Super-Thick Amniotic Membrane Graft for Ocular Surface Reconstruction

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• PURPOSE: The purpose of this study was to evaluate super-thick amniotic membrane grafts (ST-AMGs) for ocular surface reconstruction.

• DESIGN: Retrospective, interventional case series.

• METHODS: This was a single-center study of clinical practice that included select patients with typically large ocular surface abnormalities that required reconstruction. The intervention studied was surgical insertion of a ST-AMG for reconstruction or repair of the ocular surface. Main outcome measures included intraoperative handling, graft position at 1 week post implantation, graft dissolution at 3 weeks, epithelialization of the ocular surface and symblepharon.

• RESULTS: Eleven ST-AMGs were implanted after resection with cryotherapy: 5 conjunctival melanoma, 4 squamous cell carcinoma, 1 sebaceous carcinoma, and 1 atypical pterygium. In addition, 1 was implanted for scleral necrosis. ST-AMGs were up to nine times thicker than standard amniotic grafts and were therefore amenable to both running and interrupted 7-0 Vicryl sutures without cheese-wiring. All cases had a well-positioned ST-AMG at 1 week and 75% (n = 9) had partial graft dissolution at 3 weeks. Complete epithelialization without wound dehiscence was noted in all cases. However, secondary (after additional tumor treatment) symblepharon formed in 16.7% (n = 2). In all cases, the mean visual acuity and intraocular pressures remained unchanged during conjunctival reconstruction and subsequent secondary treatments. Post epithelialization adjuvant topical chemotherapy was given to extend treatment margins and treat presumed occult disease in 50% (n = 6). At mean follow-up of 25.5 months (median 10, range 3-90), 10 cases (83.3%) showed complete local tumor control, 1 showed revascularization of the scleral melt, and 1 required orbital exenteration.

• CONCLUSION: ST-AMGs were easy to suture and relatively persistent. Epithelialization of the ocular surface without primary symblepharon formation was noted. ST-AMGs should be considered an alternative for ocular surface reconstruction. (Am J Ophthalmol 2019;198:45–53. © 2018 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)

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AMNIOTIC MEMBRANES (AMNION) ARE THE INNERmost layer of the human placenta and line the amniotic cavity. The membrane itself consists of a unique combination of tissue layers. Histologically, it is a 3-layered structure that includes a single layer of cuboidal epithelial cells with microvilli, a basement membrane, and a mesenchymal layer.[1] The mesenchymal layer is subdivided into compact, fibroblast and spongy layers that exhibit amniotic sac location specific thicknesses.[2] The epithelium is firmly adherent to the basement membrane; however, the mesenchymal layer is easily separable from the underlying chorion.[3] Human amnion is neither innervated nor vascularized.

Amniotic membrane grafts (AMGs) promote epithelialization by acting as a basement membrane and facilitating conjunctival cell migration.[1] Amniotic cells secrete multiple chemical mediators: interleukin-1 and -2 receptor antagonists, pigment epithelium–derived factor, endostatin, and matrix metalloproteinase (MMP) inhibitors, and they have an extracellular matrix that is rich in laminin, fibronectin, and collagen types I, II, and V.[1] Amnion inhibits the expression of transforming growth factor-β receptors in fibroblasts and therefore impedes inflammation, scarring, and angiogenesis.[3] Amniotic membranes exhibit low immunogenicity.[3]

Human amniotic membrane grafting has been performed for more than a century.[4] In 1940, AMG was used for conjunctival replacement by Rotth.[5] Since that time it has most commonly been used for corneal and conjunctival reconstruction after pterygium excision and excision of ocular surface neoplasias.[6–9] In addition, amniotic membranes have been used to insulate and thus protect the cornea during epicorneal ophthalmic plaque radiation therapy.[10,11] However, all these reports primarily use a single thin (≈0.1 mm) layer of AMG.[5–11]

In contrast, super-thick (≈0.5–0.9 mm) AMGs (ST-AMGs) have been used to cover glaucoma drainage devices.[12] To our knowledge and in review of PubMed with the terms "amniotic," "membrane," "thick," "super," "conjunctiva," "cornea," and "Biotissue," we could find no studies evaluating a single layer of ST-AMG for reconstruction of the ocular surface. This study reports our clinical experience.

