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ORIGINAL ARTICLE

# Dehydrated human-amniotic-membrane allograft versus conjunctival autograft after pterygium excision

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## ABSTRACT

**Objective**

To compare the efficacy of dehydrated human-amniotic-membrane allograft with conjunctival autograft attached with fibrin glue following pterygium excision.

**Methods**

Forty-two patients undergoing excision of primary pterygium were enrolled in this prospective, randomized, interventional, controlled trial. After excision of pterygium, a superior bulbar conjunctival autograft was harvested and transferred onto bare sclera in 21 eyes, and dehydrated human-amniotic-membrane allograft (AmbioDry) in 21 eyes. Fibrin glue (Beriplast P) was used to attach the grafts in both groups. The patients were followed up for 3 months.

**Results**

All grafts in both groups were successfully attached. One patient in the conjunctival autograft group experienced graft dehiscence. Mean surgery time, postoperative pain, foreign-body sensation, and discomfort were not statistically different ($p = 0.16$, $p = 0.07$, $p = 0.82$, and $p = 0.31$ respectively). No recurrence was noted within the observation period. At day 1 postoperatively, mean tearing-severity scores of patients that received dehydrated amniotic-membrane allograft were statistically lower than those that received conjunctival autograft ($p = 0.024$). Cosmetic-grading results were statistically higher in the conjunctival autograft group at 3 months postoperatively ($p = 0.003$).

**Conclusion**

Dehydrated human-amniotic-membrane allograft attached with fibrin glue and anchored with nylon sutures is a safe and effective adjunct after excision of primary pterygium. It is comparable to conjunctival autograft in preventing early recurrence and can be considered as a primary grafting method after primary pterygium excision. However, conjunctival autograft has better cosmetic results than amniotic-membrane allograft.

***Keywords:*** *Pterygium, Conjunctival autograft, Amniotic-membrane transplantation, Fibrin glue*

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CONJUNCTIVAL autograft after pterygium excision is fast becoming the treatment of choice to prevent recurrences.[1-7] Conjunctival autograft as an adjunct was initially reported to have a recurrence rate of 5.3%. Since then, further studies have reported recurrence rates of up to 39% attributed to technique of pterygium excision. Although deemed safer than other adjunctive treatments, its use is limited in patients with larger defects and in patients where the superior conjunctiva has to be preserved.[4, 5] Conjunctival autograft has also been noted to be unsuccessful in suppressing postsurgical fibrosis.[6, 8, 9]

Transplantation of preserved human-amniotic membrane was recently introduced as an adjunct to pterygium surgery.[10-12] Studies have shown that the basement membrane promotes epithelial growth and differentiation, reinforces adhesion of basal epithelial cells and prevents epithelial apoptosis. The stromal matrix suppresses transforming-growth-factor-b signaling, proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts, and human conjunctival and pterygium body fibroblast, thereby inhibiting unwanted extracellular-matrix production and scarring.[10, 13] Some studies have shown it to be as effective as conjunctival autograft in preventing pterygium recurrence without the drawback of disrupting the superior conjunctiva and its inability to suppress postsurgical fibrosis.[5, 14-16]

Processing the amniotic membrane for transplantation is a tedious process that requires freezing it at –80°C.[15] The advent of dehydrated processed membranes has significantly made its use much more convenient since it is ready to use and can be stored at room temperature. Dehydrated human-amniotic-membrane allografts (AmbioDry, Okto Ophtho, IOP Inc. Costa Mesa, CA, USA) are dehydrated, decellularized, sterilized human-amniotic-membrane-tissue grafts. Using the dehydrated membrane entails hydrating it with saline solution intraoperatively. Fournier and McLachlan reported the successful use of AmbioDry in destructive lesions of the conjunctiva, acting as a reconstructive graft in nonhealing lesions of the ocular surface.[17] Battle reported the use of AmbioDry following excision of recurrent pterygium.[18]

Both conjunctival autograft and amniotic-membrane transplantation are successful in preventing pterygium recurrence in most people. Both procedures, however, cause a significant amount of discomfort for the patient due to the number of sutures needed. Thus, tissue adhesives such as the biologic and biodegradable fibrin-based adhesives have gained popularity lately. They also induce minimal inflammation.[19] A recent comparative study reported a 100% success rate for both fibrin glue and nylon sutures in conjunctival autografts after pterygium excision. [20] It also found that using fibrin glue resulted in significantly shorter surgery time and lesser postoperative discomfort.

