Amniotic membrane graft for primary pterygium: comparison with conjunctival autograft and topical mitomycin C treatment

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Abstract

Aim—To study the efficacy and safety of amniotic membrane graft as an adjunctive therapy after removal of primary pterygium, and to compare the clinical outcome with conjunctival autograft and topical mitomycin C.

Methods—80 eyes of 71 patients with primary pterygia were treated with excision followed by amniotic membrane graft. The result was compared retrospectively with 56 eyes of 50 patients receiving conjunctival autograft, and 54 eyes of 46 patients receiving topical mitomycin C. Patients were followed for at least 6 months, and the averaged follow up periods for the three groups were 13.8, 22.8, and 18.4 months, respectively.

Results—There were three recurrences (3.8%) in the amniotic membrane graft group, three recurrences (5.4%) in the conjunctival autograft group, and two recurrences (3.7%) in the topical mitomycin C group. There was no significant difference in recurrence rate among the three groups (p = 0.879). No major complications occurred in the amniotic membrane graft group or the conjunctival autograft group. One case of infectious scleritis due to scleral ischaemia occurred in the topical mitomycin C group.

Conclusion—This study showed that amniotic membrane graft was as effective as conjunctival autograft and mitomycin C in preventing pterygium recurrence, and can be considered as a preferred grafting procedure for primary pterygium.

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Pterygium is a common worldwide external eye disease, affecting populations especially in tropical and subtropical areas. To prevent recurrence, two major adjunctive therapies are usually performed: (1) the application of antimetabolites, and (2) conjunctival or limbal autograft. Although many other therapeutic modalities have been proposed, it requires extensive studies of efficiency and safety before a new procedure can be considered as ideal.

Recently, preserved human amniotic membrane has been advocated for the management of many ocular surface disorders,[1] such as after persistent corneal epithelial defects with ulceration,[2] reconstruction of ocular surface in conjunctival neoplasms or scarring,[3] chemical or thermal burns,[4 5] advanced ocular cicatricial pemphigoid and Stevens–Johnson syndrome,[6] decreasing corneal scarring following excimer laser photoablation,[7] prevention of adhesion after trabeculectomy,[8] and to reconstruct conjunctival defect after pterygium removal.[9 10] A previous study reported that amniotic membrane grafts were less effective than conjunctival autografts for pterygium surgery.[9] However, as more is understood about the molecular mechanisms by which amniotic membrane transplantation inhibits pathological neovascularisation,[11–13] scar formation,[14] and inflammation[15 16] in various ocular surface diseases, it is reasonable to hypothesise that with proper management, amniotic membrane grafts may yield better result than previously reported in reducing pterygium recurrence. We report the clinical outcome after amniotic membrane graft for primary pterygium among ethnic Chinese patients in Taiwan. The results are compared retrospectively with those patients receiving conjunctival autograft and postoperative topical mitomycin C.

Materials and methods

PATIENTS

From 1993 to 1997, 80 consecutive eyes of 71 patients with primary pterygium (M:F = 35:36, mean age 56.7 (SD 11.3), range 31–83 years) that met the inclusion criteria (see below) were enrolled prospectively in this study. To be enrolled, these patients had been questioned and medical data reviewed in detail that none had major systemic diseases such as diabetes mellitus or collagen vascular diseases, and complete ophthalmic examinations including visual acuity, intraocular pressure, slit lamp biomicroscopy, and funduscopy were performed to assure that none of them had major eye diseases such as dry eye, cicatricial pemphigoid, glaucoma, or vitreoretinal disease. In all cases in the amniotic membrane graft group, the size of the pterygium was at least 2 mm (2.0–8.0, mean 4.4 (1.4) mm) into the cornea.[17] All patients had a minimum follow up of 6 months (6–43 months, mean 13.8 (10.4) months) at the time of analysis of the data. The clinical results were compared retrospectively with those patients who received conjunctival autograft and topical mitomycin C for primary pterygium between 1990 and 1993, which were also performed by the same surgeon (DHKM). For the conjunctival autograft group, there were 56 eyes of 50 patients (M:F = 19:31, mean age 56.4 (11.9) years, range 33–76 years). For the topical mitomycin C group, there were 54 eyes of 46 patients (M:F = 21:25, mean age 57.8 (12.2)