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TABLE 1. Super-Thick Amniotic Membrane Grafting: Demographic and Historical Characteristics

Case No.	Age (y)	Sex	Systemic History	Ocular History	Pathology
1	8	Male	None	Previous excision biopsy	Melanoma
2	80	Male	None	Previous excision biopsy	Melanoma
3	59	Female	None	None	Pterygium with mild dysplasia
4	64	Female	None	None	Melanoma
5	29	Female	None	Previous excision biopsy	Elastotic degeneration
6	36	Male	None	Previous excision biopsy	SCC
7	61	Female	None	None	SCC
8	59	Male	Hypertension	None	SCC
9	39	Female	None	None	Melanoma
10	66	Male	Heart disease	Previous excision biopsy	Melanoma
11	53	Male	None	None	SG carcinoma
12	80	Male	Heart disease, COPD	Previous excision biopsy	SCC
	Mean age: 53 y; Median age: 59 y	Males: 58% (n = 7); Females: 42% (n = 5)	No systemic illness: 75% (n = 9)	Previous excision biopsy (elsewhere): 50% (n = 6)	Most common pathology: melanoma, 42% (n = 5)

COPD = chronic obstructive pulmonary disease; SG = sebaceous gland; SCC, squamous cell carcinoma.

METHODS

A RETROSPECTIVE, NONCOMPARATIVE, INTERVENTIONAL case series was evaluated at The New York Eye Cancer Center. This study adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act of 1996. New York Eye Cancer Center Internal Review Board approval was obtained to perform an anonymized retrospective chart review. Preoperative informed patient consent was obtained in all cases. This study was limited to 12 patients who were managed using single layer ST-AMGs. This included 5 patients with conjunctival melanoma, 4 with squamous cell carcinoma, 1 with sebaceous carcinoma, 1 with atypical pterygium, and 1 with scleral necrosis seen after the "I-Brite" eye whitening procedure.[13–15]

• **OPHTHALMIC EXAMINATION:** Demographic data included age, sex, and associated ocular and systemic diseases. Previous ocular surgery and histopathology findings were noted (Table 1). Ophthalmologic examinations were inclusive of, but not limited to, visual acuity with the Early Treatment Diabetic Retinopathy Study charts in Collaborative Ocular Melanoma Study–certified rooms. Slit-lamp biomicroscopy with photography, tonometry, gonioscopy with photography, and dilated indirect ophthalmoscopy was performed. High-frequency ultrasonography (20-50 MHz) was performed to rule out intraocular tumor extension.

Slit-lamp photography methods. The tumor and all conjunctival surfaces were photographed (with lid eversion) at each clinical visit and serial comparison of the pictures was performed.

High-frequency ultrasound imaging methods. High-frequency ultrasound imaging included dynamic real-time viewing of the entire anterior segment with particular attention to tissues subjacent to the tumor. Static representative longitudinal and transverse static images sectioned through the tumor were included in each report. Any evidence of angle blunting or uveal thickening (compared with normative values) suggested intraocular invasion.[16,17]

• **ORBITAL IMAGING:** Computed tomography scan of the orbit with contrast (2-mm axial and coronal cuts with sagittal reconstruction) was requested in the case of sebaceous gland carcinoma to rule out postseptal extension.

• **REGIONAL AND SYSTEMIC EXAMINATION:** Patients were examined for regional lymphadenopathy of preauricular, submandibular, and anterior cervical lymph nodes at presentation and at each follow-up examination. Patients diagnosed with conjunctival melanoma were staged using total body positron emission tomography/computed tomography.[18]

• **GRAFT PARAMETERS:** ST-AMGs were purchased from Biotissue (AmnioGuard; Model AGD, Miami, Florida, USA) and made available in the operating room. The difference in thickness of ST-AMG versus thin AMG is attributed to harvesting site. While the thin AMG is from the placenta, ST-AMG is harvested from the human umbilical cord. AmnioGuard is available in 1.0- × 0.75-cm, 2.5- × 2.0-cm, and 4.0- × 3.0-cm grafts.

Terms related to ST-AMG efficacy analysis. Terms related to ST-AMG efficacy analysis include (1) graft position at 1 week post implantation (graft in situ, displaced or not visualized); (2) graft dissolution at 3 weeks postimplantation (none, partial, or complete dissolution); (3) epithelialization of the ocular surface (complete, incomplete, or absent); (4) development of primary symblepharon; and (5) development of secondary symblepharon. Symblepharon was categorized as primary or secondary. Primary symblepharon was defined as occurring within 1 month of surgery. Secondary symblepharon was defined as occurring after a secondary treatment to the ocular surface.

Terms related to cancer persistence or recurrence. Terms related to cancer persistence or recurrence include (1) complete response (no residual tumor); (2) partial response (reduction in tumor size); and (3) tumor growth.

