

Reconstruction of conjunctiva with amniotic membrane after excision of large conjunctival melanoma: A long-term study

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PURPOSE. To evaluate, on a long-term basis, the role of amniotic membrane in the reconstruction of large conjunctival defects after excision of large conjunctival melanoma.

METHODS. Four consecutive patients with diffuse conjunctival melanoma involving both bulbar and palpebral conjunctiva were studied. Conjunctival melanoma was completely excised (with wide clinically disease-free margins) and amniotic membrane immediately sutured to the surrounding conjunctiva and sclera to cover the conjunctival defect. Minimum follow-up was 48 months.

RESULTS. Successful conjunctival surface reconstruction and physiologic fornical depth were achieved in all patients within 6 weeks. No recurrence of primary melanoma was observed during long-term follow-up.

CONCLUSIONS. Amniotic membrane transplantation is an effective alternative in ocular surface repairing surgery after removal of large conjunctival tumors. (Eur J Ophthalmol 2005; 15:446-50)

KEY WORDS. Amniotic membrane, Conjunctival melanoma, Primary acquired melanosis

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INTRODUCTION

Conjunctival melanoma is a relatively rare condition. Its incidence is 0.2 to 0.8 per million in the white population (1-3). Conjunctival melanoma is a potentially fatal neoplasm, with mortality rate of 30% at 10 years (1). It is observed most frequently in the perilimbal interpalpebral conjunctiva. Melanomas primarily located in the palpebral or fornical conjunctiva, plica semilunaris, or caruncle have a worse prognosis for survival (1, 4). The primary therapeutic approach to this tumor is extremely important, because incomplete removal is followed by tumor recurrence and distant metastasis (1, 4, 5). The gold standard in the management of malignant conjunctival melanomas is represented by wide surgical excision, cryotherapy, and conjunctival reconstruction (6, 7). When the size of the conjunctival defect is too large for primary closure, previ-

ous techniques consisted of autograft of conjunctiva from the fellow eye or autograft of oral mucosa (6). More recently a combined therapeutic method has been proposed: resection of the whole conjunctival melanoma, double freeze-thaw cryotherapy to destroy any vital malignant cell, and reconstruction with preserved amniotic membrane, to close large conjunctival defects after excision of wide tumors. The use of amniotic membrane transplantation to treat ocular surface abnormalities was first reported five decades ago, then abandoned (8, 9). The role of amniotic membrane transplantation in ocular disorders has been recently enthusiastically re-evaluated (10-16). In this study, we describe our technique and long-term experience with amniotic membrane transplantation to treat large conjunctival defect after surgical removal of diffuse conjunctival melanoma in four consecutive patients.

MATERIALS AND METHODS

Between March 1999 and October 2002, four consecutive patients with large conjunctival melanoma were referred to our institution. All cases were characterized by conjunctival melanoma with nodular and flat components involving the limbal area (Fig. 1). Flat component infiltrated both bulbar and tarsal conjunctiva in three of four cases. All cases had satellite pigmented lesions, separated from the main lesion by apparently normal conjunctiva. Main tumor and satellite lesions involved more than 50% of bulbar and more than 30% of tarsal conjunctiva in all cases. Cornea was only partially involved by the tumor. Visual acuity, intraocular pressure, and posterior segment were normal in all examined eyes. Videorhinoscopy was normal, and no pathologic head or neck lymph node was found at clinical and ultrasonographic examination. No patient had previously undergone surgery for primary ocular or cutaneous pigmented lesions. All patients were offered wide surgical excision of primary lesion and conjunctival reconstruction with amniotic membrane. A consent statement, approved by the IRB of Padova University Hospital, was signed by each patient. Amniotic membrane for ocular reconstruction was prepared as follows. Amniotic membrane was processed under sterile conditions from a fresh placenta obtained at cesarean section. Donor's serum was negative for human immunodeficiency virus, syphilis, and hepatitis B and C viruses. Under a lamellar flow hood, the placenta was first washed free of blood clots with sterile saline. The inner amniotic membrane was separated from the rest of the chorion by blunt dissection and rinsed in sterile saline and later in 4%, 8%, and 10% dimethylsulfoxide (DMSO) phosphate buffered saline (PBS) for 5 minutes each. Amniotic membrane was then flattened onto a nitrocellulose paper (with the epithelial surface up) and cut into 4x4 cm pieces. Each of them was placed in a sterile vial containing 10% DMSO medium. Vials were frozen at -70 °C. Amniotic membrane was defrosted immediately before use by warming the container to room temperature for 10 minutes, and rinsed three times in saline solution. The amniotic membrane was then removed from the nitrocellulose paper and transferred to a vial containing saline solution.

