was statistically significant ( $p = 0.009$ ).

No surgical complications were seen in either group. There was no instance of over filtration.

### Pathological findings

Light microscopic examination of the early functioning bleb at 1 week in the study eye showed scattered lympho-mononuclear cells and edematous subconjunctival tissue indicative of filtering spaces. There was subconjunctival and episcleral fibrosis in the control eye.

The remaining 8 rabbits were sacrificed at 1 month. The blebs appeared flattened and scarred in both groups. However, on light microscopy, there was more marked subconjunctival and episcleral fibrosis in the control group in comparison to the study group [Tab/Fig 1] and [Tab/Fig 2] which was further corroborated on the electron microscopy [Tab/Fig 3] and [Tab/ Fig 4]. No evidence of lysis of the AMG was seen.



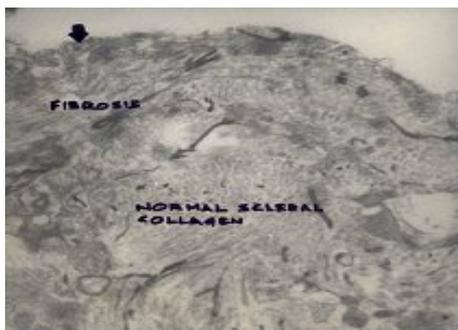
**Table/Fig 1:** Light microscopy of bleb area of study eye (with AMG) at 1 week, showing subconjunctival and episcleral fibrosis (arrow)



**Table/Fig 2:** Rabbit eye without AMG-light microscopy of bleb area showing sub conjunctival & episcleral fibrosis (arrow)



**Table/Fig 3** Electron microscopy of bleb area of study eye (with AMG) at 1 month, showing episcleral fibrosis [note the haphazard arrangement of the collagen fibrils at the top suggesting recent fibrosis (arrow)]



**Table/Fig 4** Electron microscopy of eye without AMG at 1 month, showing more marked episcleral fibrosis than in Figure 3 [note the haphazard arrangement of the collagen fibrils at the top suggesting recent fibrosis (arrow)]

## Discussion

Trabeculectomy remains the benchmark surgical procedure for glaucoma, but it is not adequate for refractory glaucoma. Attempts to enhance its success rate have revolved around the modulation of wound healing so that the chances of subconjunctival fibrosis and closure of the ostium are minimized. In the rabbit model, both the sclerostomy and the bleb have been shown to collapse due to bulk filling by granulation tissue and contraction by migratory fibroblasts (approximately 3 days) and by myofibroblasts (approximately 10 and 17 days). Clinically,

trabeculectomy in rabbit eyes fails between 1-2 weeks postoperatively.

The potential use of AMG as an adjuvant to trabeculectomy, especially in high risk cases, lies in its ability to enhance epithelialization, inhibit fibrosis, inflammation, and angiogenesis, while keeping complications to a minimum [4]. These clinical effects are a result of its ability to inhibit transforming growth factor  $\beta$  isoforms, TGF- $\beta$  receptor type-II, and differentiation of myofibroblasts in cultured human corneal and limbal fibroblasts. It has high hydraulic conductivity and poor immunogenicity, and may also function as an anatomical barrier. Significantly, no side effects have been reported. AMG compares well with Mitomycin-C (MMC), arguably the most commonly used adjuvant in high risk trabeculectomy, with respect to its ability to reduce fibroblast and macrophage numbers. But MMC is probably more effective than AMG in depressing wound healing after trabeculectomy. Nonetheless, AMG is regarded by some as possibly a safer alternative to MMC in selected cases in view of the severe adverse effects of the latter [5],[6].

Recent interest in AMG and trabeculectomy has focused on its role either alone, or in combination with Mitomycin C, suture lysis of scleral flap, and aqueous humor shunt implants [5],[6],[7]. The use of a scleral cannula to prevent occlusion of the sclerostomy has also been described. However, the reported success with all these procedures has not been consistent, especially with lengthened duration of follow up. Other modalities that have been pursued with trabeculectomy are antimetabolites and antifibrotic agents (5-FU), steroids, interferon- $\alpha$ , anti transforming growth factor antibody, disodium chromoglycate, and even photodynamic therapy [8].

An alternative technique is to replace the conjunctiva totally with amniotic membrane [9]. We decided to place the AMG underneath the superficial scleral flap, although others have also placed it between the conjunctiva-Tenons and the sclera with the assumption that it might better protect the whole surgical site. It has been recognized that direct contact of AMG to fibroblast of the Tenon's capsule might be important to inhibit effectively undesired scar formation [10],[11]. We found that the technique that we adopted increased the duration of the filtering bleb, and the duration of IOP lowering.

Our study corroborated the results of a similar study recently reported from China[12].

**Table/Fig 5. Intraocular pressure changes between study group and control group (n=16).**

Time interval	Groups	Mean $\pm$ SD (mmHg)	p-value
Preoperative	Study group	15.28 $\pm$ 1.20	
	Control group	15.11 $\pm$ 1.195	0.79
Day 1	Study group	7.61 $\pm$ 1.32	
	Control group	8.22 $\pm$ 1.37	0.37
1 week	Study group	9.30 $\pm$ 1.03	
	Control group	14.50 $\pm$ 1.58	0.00
2 weeks	Study group	14.34 $\pm$ 1.44	
	Control group	14.35 $\pm$ 1.63	0.98
1 month	Study group	15.30 $\pm$ 1.68	0.86
	Control group	15.42 $\pm$ 0.994	

An added area of concern is rejection of the xenograft in the rabbit model, where lysis was demonstrated histologically at 1 month, but not clinically. Short term effect of AMG might indicate rapid dissolution due to an immunologically induced inflammatory response, but this was not seen in our case. An alternative could be the use of rabbit AMG to overcome the possible factor of Xenograft reaction. Such a rejection of an AMG allograft had not yet been reported in any AMG augmented trabeculectomy in humans.

The parameters for defining success have varied. In terms of IOP, failure was defined in one study as a difference of IOP of 2 mm or less between the treated and the control eyes. However, others have opined that intraocular pressure may not be a reliable indicator of bleb function or of a patent sclerostomy in the rabbit model [12],[13]. In terms of bleb failure, one study on the role of subconjunctival mitomycin-C in rabbit eyes defined it as a bleb size of 3mm or less along the limbus, a reduction from cystic to minimal

conjunctival elevation, and progressive increase in vascularity. The same study considered reduction

of IOP as successful if it was 3 mm lower than the preoperative IOP or in comparison with the other eye. The most common signs of bleb failure are reduction in bleb size and reduction of conjunctival elevation. It might help to reduce the number of anti-glaucoma drug usage postoperatively[14],[15]. We noted a high incidence of encapsulated blebs ( 30% ) in our control group.

The enthusiasm for AMG augmented trabeculectomy is not universally shared[16]. There is a lack of reproducibility of technique and efficacy, and standardization of the outcome parameters, with the few studies, both animal and human, that have been reported. Small sample size and short duration of follow up in the animal model remains a limitation, as in our case. While the results of available studies are encouraging, it would seem that there is some way to go before its role as a clinical tool in contemporary glaucoma practice is adequately defined.

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**Conflict of Interest:** None.

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