In the case of scleral melt, neovascularization of the scleral defect and re-epithelialization over the defect were the goals of treatment. In addition, a subjective documentation of patient comfort was recorded along with additional parameters, such as best-corrected visual acuity, anterior segment findings, intraocular pressure, and location and extent of the lesion at presentation and follow-up. Tumor staging was performed using the 8th edition of the American Joint Committee on Cancer system (Table 2). Management—including type of surgery, adjuvant chemotherapy, adjuvant radiotherapy, best-corrected visual acuity, and intraocular pressure at each follow-up—was recorded.

• **FOLLOW-UP:** All patients were followed-up at 1 week, 3 weeks, and 2 to 3 months postsurgery. Subsequent examinations were tailored to the individual patient according to their clinical needs. Clinical evaluation and photographic documentation were performed at each visit.

RESULTS

SINCE 2007, 11 PATIENTS UNDERWENT COMBINATIONS OF excision biopsy, cryotherapy, single layer, ST-AMG, and topical chemotherapy. The most common diagnosis was conjunctival melanoma (41.7%, n = 5) followed by squamous cell carcinoma (33.3%, n = 4) and 1 case each of sebaceous gland carcinoma, atypical pterygium, and scleral melt (Table 2). The patient with scleral melt was treated solely with primary ST-AMG.

• **ST-AMG SURGERY:** Conjunctival malignancies were examined under the operating microscope to mark their extent (including 2- to 3-mm margins of normal-appearing tissue) with tissue dye. The pterygium and scleral melt were not similarly marked. Excisional biopsies included the underlying Tenon's fascia. Peripheral and deep tumor margins were treated with double freeze-thaw cryotherapy using the medium and large-sized "Fingertip" spatulated cryoprobes.[19] Cryotherapy was also used to augment the conjunctival and corneal tumor-free margins by 2 to 3 mm. The patient with scleral melt did not undergo excision or cryotherapy.

The rectangular amniotic membranes were made available within a plastic packet containing Dulbecco's modified eagle medium/glycerol (1:1; TissueTech, Inc., Miami, Florida, USA) along with ciprofloxacin 20 μg/mL and amphotericin B 1.25 μg/mL. The surgical defect was measured with calipers. The epithelial side was easily determined by its reflective property. Then, the ST-AMG was placed (epithelial side up) directly on the bare sclera or conjunctival defect to further determine the amount of trimming required to fill the defect. Though normal conjunctiva was recruited to fill defects, tractional forces were avoided. Therefore, each ST-AMG was trimmed to form with Westcott conjunctival scissors and secured to the surrounding conjunctiva with buried interrupted 7-0 Vicryl sutures (model-J575G; Ethicon Inc., Somerville, NJ, USA) (75%, n = 9). In case of large defects (defined as a conjunctival defect ≥30 mm in dimension), continuous 7-0 Vicryl sutures were used in addition to the interrupted ones (25%, n = 3). No tissue adhesives were used. No cheese-wiring or tearing of ST-AMGs was noted. After completion of surgery, topical tobramycin/dexamethasone ointment was applied to the ocular surface. In 1 case, a lateral temporary suture tarsorrhaphy was placed to limit exposure and thus protect the AMG graft.

• **GENERAL ST-AMG RESULTS:** Amniotic membrane grafting did not affect visual acuity or intraocular pressure, which remained unchanged during and immediately after conjunctival reconstruction (Tables 1 and 2). At 1 week, 100% (n = 12) of cases had a well-positioned graft in situ with intact sutures. At 3 weeks, 75% (n = 9) of cases had partial graft dissolution and 25% (n = 3) had complete

TABLE 2. Super-Thick Amniotic Membrane Grafting: Clinical Features and Management