Surgical technique may be divided into two phases. After retrobulbar anesthesia, the first phase consisted of careful surgical removal of all conjunctiva (both bulbar and tarsal) affected by melanoma, with wide clinically free margins. All satellite pigmented lesions were included in

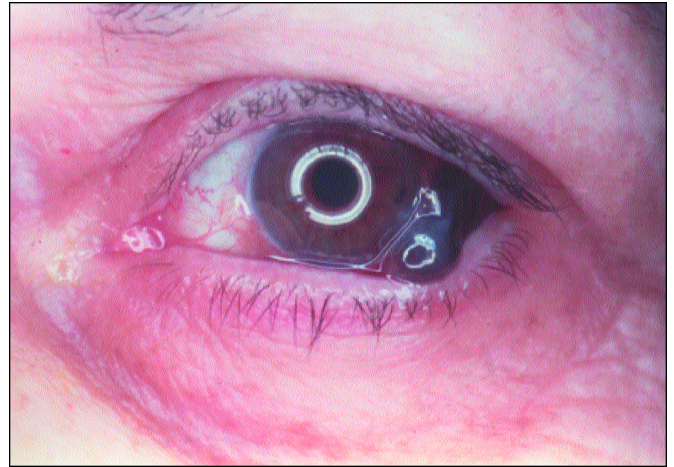


Fig. 1 - Large, nodular conjunctival melanoma.



Fig. 2 - Bulbar conjunctiva 4 years after reconstruction with amniotic membrane (same case as Fig. 1).

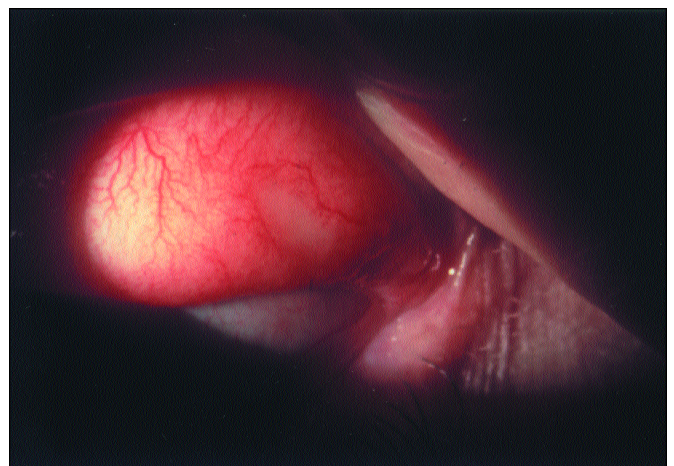


Fig. 3 - Superior tarsal conjunctiva 4 years after reconstruction with amniotic membrane: normal conjunctiva appearance (same case as Fig. 1).

