

Fresh frozen amniotic membrane for conjunctival reconstruction after excision of neoplastic and presumed neoplastic conjunctival lesions

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Abstract

Purpose Suspicious neoplastic conjunctival lesions often require wide excision with tumour-free margins, leaving significant conjunctival defects requiring reconstruction. In this study we report the results of using fresh frozen amniotic membrane grafts (AMG) after wide excision of potentially malignant lesions.

Methods Retrospective review of 53 patients; between January 2011 and April 2014. Conjunctival lesions were excised with a non-touch technique (2 mm margin) and sent for histopathological analysis. The surgical margins were treated with cryotherapy and a fresh frozen AMG was used to cover the defect. The main features examined were for any signs of recurrence, the conjunctivalisation of the AMG, complications and cosmetic appearance.

Results Fifty-three patients; 35 males and 18 females. Mean age was 54.9 (range 19–88). The mean follow up to January 2015 for all lesions was 21.4 months (range 8–48 months). The most common lesions were invasive malignant melanoma. There were no local surgical complications in 77.3% of patients; minimal scarring (11.3%), symblepharon (11.3%), and granuloma (7.5%). Five patients with conjunctival melanoma developed in-transit metastasis and orbital extension, none of it was at the site of the AMG.

Conclusion Our case series is the largest reported to date, with the largest number of melanomas. The use of fresh frozen AMG has improved the local surgical outcomes by improving healing and reducing scarring as well as allowing for a wider surgical margin.

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Introduction

The primary therapeutic aim of a suspicious neoplastic conjunctival lesion is complete eradication, successful reconstruction of the lost tissue and to avoid potential visual loss. Achieving this therapeutic goal involves excision of the lesion with tumour-free margin and reconstructing the bare area left behind by surgery.¹ Adjuvant treatments include cryotherapy, local radiation, alcohol epitheliectomy and topical agents such as mitomycin C, interferon, and topical 5-Fluorouracil.^{2–5}

The application of autologous mucosal tissues such as conjunctiva and oral mucosa, although may provide satisfactory surface coverage, have several limitations.^{6–8}

While harvesting conjunctiva may compromise the donor site, the recipient site may develop chronic inflammation, granulation tissue, scarring, and more importantly detection of recurrences can become difficult. Wide excision of lesion that avoids adjuvant therapy and autologous tissue transplantation may be ideal in managing ocular surface neoplasm.

Since January 2011, at the Ocular Oncology Centre in Sheffield, we have used fresh frozen amniotic membrane grafts (AMG) to cover defects resulting from the excision of large conjunctival lesions and in this study we report our experience of this technique.

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Materials and methods

A retrospective review of the case notes of 53 patients who had a fresh frozen AMG following the excision of large suspicious conjunctival lesions at the Royal Hallamshire Hospital, Sheffield between January 2011 and April 2014.

Preparation of AMG

Fresh frozen amniotic membrane is dissected from placentas donated during elective caesarean section deliveries, the amnion is immediately washed in isotonic citrate solution to remove blood and treated with a cocktail of antibiotics to reduce microbial load (gentamycin, imipenem, nystatin, polymyxin, and vancomycin). It is then frozen in the final packaging with 50% glycerol/Hanks solution and stored at -80°C at the National Health Service Blood and Transplant (NHSBT). The tissue is then quarantined for 180 days to retest the donor for serological markers or tested at time of donation by PCR for hepatitis B and C and HIV. They are mounted on nitrocellulose paper, supplied in 2×2 cm, 3×3 cm and 5×5 cm sheets and subsequently stored at -40°C . Once thawed they need to be used immediately.

Technique

The conjunctival lesions were excised with a non-touch technique with a 2 mm margin. All samples were sent for histopathological analysis. The edges and base of the defect was treated with triple freeze thaw cryotherapy. Fresh frozen amniotic membrane (2×2 cm from NHSBT) was used to cover the defect using either sutures (vicryl) or combination of sutures and fibrin glue (Figure 1). For patients with lesions at the limbus, localised alcohol epitheliectomy was performed. Bandage contact lens was applied when necessary and an eye pad was applied for 24 h.

Post-operatively, topical antibiotics for 2–4 weeks and topical steroids for 4 weeks were prescribed. All patients had routine follow up at 2 weeks following the procedure

then at 6 weeks. The main features that were examined was for any signs of recurrence, the conjunctivalisation of the AMG, complications and cosmetic appearance. Depending on the histopathological diagnosis and assessment of completeness of excision, further adjuvant intervention was initiated. Adjuvant therapy included mitomycin C (0.02% for squamous lesions, 0.04% for melanocytic lesions, four times a day, 1 week on and 1 week off for four cycles along with topical Betamethasone four times a day for 8 weeks), ruthenium plaque, interferon, cryotherapy, or proton beam.