Case No.	Initial BCVA	Diagnosis	Graft Location	AJCC Grading	Excision Plus Cryotherapy	Adjuvant Chemotherapy	Adjuvant Radiotherapy	Final BCVA
1	20/32	Melanoma	Bulbar conjunctiva plus cornea	Previously biopsied	Yes	Yes	No	20/25
2	20/25	Melanoma	Bulbar conjunctiva plus caruncle	Previously biopsied	Yes	No	EBRT	20/200
3	20/125	Atypical pterygium	Bulbar conjunctiva plus cornea	N/A	Yes	No	No	20/25
4	20/40	Melanoma	Bulbar conjunctiva	T1aN0M0	Yes	Yes	No	20/32
5	20/20	Scleral melt	Temporal	N/A	No	No	No	20/20
6	20/20	SCC	Forniceal conjunctiva	Previously biopsied	Yes	Yes	No	20/20
7	20/20	SCC	Bulbar conjunctiva plus cornea plus forniceal conjunctiva plus caruncle	T2N0M0	Yes	Yes	No	20/20
8	20/20	SCC	Bulbar conjunctiva plus cornea	T2N0M0	Yes	No	No	20/20
9	20/20	Melanoma	Bulbar conjunctiva plus forniceal conjunctiva	T2bN0M0	Yes	Yes	No	20/20
10	20/25	Melanoma	Bulbar conjunctiva	Previously biopsied	Yes	No	No	N/A
11	20/25	SG carcinoma	Lower lid plus medial canthus	T3bN0M0	Yes	No	EBRT	20/25
12	20/25	SCC	Bulbar conjunctiva plus cornea	Previously biopsied	Yes	Yes	No	20/20
	Mean: 20/32; median: 20/25	Most common: melanoma, 42% (n = 5)	Most common location: bulbar conjunctiva, 67% (n = 8)	Regional lymph node positive: none	Excision plus cryotherapy: 92% (n = 11)	Adjuvant chemotherapy: 50% (n = 6)	Adjuvant radiotherapy: 17% (n = 2)	Mean: 20/25; median: 20/20

AJCC = American Joint Committee on Cancer, 8th edition; BCVA = best corrected visual acuity; EBRT = external beam radiotherapy; N/A = not applicable; SCC = squamous cell carcinoma; SG = sebaceous gland.

TABLE 3. Super-Thick Amniotic Membrane Graft Outcomes

Case No.	Suture Technique	Postoperatively		Epithelialization	Secondary Symblepharon	Disease Recurrence
		1 week	3 weeks			
1	Interrupted plus continuous	GIS	Partial GD	Complete	No	No
2	Interrupted	GIS	Partial GD	Complete	Yes	No
3	Interrupted	GIS	Partial GD	Complete	No	No
4	Interrupted	GIS	Partial GD	Complete	No	No
5	Interrupted plus continuous	GIS	Complete GD	Complete	No	No
6	Interrupted	GIS	Complete GD	Complete	No	No
7	Interrupted	GIS	Complete GD	Complete	Yes	No
8	Interrupted	GIS	Partial GD	Complete	No	No
9	Interrupted plus continuous	GIS	Partial GD	Complete	No	No
10	Interrupted	GIS	Partial GD	Complete	No	Yes
11	Interrupted	GIS	Partial GD	Complete	No	No
12	Interrupted	GIS	Partial GD	Complete	No	No
	Interrupted: 100% (n = 12); Continuous: 25% (n = 3)	GIS at 1 week: 100% (n = 12)	Partial GD at 3 weeks: 75% (n = 9)	Complete epithelialization: 100% (n = 12)	Secondary symblepharon: 17% (n = 2)	Disease recurrence: 8% (n = 1)

GD = graft dissolution; GIS = graft in situ.

graft dissolution. All cases achieved complete conjunctival epithelialization without wound dehiscence (Table 3). No primary symblepharon was noted in this series. At last follow-up, secondary symblepharon was noted in 16.7% (n = 2) of patients. One of these patients developed symblepharon after external beam radiotherapy, and the other had a severe reaction to topical 5-fluoroucil 1% resulting in inferior forniceal shortening with conjunctival attachment to the cornea. No postseptal tumor extension (into the orbit) was noted in this series.

• **SELECT CASE SUMMARIES:** *Case 1.* An 8-year-old male was referred for the management of a recurrent pigmented conjunctival tumor on his left eye. He had a previous excision biopsy at 4 years of age where histopathology revealed a compound cystic nevus. The tumor subsequently grew to extend from 3 to 10 o'clock at the limbus onto the cornea and into the superior fornix (Figure 1). He was treated by excision with adjuvant "finger-tip" cryotherapy to the peripheral and deep tumor margins. Conjunctiva was mobilized and the large conjunctival defect was closed with a ST-AMG anchored at the limbus and sutured to the posterior, temporal, and nasal conjunctival edges. Histopathology revealed a melanoma in situ. The ST-AMG facilitated epithelialization of the superior bulbar surface, then the patient received 4 weeks of adjuvant topical mitomycin chemotherapy.[20,21] At 2 months of follow up, light persistent perilimbal pigmentation remained. At 9 months of follow-up, there was with no symblepharon and only slight residual pigmentation (Figure 1).