Our study compared the transplantation of dehydrated human-amniotic-membrane allograft with conjunctival autograft after pterygium excision using fibrin glue.

## METHODOLOGY

Forty-two consecutive patients with primary pterygia undergoing pterygium excision at the University of the Philippines-Philippine General Hospital from November 2004 to March 2005 were prospectively enrolled. General data, demographic factors, medical and ocular history were obtained. All patients underwent a complete ophthalmologic examination including visual-acuity testing, refraction, intraocular-pressure measurement, slitlamp examination, and anterior-segment photography. Patients with a history of ocular pathology (other than error of refraction), ocular surgery or trauma, severe ocular-surface disease, chronic conjunctivitis, glaucoma, family history of glaucoma, ocular hypertension, narrow occludable angles, glaucomatous or physiologic disc cupping, or known hypersensitivity to the components of commercial fibrin glue (Beriplast P, CSL Behring, King of Prussia, PA, USA) were excluded. Informed consent was obtained from all patients. The institutional review board approved the protocol and informed consent form.

### Pterygium Grading

Pterygia were measured and graded according to the grading scheme proposed by Tan et al. in 1997. The pterygia were classified into grades 1, 2, or 3 based on slitlamp biomicroscopy evaluation. Grade 1 (atrophic) included pterygia in which episcleral vessels under the body of pterygium are not obscured and clearly distinguished. Grade 3 (fleshy) included pterygia in which episcleral vessels underlying the body of the pterygium are totally obscured. Grade 2 (intermediate) included all other pterygia that did not fall under the other two grades.[21]

### Treatment Assignment

Using a table of random numbers, patients were randomized to receive either pterygium excision with amniotic-membrane transplantation or pterygium excision with conjunctival autograft. One investigator examined all patients pre- and postoperatively. All surgeries were performed by two surgeons (KTL and RLBS).

### Fibrin Glue

Beriplast P is a fibrin sealant that imitates the final stage of the coagulation process. Fibrinogen is converted into fibrin on the tissue surface by the action of thrombin. The fibrin formed is then cross-linked by factor XIIIa, thus creating a firm, mechanically stable fibrin network.

Aprotinin from bovine lungs is then added to the fibrin sealant to prevent rapid fibrinolysis. The potential transmission of contaminants is mitigated by screening of plasma donors, virus removal and inactivation by the manufacturing process, and pasteurization and clinical virus-safety measures.[20]

**Surgical Technique**

One drop each of 2.5% phenylephrine (Mydfrin, Alcon Lab Inc, Fort Worth, TX, USA), 0.15% brimonidine (Alphagan P, Allergan Inc., Irvine, CA, USA), and 0.5% antazoline phosphate, 0.05% naphazoline HCl, 0.125% Zn sulfate (Zincfrin-A, Alcon Lab Inc, Fort Worth, TX, USA) were instilled thirty minutes prior to surgery. After instillation of topical proparacaine HCl (Alcaine, Alcon Lab Inc, Fort Worth, TX, USA), standard preparation and draping of the involved eye were done. Prior to excision, the widest dimensions of the pterygium were measured using a caliper (head to limbus and width at limbal area). The head of the pterygium was carefully dissected from the cornea using a surgical blade (No.15). Lidocaine with epinephrine solution (Xylocaine 2%,Astra-Zeneca, Sweden) was injected subconjunctivally to balloon the conjunctiva and delineate the fibrovascular tissue underneath. The pterygium body was then dissected from the underlying sclera using Wescott scissors. Subconjunctival fibrovascular tissue was dissected away from the overlying conjunctival epithelium. The pterygium was then excised at the base. Atrophic edges of the conjunctiva were removed. Tenon's capsule was removed to provide a clean scleral bed for the placement of the graft. The greatest vertical and horizontal diameters of the bare sclera were then measured using a caliper.

The dehydrated human-amniotic-membrane allograft was prepared according to the manufacturer's instructions. The dry allograft was trimmed to be oversized by 1 mm at all sides and then hydrated while on the surgical site with sterile saline solution orienting the graft basement matrix side up. Several drops of sterile solution were applied to the allograft at one- to two-minute intervals for a period of 5 to 10 minutes. One drop of the fibrinogen solution was applied to the bare sclera coating the whole surface. Thrombin solution was then applied over the fibrinogen solution. The amnion graft was then slid onto the bare sclera making sure that the graft was completely adherent or apposed to the recipient conjunctiva. The hydrated amnion graft was noted to be mobile even with the application of the fibrin glue. Four anchor sutures (nylon 10-0 Alcon Lab Inc, Fort Worth, TX, USA) were added to secure the amnion graft to the underlying sclera. The suture knots were trimmed and buried.