Further follow-up was arranged at 3 monthly reviews for first year then 6 monthly thereafter. Repeat conjunctival map biopsies and regular imaging (head and neck/orbit MRI) were performed if required.

Results

Since January 2011 to April 2014, 53 patients underwent excision of conjunctival lesions who required fresh frozen AMG. The cohort included 35 males and 18 females. The mean age was 54.9 (median 59, range 19–88); 26 left eyes and 27 right eyes. The mean follow up to January 2015 for all lesions was 21.4 months (range 8–48 months). Of the 53 patients, 1 patient was lost to follow up (benign lesion), 9 patients were discharged (all benign lesions between 1–24 months) and 3 patient died (all invasive malignant melanoma).

The histopathological diagnosis of all the conjunctival lesions, location, adjuvant measures and complications are summarised in Table 1. The most common lesion treated was invasive malignant melanoma. All the patients who had excision of the lesion had intraoperative cryotherapy that was followed by AMG. Additional corneal alcohol epitheliectomy was performed in two of the patients with invasive malignant melanoma, both occurring in the temporal limbal locations.

Three patients in the malignant melanoma group, required further proton beam therapy for in-transit metastases (Figure 2). Two others underwent exenteration for anterior orbital spread at 8 months and 22 months.

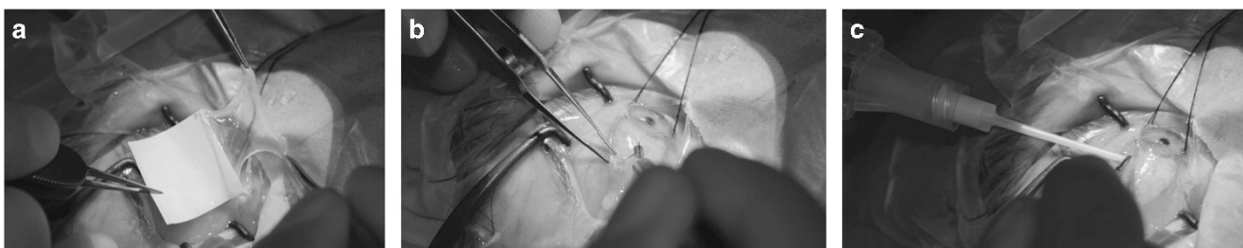


Figure 1 Intraoperative photographs of the use of fresh frozen AMG to cover defect. (a) Graft material being removed from nitrocellulose paper. (b) Application of graft to the surgical bed with sutures. (c) Fibrin glue being applied to the surgical bed.

Table 1 Summary of results

	<i>Invasive malignant melanoma</i>	<i>In situ malignant melanoma</i>	<i>Invasive squamous cell carcinoma</i>	<i>In situ squamous cell carcinoma</i>	<i>Sebaceous gland carcinoma</i>	<i>Benign lesions^a</i>
No. of pts	22 (8 males, 14 females)	5 (3 male, 2 female)	1 (Female)	7 (6 males, 1 female)	1 (Male)	17 (11 male, 6 female)
Mean age	62.7 (Range 19–88)	51.6 (Range 30–76)	69	62.1 (Range 40–80)	73	41.1 (Range 19–82)
Mean follow up (up to January 2015)	22.4 months Range 14–43 months	26.2 months Range 12–48 months	8 months	27.5 months Range 12–14 months	34 months	10.3 months Range 9–12 months
Site	10 Temporal limbus 3 Temporal conjunctiva 2 Nasal conjunctiva 1 Superior fornix 1 Inferior limbus 1 Superior conjunctiva 3 Nasal limbus 1 Inferior conjunctiva	2 Temporal conjunctiva 3 Nasal conjunctiva	Temporal conjunctiva	1 Temporal conjunctiva 2 Nasal limbus 4 Temporal limbus	Inferior fornix	7 Nasal conjunctiva 3 Temporal limbus 6 Temporal conjunctiva 1 Inferior conjunctiva
Additional treatment/procedures	5 Mitomycin C 3 Proton beam 11 Ruthenium plaque 1 Cryotherapy 2 Exenteration 2 None	3 Mytomycin C 2 None	None	6 Mitomycin C	Lower eyelid and inferior fornix reconstruction, scarring releasing procedures	None
Recurrence	17 None 5 Yes, not at the graft site	None	None	None	None	None
Post-operative complication	17 None 2 Symblepharon 3 Minimal scarring	None	Symblepharon	4 None 2 Minimal scarring 1 Granuloma, and symblepharon	Symblepharon, scarring, granuloma, restriction of eye movements with resolved	15 None 1 Granuloma 1 Symblepharon
Deaths	3	None	None	None	None	None

^aType of lesion: pyogenic granuloma in capillary haemangioma (1), inflamed cystic conjunctival naevus (1), benign compound naevus (2), benign cystic naevus (6), atypical naevus (2), other naevus-inflammatory (5).