Case 6. A 36-year-old man was referred for the management of a recurrent squamous cell carcinoma of the conjunctiva. The tumor involved the medial lower forniceal conjunctiva (Figure 2). He was started on 3-month course of topical interferon-α2b (1 million IU/mL) chemotherapy, but only partial regression was noted.[22,23] Surgical treatment involved complete excision with 2- to 3-mm margins and adjuvant "Finger-tip" cryotherapy. The resulting defect was closed with a ST-AMG. A Mini Monoka stent (SKU S1.500; FCI Ophthalmics, Pembroke, Massachusetts, USA) was placed in the inferior canaliculus to prevent any secondary stenosis resulting from cryotherapy. At 10 months of follow-up, there is no evidence of recurrence or symblepharon and the inferior fornix remained deep (Figure 2).

Case 7. A 61-year-old woman was referred for treatment of a conjunctival tumor on her left eye. Primary tumor excision extended from the medial orbit and caruncle to the cornea (Figure 3). Deep exploration revealed that the medial inferior forniceal tumor was adherent to the medial rectus. Excision was combined with "Finger-tip"

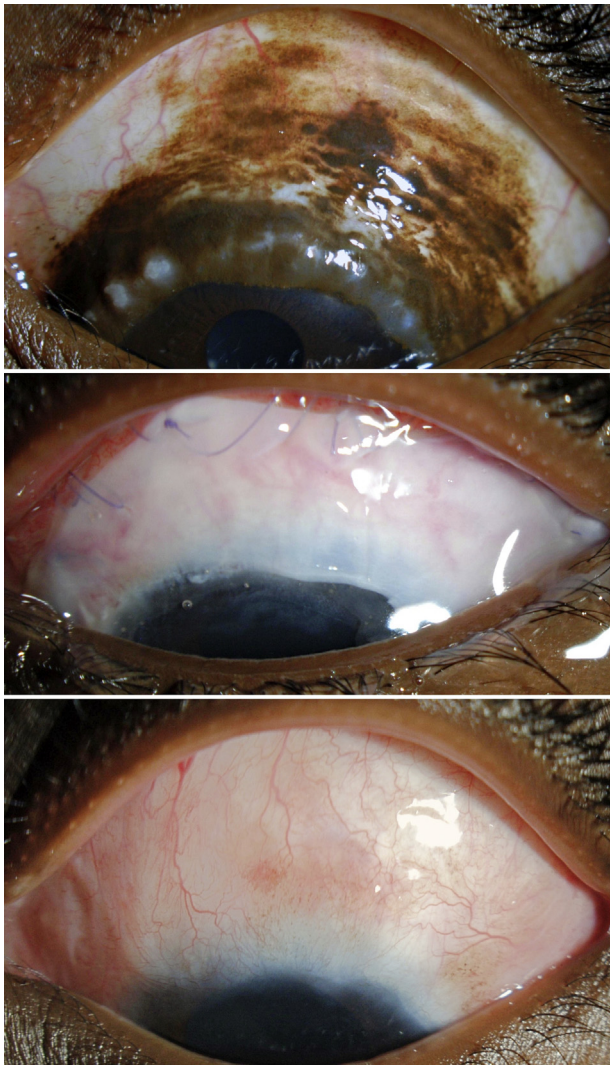

FIGURE 1. Super-thick amniotic membrane grafting. (Top) Slit-lamp photography reveals a ≈20- × 10-mm conjunctival melanoma involving the cornea of the left eye. (Middle) One week postoperatively. Note the super-thick amniotic membrane graft secured with interrupted and continuous 7-0 Vicryl sutures. (Bottom) Nine months postoperatively. Note the wound healing with minimal scarring and no symblepharon.