Free conjunctival grafts oversized by 1 mm at all sides marked with gentian violet were harvested from the superotemporal or superonasal conjunctiva of the same eye for nasal and temporal pterygium, respectively. Lidocaine with epinephrine solution was injected subconjunctivally to balloon the area of the graft and separate it from the underlying Tenon's capsule. Using atraumatic conjunctival forceps and Vannas scissors, the conjunctiva was dissected from Tenon's capsule taking care to include as little Tenon's tissue as possible in the graft. The graft was then slid carefully on top of the cornea and kept moist using sterile normal saline solution. Fibrin glue was applied as mentioned previously. The graft was then slid onto the bare sclera making sure that it was completely adherent or apposed to the recipient conjunctiva.

Both grafts were then smoothed, gently pressing them onto the scleral bed for five minutes. Excess glue was removed and the graft trimmed if necessary. The speculum was removed and the patient was asked to blink to test graft adherence and mobility.

Steroid-antibiotic ointment was then applied on all eyes and a pressure patch applied for 24 hours.

**Postoperative Regimen and Follow-up Examination**

After removal of the patch, visual-acuity testing, biomicroscopic examination, fluorescein staining, and anterior-segment photography were done. The presence, appearance, and integrity of the graft were noted. All cases received identical postoperative regimen of topical steroid-antibiotic drops and topical steroid-antibiotic ointment (Tobradex, Alcon Lab Inc, Fort Worth, TX, USA) tapered over two months.

All patients were followed up weekly for 2 weeks, then at one month, two months, and three months. At each postoperative visit, the same examinations were performed by a single investigator.

**Outcome Measures**

This study compared surgery time, efficacy, early recurrence, postoperative discomfort, and cosmesis in both groups.

Surgery time was recorded from the start of graft preparation to the end of surgery.

Graft success was defined as incorporation of the graft into the surrounding conjunctiva with complete epithelialization over it; graft failure was defined as absence of the graft at 2 weeks or less postoperatively.

Pterygium recurrence was defined as the presence of fibrovascular conjunctival reencroachment extending beyond the limbus.

Subjective sensations such as pain, foreign body sensation, tearing, and discomfort were determined and measured using a 5-point scale adapted from Lim-Bon-

Siong et al:[22] NONE = no pain at all, VERY MILD = presence of pain but easily tolerated, MILD = presence of pain causing some discomfort, MODERATE = presence of pain that interferes with usual activity or sleep, SEVERE = presence of pain that completely interferes with usual activity or sleep. The questionnaire was administered at each follow-up visit.

Three separate masked observers were given photographs of the operated eye taken at 3 months postoperatively and were asked to grade the cosmetic appearance using a 5-point scale: POOR = very congested graft with numerous radial vessels, FAIR = congested graft with few radial vessels, GOOD = slightly congested graft with minimal radial vessels, VERY GOOD = graft not congested with very minimal conjunctival vessels and EXCELLENT = pristine, untouched appearance.

Postoperative complications such as graft ischemia and necrosis, infection, graft dehiscence, persistent epithelial defect, steroid-induced glaucoma, and granuloma formation were likewise noted.

**Statistical Analysis**

All continuous numerical data were summarized using descriptive statistics (percentages and frequency distribution). Tests for homogeneity of sample included comparing for significant differences in all numerical continuous data. Data filtration and test for normality of data revealed skewness values greater than 1. Owing to the small sample size, nonparametric statistics were employed. Mann Whitney U Test was used to compare the significant change in mean ranks between the two procedures while chi-square was used to detect independence of the groups and the discrete categories.

Spearman's rho correlation was used to investigate the association between pterygium grade and cosmesis.

In order to compare the proportion of ratings for a specific scale category, the Z-test for two proportions was utilized.

Comparison of inter-rater agreement was facilitated using kappa statistics and *p*-values compared using Statistica version 1999 (Stat Soft Inc., Tulsa, OK, USA).

To compare for the change in pain, foreign body sensation, tearing and discomfort severity at baseline and 3 months postsurgery, Wilcoxon signed rank test for paired data was used for each treatment group while the Mann Whitney U test was utilized to compare the mean ranks of scores between the two groups at each period of observation.