Table 1 Comparison of patients' demographic data among amniotic membrane graft group, conjunctival autograft group, and topical mitomycin C group

	Amniotic membrane graft	Conjunctival autograft	Topical mitomycin C	p Value
No (eye) of patients	71 (80)	50 (56)	46 (54)	—
Sex (M:F)	35:36	19:31	21:25	0.466
Mean age (years) (SD)	56.7 (11.3)	56.4 (11.9)	57.8 (12.2)	0.836
(range)	(31–83)	(33–76)	(39–91)	
Laterality (R:L)	39:41	19:37	23:31	0.350
Mean follow up (months) (SD)	13.8 (10.4)	22.8 (18.2)	18.4 (18.3)	0.004
(range)	(6–43)	(6–50)	(6–76)	

years, range 39–91 years). The age and sex distribution of both groups were similar to those in the amniotic membrane graft group (Table 1). The mean follow up period for the conjunctival autograft group was 22.8 (18.2) months (6–50 months), and that for topical mitomycin C group was 18.4 (18.3) months (6–76 months).

Surgical procedures

AMNIOTIC MEMBRANE PREPARATION

The approval for the use of cryopreserved human amniotic membrane for the pterygium operation was obtained from the committee of medical ethics and human experiment of Chang Gung Memorial Hospital, Taoyuan, Taiwan. Isolation and preservation of human amniotic membrane obtained following a caesarean section have been described previously.[2] [9] Briefly, after thorough washing in normal saline, amniotic membrane was cut into about 3×3 cm pieces and put into vials containing 1:1 mixture of Dulbecco's modified Eagle's Medium (DMEM; Sigma, St Louis, MO, USA) and sterilised glycerol, and then was preserved in a refrigerator at −70°C until use. The membranes were kept frozen for 2–3 months before use. According to the rate of consumption, the duration of cryopreservation ranged from 2 to 6 months. Upon use, the membrane was thawed and soaked in normal saline containing gentamicin (3 mg/ml) for at least 3 minutes, and the jelly-like material over the stromal side was meticulously removed. During the study period, a total of four amniotic membranes were used.

PTERYGIUM EXCISION

Before surgery, written informed consent for the procedure was obtained from each patient. All of the surgeries were performed by the same surgeon (DHKM). Subconjunctival anaesthesia with 4% lignocaine (Xylocaine) containing 1:10 000 adrenaline (epinephrine) was used for most patients. Peribulbar anaesthesia was reserved for those patients who could not cooperate. For nasal pterygia, the patients were asked to look temporally. The head of pterygium was first separated at the limbus and dissected towards the central cornea with a spring scissors. After excising the head and most of the body of the pterygium, subconjunctival Tenon's tissue was separated from overlying conjunctiva, undermined, and excised extensively upward and downward towards the fornices, and medially towards but not reaching the caruncle. Caution was taken not to damage the medial rectus muscle. Cautery was

applied to bleeders. The conjunctiva above and below the pterygium was trimmed to create a rectangular area of bare sclera. Residual fibrovascular tissue over the cornea was removed either by toothed forceps or by gentle scrapping with a No 15 Baird-Parker blade.

AMNIOTIC MEMBRANE GRAFT

A rectangular conjunctival defect of approximately 5×7 to 6×8 mm or even larger was created. This bare scleral area was then covered with amniotic membrane, which was oriented with basement membrane side up. The amniotic membrane was sutured through the episcleral tissue to the edge of conjunctiva along the bare sclera border with seven to eight interrupted stitches of 8-0 Vicryl suture.

CONJUNCTIVAL AUTOGRAFT

The technique of conjunctival autograft has been described previously.[18–21] A conjunctival graft matching the bare sclera was harvested from superotemporal bulbar conjunctiva 3 mm from the limbus. The donor site was later closed with a continuous suture of 8-0 Vicryl. The graft was then sutured to bare sclera with interrupted 8-0 Vicryl as mentioned above.

TOPICAL MITOMYCIN C

After removal of the pterygium and Tenon's tissue, the adjacent conjunctiva was fixed to the upper and lower corner of bare sclera with 8-0 Vicryl. Mitomycin C solution 0.02% made by resuspending mitomycin C powder (Kyowa Hakko Kogyo Co, Tokyo, Japan; 2 mg/vial) with 10 ml of Tear Naturale (Alcon-Couvreur, Puurs, Belgium) was applied three times daily for 1 week. The patients were cautioned that they should not use the eye drop more frequently, and the drug should be discarded after 1 week.