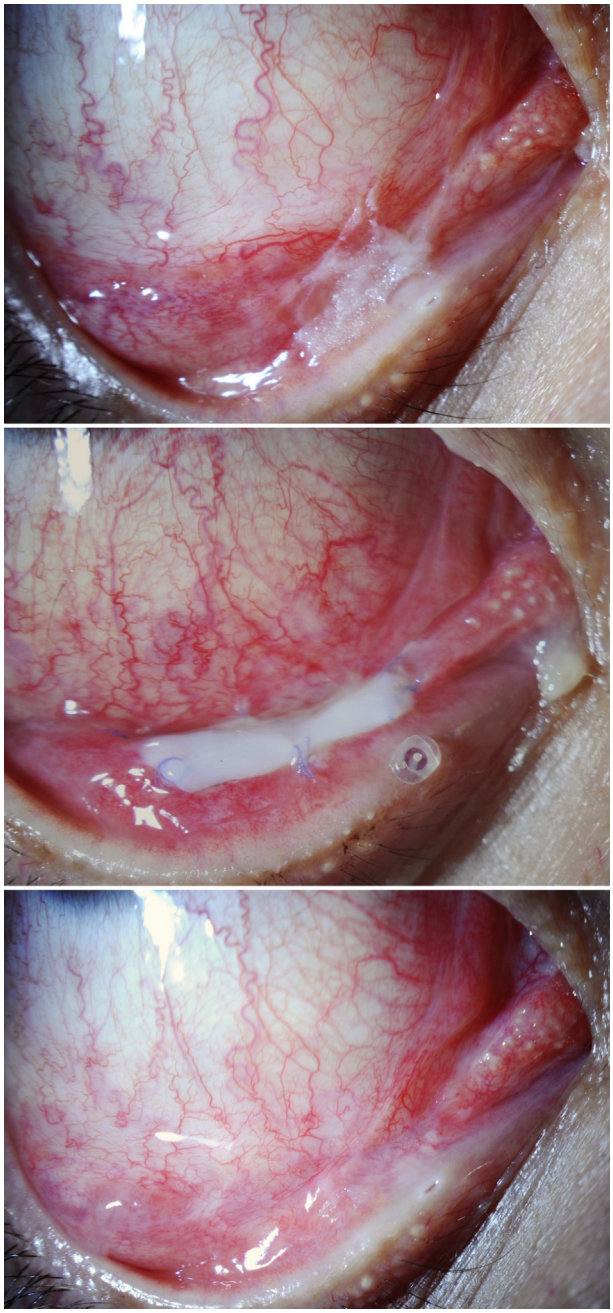

FIGURE 2. Super-thick amniotic membrane grafting. (Top) Slit-lamp photography reveals an ocular surface squamous malignancy of the lower forniceal conjunctiva of the right eye. (Middle) One week postoperatively. Note the super-thick amniotic membrane graft and Mini Monoka stent in situ. (Bottom) Ten months postoperatively. Note that there is no forniceal shortening nor symblepharon.

cryotherapy to the peripheral and deep tumor margins (including the medial rectus). An ST-AMG was sutured onto the nasal bulbar surface to fill the defect. Traction sutures were placed in the graft and through the eyelid to reconstruct the inferior fornix. Histopathology revealed an invasive squamous cell carcinoma. After epithelialization, a 2-week course of adjuvant topical 5-fluoroucil 1% chemotherapy was applied.[24] However, the patient did not tolerate topical 5-fluoroucil 1% (she had a severe reaction) and was switched to topical interferon-(1 million units/cc) chemotherapy. She developed secondary symblepharon (Figure 3). There was no evidence of tumor recurrence at 10 months of follow-up.

Case 9. A 39-year-old woman was referred for treatment of a right conjunctival tumor that extended from the temporal bulbar conjunctiva into the inferior fornix (Figure 4). She underwent primary excision combined with "Finger-tip" cryotherapy to the peripheral and deep