All statistical tests were carried out to reject the null hypothesis assumed for each study objective at .05 level of significance and were carried out using the Statistical Package for the Social Sciences ver. 10 (SPSS, Inc., Chicago, IL, USA) and the Statistica version 1999.

## RESULTS

All 42 patients met the inclusion criteria. They were randomized to receive either dehydrated human-amniotic-membrane allograft (n = 21) or conjunctival autograft (n=21). One patient in the dehydrated-human-amniotic-membrane-allograft group was lost to follow-up. A total of 41 subjects were included in the final analysis. The baseline clinical profile and preoperative evaluation are summarized in Table 1. The youngest patient in this study was 20 years while the oldest was 74 years (mean of 43 ± 13, median age of 43 years). There were 22 males (54%). The left eye was most commonly involved (23 or 56%) and a nasal location was most common. All patients had a best-corrected visual acuity of ≥ 6/30 except for 1 patient in the autograft group who had ≥1/60.

Tests of homogeneity revealed no significant difference between the two groups at baseline in terms of age, sex

**Table 1. Demographic and preoperative clinical profile of study patients.**

| Characteristic | Amniotic Membrane Allograft N= 20, (%) | Conjunctival Autograft N=21, (%) | *p*[a] |
|---|---|---|---|
| *Age* | | | |
| Range | 21 to 74 | 20 to 69 | |
| Mean ± SD (years) | 43 ± 16 | 43 ± 13 | 0.86[b] |
| Median | 42 | 43 | |
| *Sex* | | | |
| Male | 10 (50) | 12 (57) | 0.64[c] |
| Female | 10 (50) | 9 (43) | |
| *Affected eye* | | | |
| Right | 9 (45) | 9 (43) | 0.89[c] |
| Left | 11 (55) | 12 (57) | |
| *Location of pterygium* | | | |
| Nasal | 18 (90) | 16 (76) | 0.32[c] |
| Temporal | 2 (10) | 3 (14) | |
| Bipolar | 0 | 2 (9) | |
| *Duration* | | | |
| < 1 year | 3 (15) | 1 (5) | 0.20[c] |
| 1- 5 years | 15 (75) | 15 (71) | |
| 5-10 years | 1 (5) | 0 | |
| >10 years | 1 (5) | 5 (24) | |
| *Grading* | | | |
| I | 4 | 2 | 0.55[c] |
| II | 13 | 14 | |
| III | 3 | 5 | |
| *Measurements* | | | |
| Horizontal | | | |
| Range | 1.5 to 7 | 2 to 8 | 0.16[b] |
| Mean ± SD (mm) | 3.4 ± 1.5 | 4.4 ± 2 | |
| Median | 3 | 4 | |
| Vertical | | | |
| Range | 3 to 8 | 2.5 to 11 | 0.69[b] |
| Mean ± SD (mm) | 5 ± 1.3 | 5.7 ± 2 | |
| Median | 5 | 5 | |

[a]Significant difference if *p* value<.05
[b]Computed using Mann Whitney U Test, SPSS 10
[c]Computed using Chi-square, SPSS 10

distribution, affected eye, location of pterygium, duration of the pterygium based on history, use of topical medications, baseline pterygium grading, horizontal and vertical measurements of pterygium size and intraoperative defect size ($p > .05$ for all).

Mean surgery times (from application of graft to end of surgery) were not significantly different between the two groups ($p = 0.16$) (Table 2).

All patients in both groups had good graft survival from day 1 to 3 months postoperatively (Table 2). One patient in the conjunctival autograft group had graft dehiscence at day 1 postoperatively, which had to be sutured, but had subsequent good graft survival thereafter. One patient in the conjunctival autograft group developed a granuloma at the junction of the graft and recipient conjunctiva, and underwent excision of granuloma.

No recurrences of pterygia were noted in both groups at three months postoperatively (Table 2).

We compared the severity of pain, tearing, foreign-body sensation, and level of discomfort between the two groups across the specified periods of observation. Among all patients, only 1 in the conjunctival autograft group complained of severe pain noted at day 1 postsurgery. Most of the subjects had mild to moderate pain that gradually abated at 2 weeks and beyond. Pain severity scores were not significantly different between the two procedures across the periods of observation ($p = 0.07$). The mean tearing-severity scores of patients who received dehydrated human-amniotic-membrane allograft were statistically lower compared with those who received conjunctival autograft at day 1 postoperatively ($p = 0.024$). Foreign-body-sensation severity was similar for both groups at baseline, but the rate of decrease over the ensuing weeks was statistically significant ($p < .001$). The degree of severity change, however, was not significantly different between the two procedures ($p = 0.82$). The degree of change in discomfort severity was also similar ($p = 0.31$).