POSTOPERATIVE FOLLOW UP

Postoperatively, Maxitrol ointment (Alcon) twice daily to four times daily with oral indomethacin 25 mg and antacid four times daily were given, and if there was excessive irritation from the stitches, lubricants such as Tear Naturale or Balanced Salt Solution (Alcon) were given. Patients were examined at 1 week, and most of the stitches were removed at 2 weeks. Thereafter, the eye drops were changed to 0.1% fluorometholone (Ciba Vision, Hettlingen, Switzerland) four times daily then decreased to twice daily 1 month later, and then changed to 0.02% fluorometholone (Santen, Osaka, Japan) four times daily to twice daily for 3 months. Patients were followed monthly from the first month to the fourth month, then bimonthly from the fourth month to 1 year. Thereafter, regular follow ups at 3 month interval were encouraged. External ocular photographs were taken preoperatively, postoperatively at 1 month, and every 3 months. Complications such as pyogenic granuloma, inclusion cyst, or scleral thinning were recorded. Recurrence was defined as any fibrovascular growth beyond the limbus onto the cornea, and was assessed by another

Table 2 Comparison of cumulative non-recurrence rate among amniotic membrane graft group, conjunctival autograft group, and topical mitomycin C group

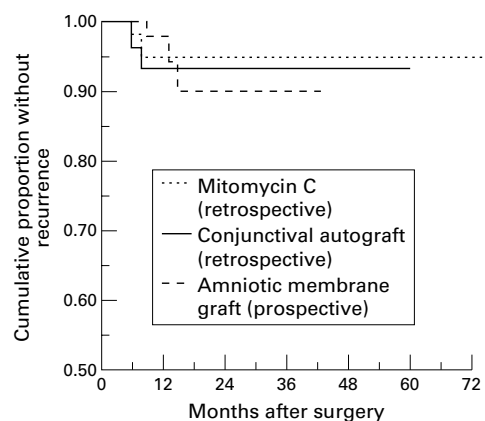|	No (%) of recurrences	Cumulative non-recurrence rate (%)			
		6 months	1 year	2 years	p Value
Amniotic membrane graft	3/80 (3.8%)	100%	97.7%	90.0%	
Conjunctival autograft	3/56 (5.4%)	96.4%	93.4%	93.4%	0.937
Topical mitomycin C	2/54 (3.7%)	98.2%	94.6%	94.6%	

Figure 1 Kaplan–Meier survival curve of recurrence after pterygium excision followed by amniotic membrane graft, conjunctival autograft, and topical mitomycin C treatment.

observer (SBL) by slit lamp examination or by postoperative photographs.

As the recurrence rate of pterygium excision without any adjunctive therapy is unacceptably high, for ethical reasons, we did not use a control group without any adjunctive treatment.[22][23] Comparative data from the literatures were used.

STATISTIC ANALYSIS

Eyes rather than people were used as an unit for statistical analysis, because there were only a small number (13.8%) of patients who had both eyes operated. Data were presented as mean (SD) or frequency (%). The χ^2 test was done to compare categorical data among the three groups. One way analysis of variance (ANOVA) was used to compare continuous data among the three groups. Scheffe's multiple comparison was applied for those with significant findings in one way ANOVA. Recurrence rate was computed using the Kaplan–Meier method and compared among the three groups using the log rank test. All p values reported were two sided. Statistical significance was considered when the p value was <0.05.

Table 3 Comparison of cumulative non-recurrence rate for all patients stratified by age and sex only

	No (%) of recurrence	Cumulative non-recurrence rate (%)			
		6 months	1 year	2 years	p Value
Age (years)					
<50	1/48 (2.1%)	97.9%	97.9%	97.9%	
50–59	3/62 (4.8%)	100%	92.7%	92.7%	0.834
⩾60	4/80 (5.0%)	97.5%	97.5%	91.9%	
Sex					
M (eye)	6/81 (7.4%)	96.2%	92.4%	92.4%	0.073
F (eye)	2/109 (1.8%)	100%	98.2%	95.8%	

Results

The characteristics of patients in the three groups were compared in Table 1. There were no statistically significant difference regarding sex (p = 0.466), age (p = 0.836), and laterality (p = 0.350) among the three groups. Averaged follow up time was different in the three groups (p = 0.004), where the conjunctival autograft group (22.8 (SD 18.2) months) was significantly longer than that in the amniotic membrane graft group (13.8 (10.4) months) as indicated by Scheffe's multiple comparison.