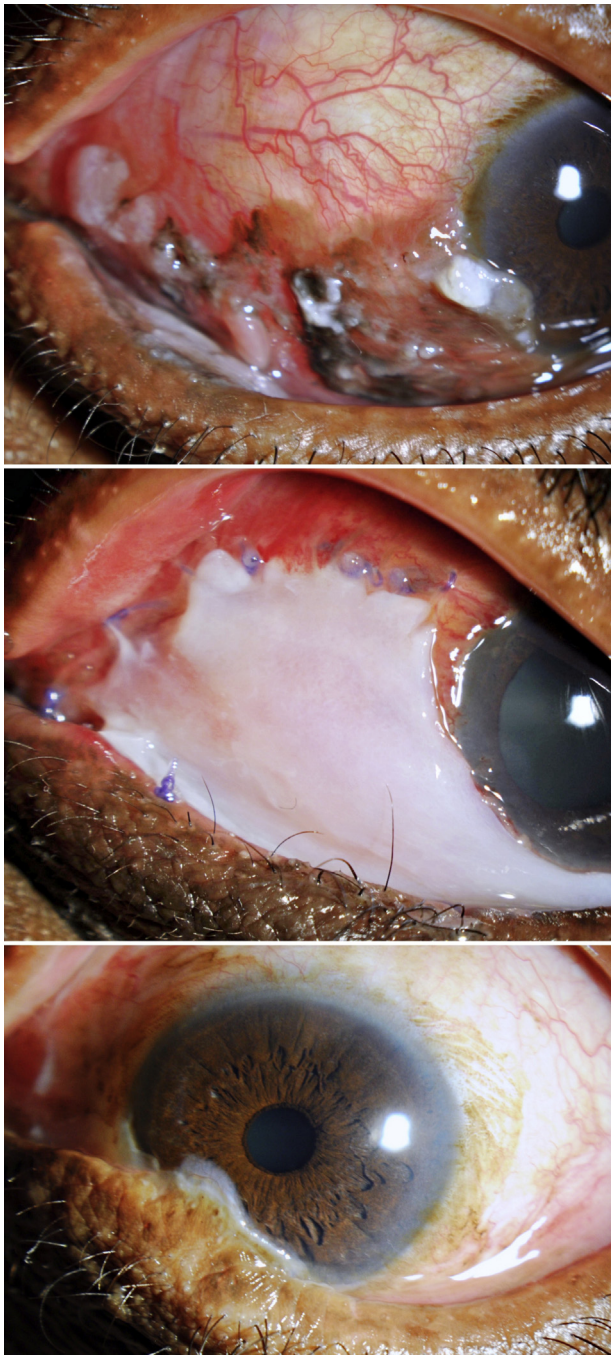

FIGURE 3. Super-thick amniotic membrane grafting. (Top) Slit-lamp photography reveals an ocular surface squamous malignancy of the left eye involving the limbus, bulbar conjunctiva, and lower fornix. (Middle) One week postoperatively. Note the super-thick amniotic membrane graft in situ. (Bottom) Ten months postoperatively. Note contraction of the inferior fornix with symblepharon onto the cornea.

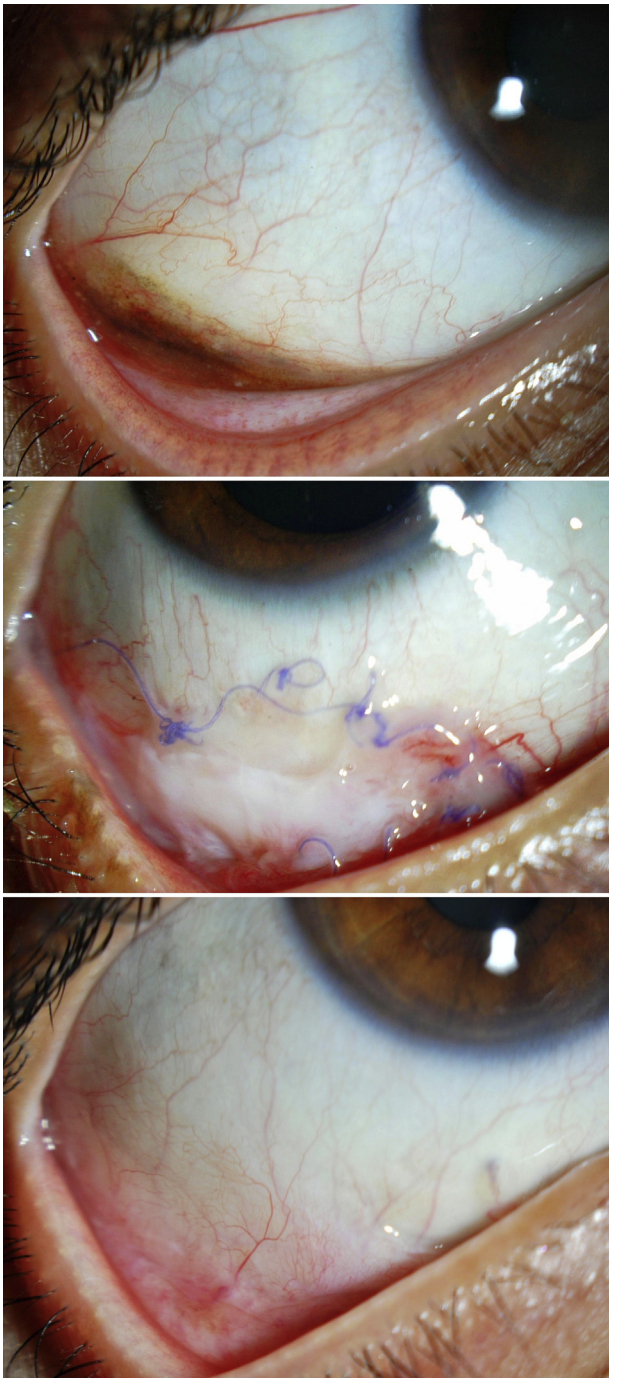

FIGURE 4. Super-thick amniotic membrane grafting. (Top) Slit-lamp photography reveals a conjunctival melanoma in the lower fornix of the right eye. (Middle) One week postoperatively. Note the ST-AMG secured in situ. (Bottom) Ten months postoperatively. Note that there is no forniceal shortening nor symblepharon.