the removed tissue. One of the patients had limited corneal involvement, which was treated, before conjunctival excision, with ethanol-assisted epithelium removal. Adjuvant cryotherapy over the basis and along the borders of excised tissue was used to prevent tumor cells seeding into a new area. Accurate hemostasis was obtained. In the second (surgical) phase, both bulbar and tarsal conjunctival defect was immediately covered by amniotic membrane with epithelial side up. Stromal side of the amniotic membrane was directly applied over sclera and tarsus. Amniotic membrane was secured with interrupted 8/0 polyglactin sutures (Vicryl, Ethicon, Italy) to the sclera and tarsus. Scleral sutures were positioned along the limbus and the borders of removed conjunctiva; a few scleral sutures were given 5 mm from the limbus toward the conjunctival fornix. Tarsal sutures were positioned at the mucosal eyelid border. No suture was positioned at the fornix level. The contact of the membrane to the remaining conjunctival edge is particularly important. It provides a firm base for attachment of the amniotic membrane and allows for the migration of healthy conjunctival epithelial cells onto it. No symblepharon ring was used. The eye was patched for 24 hours. Postoperative care consisted of topical antibiotic eyedrops (three times a day for 2 weeks, and two times a day for 2 weeks) and lubricating eye drops four times a day for 2 months. Patients were followed according to a standardized protocol: 2 days, 1 and 2 weeks after surgery, and then monthly up to 6 months, then every 6 months. Patients were evaluated for tumor persistence and or recurrence, conjunctival re-epithelization, and anterior segment complications.

RESULTS

Four patients (three female, one male; mean age 67 years) underwent wide amniotic membrane transplantation to reconstruct a wide conjunctival defect secondary to the removal of large conjunctival melanoma. Follow-up time ranged from 51 to 64 months. Examination of pathologic specimens confirmed clinical diagnosis of malignant melanoma, with free margins. Three of four cases showed, in the excised conjunctiva, patchy areas of primary acquired melanosis separated from the primary lesion by normal conjunctiva. No patient had melanoma recurrence during long-term follow-up. No patient had signs of metastatic disease, evaluated at 6 months interval with ophthalmologic, otorhinolaryngologic, and oncologic

evaluation. Successful conjunctival surface reconstruction was achieved in all patients. The amniotic membrane dissolved within 1 month in all cases (mean 3 weeks), and conjunctival epithelium replaced the amniotic membrane. We documented a good reconstruction of normal conjunctival epithelium and an adequate fornix depth in all eyes between 1 and 6 weeks (Figs. 2 and 3). Ocular surface was stabilized between 6 and 8 weeks; no symblepharon or other anterior segment complications were documented.

DISCUSSION

One of the major problems after wide surgical resection of a large conjunctival lesion is the reconstruction of remaining wide tissue defect. When the defect is too large to be closed by single margin apposition, commonly used surgical techniques are: conjunctival translocation flap, mucous membrane graft from the fellow eye, or oral mucosa flap (6, 7). Although autologous conjunctival graft may be considered the best choice, it involves not only psychological problems (patients refuse to have their unaffected eye operated), but also severe donor site morbidity, as symblepharon or restricted ocular or eyelid motility. On the other hand, the major limiting factor of physiologically thick mucosal grafts (mouth or inner lip) is a cosmetic discomfort and the possibility to hide tumor regrowth. For this reason amniotic membrane transplantation has been successfully used for anterior segment reconstruction after surgical treatment of symblepharon, pterygium, chemical or thermal burns, and neurotrophic corneal ulceration (12-17). Tsubota et al described the treatment of 11 patients with ocular cicatricial pemphigoid and Stevens-Johnson syndrome with a combination of allograft limbal transplantation, amniotic membrane transplantation, and tarsorrhaphy, followed every 15 minutes by artificial tears obtained from autologous serum. They achieved successful ocular surface reconstruction in 12 eyes, with minimal recurrence of symblepharon (13). Azuara-Blanco et al reported treatment with amniotic membrane transplantation of 10 consecutive patients with persistent epithelial defect secondary to corneal abscess or chemical burns (15). They observed that amniotic membrane transplantation is effective in promoting corneal healing in patients with persistent epithelial defect and is helpful after surgery to release corneo-conjunctival adhesions (15). Amniotic membrane transplantation was