Three cases (3.8%) in the amniotic membrane graft group developed recurrence which occurred at 9, 13, and 15 (mean 12.3) months postoperatively (Table 2). Three cases (5.4%) in the conjunctival autograft group developed recurrence, which occurred at 2, 3, and 4 (mean 3.0) months postoperatively. Two cases (3.7%) in the topical mitomycin C group developed recurrence, which occurred at 2 and 9 (mean 5.5) months postoperatively. There was no significant difference in the recurrence rate among the three groups (p = 0.879). To further consider the time of recurrence, a Kaplan–Meier curve was made. The non-recurrence rate at 2 years postoperatively for the amniotic membrane graft group was 90.0%, which was not significantly different from that for the conjunctival autograft (93.4%) and the mitomycin C groups (94.6%, p = 0.937) (Table 2 and Fig 1). When stratified by age regardless of operation groups, there was no significant difference in the recurrence rate among patients below 50 years (one case, 2.1%), between 50 and 59 years (three cases, 4.8%), and above 60 years (four cases, 5.0%, p = 0.834). Likewise, when stratified by sex only, there was no significant difference in the recurrence rate between male (six cases, 7.4%) and female patients (two cases, 1.8%, p = 0.073; Table 3).

Because there were 13.8% patients who had both eyes operated, we had performed the entire statistical analysis on data with one eye from each patient only (data not shown). No obvious difference between the two results was seen, indicating that the repeated observations did not alter our findings.

In the amniotic membrane graft group, one case (1.25%) of pyogenic granuloma and one case (1.25%) of iatrogenic microhyphaema were experienced. The latter was probably induced by an inadvertent deep suture at the limbus which completely resolved 3 days later. In the conjunctival autograft group, two cases of pyogenic granuloma (3.6%) and four cases (7.3%) of conjunctival inclusion cyst occurred. Two of the cysts were punctured to drain the subconjunctival fluid, and the others resolved spontaneously. One case (1.8%) in the mitomycin C group developed pyogenic granuloma, which had to be excised 1 month later. Although only one case (1.8%) in the mitomycin C group developed scleral ischaemia postoperatively, this case unfortunately suffered from infectious scleritis caused by *Pseudomonas aeruginosa* 1 year later.

Discussion

It is believed that surgical trauma and subsequent postoperative inflammation activates subconjunctival fibroblasts, and the proliferation of fibroblasts and vascular cells, and deposition of extracellular matrix (ECM) proteins in turn contribute to the pterygium recurrence.[17 24 25] Alternatively, pterygium fibroblasts were reported to exhibit some characteristics of transformed cells such as hyperproliferation[26] and overexpression of matrix metalloproteinases,[27] which may partially explain the invasive nature of pterygium tissue.

As a natural basement membrane, amniotic membrane (AM) contains various matrix proteins[28] which facilitate the adhesion,[29 30] migration,[31] differentiation,[32 33] and prevention of apoptosis of epithelial cells.[34 35] The AM is also capable of binding growth factors which may help to promote wound healing.[36–39] However, these characteristics of AM may not fully explain why AM graft prevents pterygium recurrence. For example, it has been shown that a supernatant of homogenised AM promotes rather than inhibits proliferation of conjunctival fibroblasts.[38] In addition, factors contained in AM may also change after preservation. It has been reported that after preservation at −80°C for one month, activities of transforming growth factor β (TGFβ) 1 and 2, basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF) in AM decreases by 50%.[38] However, recent finding indicated that preserved AM suppresses the expression of TGFβ-I, TGFβ-II, TGFβ-III, TGFβ receptor type II, and myofibroblast differentiation in corneal and limbal fibroblasts.[14] Likewise, preserved AM also suppresses the signalling pathway of TGFβ, CD-44, β-1 integrin, and FGFR1/flg of pterygium fibroblasts.[40] Subsequently, AM matrix inhibits ECM production and scar formation by these fibroblasts. Therefore, promotion of conjunctival epithelial wound healing and suppressing activation and ECM production by pterygium fibroblast are thought to be the major mechanisms by which an AM graft inhibits pterygium recurrence. Other possible mechanisms include inhibition of inflammation by inhibiting chemokines expression by fibroblasts[15] and interleukin-1 expression by epithelial cells,[16] inhibition of neovascularisation by inhibiting vascular endothelial cell growth,[13] presence of anti-angiogenic/anti-inflammatory proteins,[12] and protease inhibitors.[41] Among various protease inhibitors, tissue inhibitors of metalloproteinase (TIMPs) are remarkable in that TIMP activity in AM is preserved following cryopreservation.[12 42] Possibly, inhibition of postoperative inflammation and inhibition of vascular cells activation and invasion by AM may also contribute to reduce pterygium recurrence.