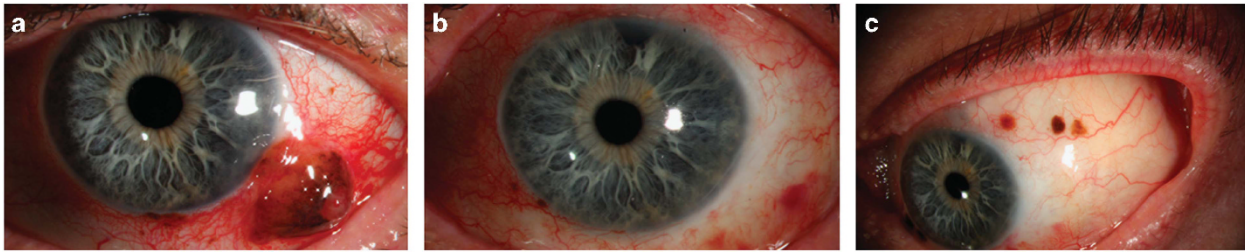


Figure 2 Slit lamp photograph: Invasive malignant melanoma. (a) Pre-operative image of the lesion inferior temporally in the left eye. (b) 3 month post-operatively. (c) Superior in-transit metastases.

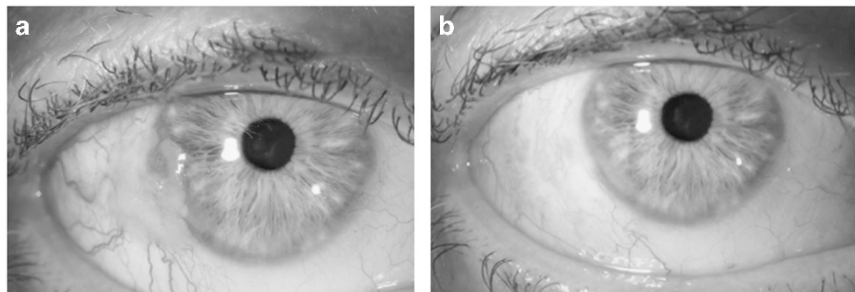


Figure 3 Slit lamp photograph: *in situ* squamous cell carcinoma. (a) Pre-operative photograph showing lesion temporally. (b) 3 months post-operative, good cosmetic result.

The recurrence of the melanoma did not occur at the site of the AMG. Three patients died, two from metastatic disease and one of unknown cause. In the *in situ* malignant melanoma group, three out of the five patients had additional mitomycin C treatment.

The one patient who had invasive squamous cell carcinoma (SCC) previously had treatment with topical interferon, topical mitomycin C and photodynamic therapy (PDT) during the presumed non-invasive stage of the disease. Failure to control growth led to surgical excision. In the *in situ* SCC group (Figure 3), all patients except one had adjuvant mitomycin C treatment. One patient had topical interferon treatment before excision with AMG.

The patient with sebaceous gland carcinoma required further surgical procedures to reconstruct the lower eyelid, inferior fornix and bulbar conjunctiva including division of scar tissue, due the area of destruction caused by the lesion. The significant restriction of ocular movements and diplopia resolved but he was left with scarring, granuloma and symblepharon.

Of the benign lesions, 52.9% of patients were discharged after a range of 1–24 months follow up. One patient was lost to follow.

In 89% of patients, the AMG was sutured directly using continuous and interrupted 8.0 vicryl \pm 10.0 vicryl. The rest were sutured with 7.0 vicryl sutures and fibrin glue was used.

Overall, there were no local surgical complications in 77.3% of patients. There was minimal scarring in 11.3% of patients, symblepharon in 11.3% patients, a granuloma in 7.5% patients, and restriction of eye movement in one patient which was secondary to complicated lid excision for sebaceous gland carcinoma. There was no recurrence at the site of AMG. Of the five patients that developed in-transit metastasis and orbital extension, none of it was at the site of the AMG.