also performed by Tseng et al in six consecutive patients (seven eyes) during removal of large conjunctival lesions and in nine patients (nine eyes) during removal of conjunctival scars or symblepharon (12). During follow-up (mean 10 months), 10 patients (11 eyes) showed successful surface reconstruction without recurrences. Five patients (five eyes) showed improved visual acuity, and one patient showed complete re-epithelization and resolution of motility restriction (12). Successful results were also obtained by Gris et al using an amniotic membrane graft in two patients with neurotrophic corneal ulceration resistant to medical treatment (one with and one without stromal vascularization) (18). Histopathologic findings revealed that amniotic membrane acts either as a mechanical substrate for epithelization or biologically contributes to restore damaged stroma (19, 20). During healing process, amniotic membrane is replaced by fibroblasts and collagen, partially maintaining stromal thickness (18). Tseng et al also showed that amniotic membrane can be considered an alternative substrate for conjunctival surface reconstruction during removal for large tumors, disfiguring scars, or symblepharon (12). Shields et al reported one case of extensive conjunctival and corneal melanoma in a 40-year-old woman (21). The conjunctival tumor was completely resected and treated with double-freeze cryotherapy, as flat acquired melanosis. Corneal melanosis was treated with topical mitomycin C eyedrops. The extensive conjunctival defect, involving half of the bulbar conjunctiva, was reconstructed with an amniotic membrane allograft. Preliminary evidence suggests that combined therapeutic approaches can be effective in the management of diffuse conjunctival melanoma arising from primary acquired melanosis. Recently, Paridaens et al described the efficacy of amniotic membrane transplantation for the management of conjunctival malignant melanoma and primary acquired melanosis (22). They treated four consecutive patients. A satisfactory result was noted in three patients with limbal melanoma, while in one patient with epibulbar, forniceal, and palpebral conjunctival melanoma, the amniotic membrane transplantation was complicated by symblepharon formation and restricted ocular motility.

Espana et al evaluated the clinical outcome in 16 eyes of 16 patients using amniotic membrane transplantation after a wide excision (>20 mm²) of conjunctival intraepithelial neoplasia, primary acquired melanosis, or malignant melanoma. Ocular surface healing was fast and complete in all cases (mean follow-up: 23.7 months) (23).

One complication (a pyogenic granuloma) was documented. Recurrence rate was 10% in patients with conjunctival intraepithelial neoplasia; no other recurrences were observed (23). When resecting large and diffuse conjunctival tumor, the surgeon has two major problems: total surgical excision, to obtain clinically and pathologically free margins, and the lack of residual tissue to cover a very large iatrogenic conjunctival defect. Surgical substitutes such as mucosal graft from the conjunctiva of the fellow eye or mouth are unsatisfactory for dimensional, surgical, and cosmetic reasons. Moreover, using these tissues, fair reconstruction of conjunctival fornix is extremely difficult, and relevant side effects are commonly documented.

The satisfactory long-term results we achieved in the reconstruction of conjunctiva after removal of large conjunctival malignant tumors confirm previous results obtained in the reconstruction of conjunctiva with amniotic membrane. Moreover, our results confirm the hypothesis that amniotic membrane transplantation can be considered an effective alternative surgical procedure for conjunctival reconstruction after excisions of large and/or diffuse conjunctival malignant neoplasia. Compared to previously reported techniques, our technique is easier to perform, avoids potential complications, and offers a better cosmetic appearance. Several characteristics of the amniotic membrane explain clinical success (19, 20). Its epithelium produces different growth factors, and basement membrane facilitates migration of epithelial cells, adhesion of basal cells, and may promote epithelial differentiation. Biologic properties of the amniotic membrane promote rapid conjunctival reconstruction with no symblepharon. After a long follow-up, no patient had signs of local recurrences and amniotic membrane does not prevent examination of treated areas. The use of amniotic membrane as support for physiologic conjunctival re-epithelization may also prevent limbal stem cell deficiency. In conclusion, amniotic membrane transplantation seems to be a safe and effective way to reconstruct bulbar and palpebral conjunctiva after excision of large conjunctiva melanomas and probably other conjunctival malignant tumors.

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