Cosmetic-grading results showed a statistically higher proportion of "GOOD" ratings by patients randomized to the conjunctival autograft compared with those who received dehydrated human-amniotic-membrane allograft (Table 2). Although there was a slightly higher proportion of "POOR" ratings in the amniotic-membrane group (12% versus 3%), this was not statistically significant ($p = .06$) (Table 2).

**Table 2. Outcome measures.**

| Outcome | Amniotic Membrane Allograft N= 20, (%) | Conjunctival Autograft N=21, (%) | p[a] |
|---|---|---|---|
| *Surgery Time* | | | |
| Application of graft to end | | | |
| Range (minutes) | 8 to 25 | 8 to 36 | 0.16[b] |
| Mean | 14 ± 4 | 17 ± 7 | |
| *Graft Survival* | | | |
| Intact graft | 20 | 20 | |
| Graft dehiscence[d] | 0 | 1 | |
| *Recurrence* | 0 | 0 | |
| *Complications* | 0 | 1[e] | |
| *Cosmesis Rating* | | | |
| Excellent | 10 (17) | 7 (11) | 0.34[c] |
| Very Good | 22 (36) | 18 (29) | 0.41[c] |
| Good | 13 (22) | 25 (40) | 0.03[c] |
| Fair | 8 (13) | 11 (17) | 0.54[c] |
| Poor | 7 (12) | 2 (3) | 0.06[c] |
| Number of ratings made | 60 | 63 | |

[a]Significant difference if p < .05
[b]Computed using Mann Whitney U Test, SPSS 10.
[c]Computed using Z-test of 2 proportions, Statistica version 1999.
[d] Occurred 1 day postoperatively
[e] Granuloma 2 months postoperatively

## DISCUSSION

This study compared suture-anchored AmbioDry graft with conjunctival autograft using fibrin glue to secure both grafts. Mean surgery time was noted to be similar in both groups. Both treatment groups also exhibited similar graft survival rates. No recurrences occurred by the end of the three-month observation period. No conclusion, however, can be made from our data regarding long-term recurrence rates. Among the postoperative symptoms, only tearing scores showed a significant difference between the 2 groups.

To the best of our knowledge, no clinical studies have yet been published comparing dehydrated human-amniotic-membrane allograft with conjunctival autograft after pterygium excision. Battle reported one case where AmbioDry was used following excision of recurrent pterygium.[18]

We noted a significant learning curve in using AmbioDry. In its dry state, the graft was difficult to handle and to orient. Handling the translucent, hydrated graft also proved to be a challenge especially in the presence of blood.

In this study, anchoring sutures had to be placed in the AmbioDry group because of residual graft mobility after fibrin-glue application. Preliminary results of a similar study using a different preparation of fibrin glue (Tisseel, Baxter, Vienna, Austria) showed complete adherence of the graft to the bare sclera (personal communication with Roy Chuck, MD, Wilmer Eye Institute, Baltimore, MD, USA). Differences in the molecular component of the fibrin-glue preparations may be the reason for the disparity in results.

There were no serious complications related to both procedures observed in this study. One patient developed a granuloma 2 months postoperatively, which was subsequently excised. One patient had a partial graft

dehiscence noted on the first postoperative day that had to be sutured. All sutures were released 1 month postsurgery.

Although cosmetic appearance of patients in the conjunctival autograft group was significantly better, AmbioDry remains to be a viable alternative for patients with advanced or bipolar pterygium, scarred conjunctiva, and glaucoma patients who may need filtering procedure in the future.

The limitations of this study include the short follow-up period, the lack of an independent investigator to evaluate recurrence and other complications, and the lack of a more objective grading scale for cosmetic appearance.

In summary, dehydrated human-amniotic-membrane allograft anchored with nylon sutures and attached with fibrin glue (Beriplast P) is a safe and effective adjunct after excision of primary pterygium. It is comparable to conjunctival autograft in preventing early recurrence and can be considered as a primary grafting method for primary pterygium excision. However, conjunctival autograft may be superior cosmetically. Further studies are needed to evaluate the long-term efficacy of dehydrated amniotic-membrane allograft and to determine recurrence rates.

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