A recurrence rate of 10.9% following AM graft for primary pterygium has been reported,[9] together with rates of 25% to 37.5% for recurrent pterygia.[9 10] In this study, the recurrence rate following AM graft for primary pterygium was 3.8%, which was about the same as the topical mitomycin C group (3.7%), and was slightly better than the conjunctival autograft group (5.4%). Compared with a previous report,[9] in this study more patients were enrolled (80 eyes *v* 46 eyes), and the average follow up period was also longer (13.8 months *v* 10.4 months). The minimal follow up period was 6 months, in contrast with only 2.5 months in the previous report.[9] Nevertheless, the recurrence rate was still lower (3.8% *v* 10.9%), and we suggest that this was due to removal of more conjunctiva, especially the conjunctiva adjacent to pterygium at the limbus, which might also be abnormal.[43] The coverage of a larger area by amniotic membrane in turn may promote the proliferation and differentiation of the remaining normal limbal epithelial cells,[44] which may have an inhibitory effect on fibrovascular ingrowth.[45] However, some drawbacks exist in this study: (1) the retrospective nature of comparison with conjunctival autograft and topical mitomycin C group, (2) non-randomisation between the two control groups, and (3) lack of a grading system to describe the extent and severity of inflammation associated with pterygium.[46] Nevertheless, compared with previously reported recurrence rates following topical mitomycin C treatment (0% to 12.0%)[47 48] and conjunctival autograft (0% to 39%),[46 49 50] the results reported in this study were still comparable. Furthermore, during the study period the author performed only AM graft on consecutive cases; therefore, there was no personal preference in patient selection. However, a randomised and prospectively based study is still needed for more precise comparison.

Compared with the bare sclera method, conjunctival autograft is a more technically demanding procedure, surgeon factors (experience, technique, etc) may have a profound influence on the recurrence rate. Moreover, conjunctival grafts including limbus generally yield better results than those that do not, because in addition to the contact inhibition effect on residual abnormal tissue by conjunctival graft,[51] the former may also contain limbal stem cells which help to restore the limbal barrier, and this in turn inhibits pterygium recurrence and retards recurrence time.[9 51 52] In this study we used conjunctival grafts without limbus, although the recurrence rate was satisfactory, it was still less effective than the best result from grafts containing the limbus (0%, Kenyon *et al*, Tan *et al*).[46 49]

Although topical mitomycin C was more effective than conjunctival autograft in reducing recurrence in several comparative studies,[21 50 53 54] some vision threatening side effects such as scleral ulceration, cataract formation, and glaucoma have been reported.[55–58] To avoid complications, many authors now advocate intraoperative application.[48 59–61] In addition, mitomycin C should be very cautiously applied to those patients with conditions predisposing to poor wound healing such as dry eye, blepharitis, or herpetic keratitis. To date, there is no report of sight threatening complication following conjunctival autograft or amniotic membrane

graft. Minor complications such as conjunctival epithelial inclusion cysts occurred more frequently in the conjunctival autograft group in both this and previous studies,[9] which may be caused by embedded conjunctival epithelium underneath the graft or recipient bed. In addition, amniotic membrane contamination is a potential risk and cannot be overlooked.

In summary, we have compared the surgical result of primary pterygium removal followed by amniotic membrane graft, conjunctival autograft, and topical mitomycin C treatment. We have shown that amniotic membrane graft was as effective as the other two methods, and was safe with no major complications. This suggests that amniotic membrane graft may be a preferred procedure for primary pterygium, and is especially suited for pterygium with diffuse conjunctival involvement or glaucoma patients waiting for filtering operations.

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