Discussion

Large conjunctival lesions require excision with clear surgical margins which often leave large conjunctival defects that require reconstruction. Defects larger than 10mm result in unsatisfactory direct closure and have the potential to cause scarring, restriction of eye movements or discomfort for the patient.^{8,9}

After complete excision of tumour or lesion the goal of the reconstruction is to achieve good functional and cosmetic appearance. A suitable 'filler' should not induce inflammation, vascularisation or scarring. The grafted tissue should be resistant to ischaemic changes and necrosis. The resultant healing of the grafted tissue needs to remain transparent as to not mask any recurrence. In addition it should be easy to handle and should allow population of native cells, such as goblet cells and conjunctival epithelial cells. If allogenic, the graft should

be non-immunogenic and ideally should have anti-cancer properties. Amniotic membrane possesses several of the desirable requirements and may be a superior option in the reconstruction of the large conjunctival defects created by excision.

De Roth first introduced amnion to conjunctival reconstructive surgery in the 1940.¹⁰ However, the concept did not take hold until the 90s when Tseng with other investigators described the use of AM in ophthalmology.^{11,12} They used the AM to treat corneal epithelial defect, neoplasia, pterygium, and symblepharon.^{11,12} AM is versatile tissue that has found multiple uses in corneal and conjunctival surgery.¹³

AM consist of a stromal and basement membrane which acts as a scaffold for conjunctival epithelial migration. Further the stromal component of the membrane is incorporated into the donor site that facilitate long term anchoring of conjunctival derived cells including goblet cells. Goblet cells allow wetting of the surface and prevents the development of dry spots and aid in the healing process. In addition, AM downregulates inflammation and fibrosis.¹³ These along with anti-angiogenic properties prevents chronic inflammation, granulation tissue formation and scarring.¹⁴

Previous studies have reported the successful use of AMG in the reconstruction of conjunctival defects following different types of conjunctival tumours.^{4,15,16} Shields *et al*,⁴ first reported successful use of AMG in a single case report of diffuse conjunctival and corneal melanoma arising from primary acquired melanosis. They also applied cryotherapy and topical mitomycin C, however, the follow up period was short.

Paridaens *et al*¹⁶ series reported four case conjunctival melanoma with one recurrence, which was treated with mitomycin C. During a 30 month follow up of four cases, Espana *et al* found no recurrences.¹⁵ Similar to results reported by Dolla Pozza *et al*.¹⁷ Our paper reports the largest number of conjunctival melanomas treated with excision and AMG. In our series, there were no recurrences in the *in situ* melanoma group following a mean follow-up period of 26.2 months. However, in the 22 invasive conjunctival malignant melanoma cases there was recurrence in 5 patients, 2 of which required exenteration for orbital spread, 2 had in-transit metastases treated with proton beam therapy but died of metastatic disease and 1 in-transit metastases was treated with ruthenium plaque treatment and interferon injections. The recurrence did not occur at the site of the AMG in all cases. There were minimal complications and all had good cosmetic results.

The safe and successful application of excision and AMG for non-melanotic tumours have been reported by previous authors.^{9,15} Palamar *et al*⁹ treated 21 cases of ocular surface neoplasia, 10 of which were invasive squamous cell carcinoma. In a mean follow-up period of

30 months there were no cases of recurrences. The reconstruction had good cosmetic and functional results in the majority of cases and allowed a much more generous margin of excision.⁹ Our results are also similar to that of Palamar *et al*.⁹

We documented post-operative complications, such as granuloma formation and minimal scarring in a minority of the patients. The absence or very low recurrence observed by us and other authors of tumours especially the malignant ones is striking. Studies into the anti-cancer property of AM is beginning to emerge in the literature.¹⁸⁻²⁰ These *in vitro* studies suggest the anti-cancer properties of AM is mediated by various mechanisms that include induction of apoptosis and cell arrest. These molecular mechanisms are still under investigations.

To our knowledge, our case series has the largest number of melanomas. The use of fresh AMG has improved the local surgical outcomes by improving healing and reducing scarring as it allows for a wider surgical margin. We noted that a wider, complete excision of lesions with non-touch technique reduces the risk of local recurrences. Perhaps as expected, large conjunctival melanomas with in-transit conjunctival metastases have a poorer prognosis.

In summary, our study supports the use of fresh frozen AMG in the management of suspicious conjunctival lesions requiring wide surgical excision.

Summary

What was known before

- Large conjunctival lesions require excision with clear surgical margins which often leave large conjunctival defects that require reconstruction. Amniotic membrane grafts (AMG) is versatile tissue that has found multiple uses in corneal and conjunctival surgery.

What this study adds

- The use of fresh AMG has improved the local surgical outcomes by improving healing and reducing scarring as it allows for a wider surgical margin.
 - Wider, complete excision of lesions with non-touch technique reduces the risk of local recurrences.
 - Large conjunctival melanomas with in-transit conjunctival metastases have a poorer prognosis.
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Conflict of interest

The authors declare no conflict of interest.

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