tumor margins. A ST-AMG was sutured onto the ocular surface to fill the defect. A Frost suture lateral tarsorrhaphy was placed to close the lateral aspect of the eyelids to cover the ST-AMG. Histology of the excised specimen revealed an invasive malignant melanoma. After epithelialization of the ocular surface, surgery was followed by a course of adjuvant topical mitomycin 0.02% chemotherapy and a course of adjuvant topical

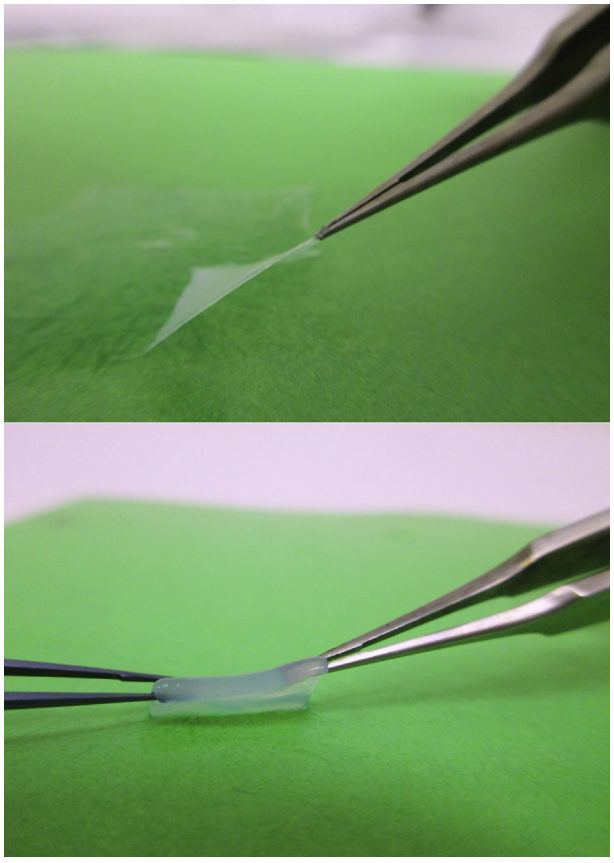

FIGURE 5. Photographic comparison showing the thin (≈0.1 mm) amniotic membrane (top) and the super thick (≈0.5–0.9 mm) amniotic membrane (bottom).

interferon-chemotherapy.[20,21,25] Now, 6 months after completion of topical chemotherapy, there is no evidence of tumor recurrence (Figure 4).

• **LONG-TERM RESULTS:** At a mean (±SD) follow-up of 25.5 ± 26.9 months (median 10, range 3-90 months), 10 cases (83.3%) showed complete tumor response, and the patient with scleral melt had successful revascularization of the scleral melt along with rejection of calcified components. However, case 10 required secondary eyelid-sparing orbital exenteration for tumor progression (Tables 1 and 2). No patient with malignant conjunctival neoplasia had palpable regional lymphadenopathy or evidence of systemic metastatic cancer throughout the duration of this study.

DISCUSSION

WE REPORT ON THE USE OF SINGLE LAYER ST-AMG FOR ocular surface reconstruction. ST-AMGs demonstrated excellent intraoperative handling, suturability, and persistence to enable epithelialization of the ocular surface. Though no wound dehiscence was noted; graft dissolution appeared to be sensitive to graft size and exposure. Learning from these findings, we used temporary paramedian suture tarsorrhaphy to keep 1 graft from exposure for 4 weeks. This case developed no symblepharon (Figure 4).

Our literature review revealed that single-layer, similarly thick, albeit small AmnioGuard has been used for the management of glaucoma surgery complications and to cover explants.[12] In contrast, our study shows that extra-large ST-AMG can be useful for conjunctival replacement and thus ocular surface reconstruction.

This technique offers several advantages over the use of conventional thickness single and multilayer AMG. Firstly, (≈0.5–0.9 mm) ST-AMG was easier to suture and handle and did not suffer a tear, cheese-wiring, or dehiscence. It has been our experience that conventional (≈0.1 mm) AMGs are smaller, less durable, and more difficult to sew into place (Figure 5). The larger, thicker ST-AMG remained intact and well positioned for up to 3 weeks, allowing for large defect conjunctival epithelialization. This property is especially important in cases involving opposing denuded surfaces.

The main limitation of our study was the small sample size and its retrospective noncomparative nature. However, we present unique experience with ST-AMG for ocular surface reconstruction, even in difficult cases that involved large surface areas and subsequent topical chemotherapy. This series revealed the ease of intraoperative ST-AMG use, postoperative persistence of graft material, and resultant ocular surface reconstruction.

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