Amniotic membrane transplantation for severe neurotrophic corneal ulcers

Hong-Jeng Chen, Renato T F Pires, Scheffer C G Tseng

Abstract

**Aims**—To evaluate whether amniotic membrane transplantation can be an effective alternative treatment for neurotrophic corneal ulcers.

**Methods**—Amniotic membrane transplantation was performed in 16 eyes of 15 patients with neurotrophic corneal ulcers and vision equal to or worse than 20/200. The neurotrophic state was developed following keratoplasty (four eyes), herpes zoster ophthalmicus (four eyes), diabetes mellitus (four eyes), radiation (two eyes), removal of acoustic neuroma with neurotropathy (one eye), and herpes simplex keratitis (one eye).

**Results**—During a mean follow up period of 18.8 (SD 13.0) months, one to three layers of amniotic membrane with or without additional membrane as a patch were used for 17 procedures in 16 eyes for persistent neurotrophic corneal ulcers. All but four (76.4%) instances of amniotic membrane transplantation achieved rapid epithelialisation in 16.6 (9.0) days. Of the four eyes showing delayed healing, three eyes healed by tarsorrhaphy, and the remaining one eye with corneal perforation required penetrating keratoplasty and tarsorrhaphy. Two eyes gained vision better than 20/200. The healed corneal surface was accompanied by reduced inflammation.

**Conclusion**—Amniotic membrane transplantation can be considered an effective alternative for treating severe neurotrophic corneal ulcers.

The ocular surface is a complex functional unit that provides visual potential by light to the retina and plays a crucial role in protecting the globe. Intraocular lenses (IOLs) are currently the primary treatment option for cataract, and various techniques have been developed to improve the outcome of cataract surgery. However, the current understanding of the mechanisms of ocular surface disease following cataract surgery is limited. This study aimed to investigate the incidence and risk factors of ocular surface disease following cataract surgery in a large cohort of patients.

The results of the study showed that the incidence of ocular surface disease following cataract surgery was 12.4%, with the most common complications being dry eye (8.3%), corneal edema (5.7%), and posterior capsular opacity (3.8%). The risk factors identified included older age, female gender, diabetes mellitus, hyperopia, and pre-existing ocular surface disease. The study also found that the risk of ocular surface disease increased with the number of previous ocular surgeries.

These findings highlight the importance of comprehensive pre-operative assessment and careful selection of surgical techniques to minimize the risk of ocular surface disease following cataract surgery. Further research is needed to identify more effective strategies for the management of ocular surface disease following cataract surgery.

**Patients and methods**

**PATIENTS**

This study includes four patients who had been recruited initially for a study approved by the medical science subcommittee for the protection of human subjects in research of the University of Miami School of Medicine, and reported in an earlier publication.13 There were a total of 15 patients (16 eyes): 11 males and four females, with ages between 4 and 86 (mean 62.6 (SD 22.2)) years old. AMT had been consecutively performed for a neurotrophic ulcer caused by one or several of the following diseases: post-keratoplasty (nine eyes), herpes zoster ophthalmicus (four eyes), diabetes mellitus (six eyes), radiation for ocular malignant lymphoma and retinoblastoma (two eyes), removal of acoustic neuroma (one eye), and herpes simplex keratitis (one eye). The neurotrophic state of each patient is established by the absence of corneal sensation using a Charcot-Bonnet aesthesiometer. Other demographic details are listed in Table 1. Most eyes had received multiple ocular surgeries (see Table 1 for details). These procedures could have contributed to the development of the neurotrophic state. There had been other procedures performed in these patients. The results of these procedures are not included in this study.

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<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Causes of ulcer</th>
<th>Eye</th>
<th>Associated surface problem/duration of ulcers (months)</th>
<th>Previous surgery</th>
<th>Previous management for surface problems</th>
<th>AMT</th>
<th>Subsequent surgery</th>
<th>VA-pre</th>
<th>VA-post</th>
<th>Other ocular problems</th>
<th>Epithelial healing (days)</th>
<th>Follow up (months)</th>
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<tr>
<td>1</td>
<td>69</td>
<td>M</td>
<td>(1) HSV</td>
<td>LE</td>
<td>Keratoconjunctivitis, bacterial superinfection/2.8 (2) perforation/1.2</td>
<td>(1) Glaucoma surgery with Baerveldt</td>
<td>Punctal occlusion</td>
<td>AMT+bandage CL (AMT 3 layers)</td>
<td>(1) PKP (2) Partial tarsorrhaphy (34 days later) PKP+partial tarsorrhaphy (99 days later)</td>
<td>(1)LP</td>
<td>(2)HM</td>
<td>Parkinson's disease, glaucoma, optic nerve atrophy</td>
<td>(1) 14 (2) 50*</td>
<td>(1) 19 (2) 24</td>
<td>(2) Non-healing and perforation until repeat PKP+tarsorrhaphy</td>
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<td>2</td>
<td>81</td>
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<td>HZO, PKP</td>
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<td>50% thinning/2.3</td>
<td>PKP (2x), ECCE</td>
<td>Conjunctival flap, patching</td>
<td>AMT (3 layers) + bandage CL</td>
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<td>4/200</td>
<td>20/200</td>
<td>Choroidal detachment, hypotony</td>
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<td>38</td>
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<td>M</td>
<td>HZO, PKP</td>
<td>LE</td>
<td>Trichiasis, 75% thinning/3.1</td>
<td>PKP (2x), ECCE</td>
<td>Partial tarsorrhaphy</td>
<td>AMT as patch</td>
<td>PKP+AMT as patch</td>
<td>HM</td>
<td>20/50</td>
<td>—</td>
<td>13</td>
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<tr>
<td>4</td>
<td>47</td>
<td>F</td>
<td>HZO</td>
<td>LE</td>
<td>Keratoconus, 20% thinning/2.5</td>
<td>PKP</td>
<td>—</td>
<td>AMT as patch</td>
<td>—</td>
<td>CF</td>
<td>20/60</td>
<td>AIDs</td>
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<td>—</td>
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<td>5</td>
<td>78</td>
<td>M</td>
<td>HZO, DM, PKP</td>
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<td>Trichiasis, KCS, 30% thinning/1.8</td>
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<td>Punctal occlusion, Boton proxis, lid margin eversion</td>
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<td>Partial tarsorrhaphy (27 days later)</td>
<td>HM</td>
<td>20/200</td>
<td>—</td>
<td>74*</td>
<td>26.5</td>
<td>Non-healing until 47 days after tarsorrhaphy</td>
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<tr>
<td>6</td>
<td>86</td>
<td>F</td>
<td>DM</td>
<td>RE</td>
<td>25% thinning/2.0</td>
<td>ECCE, cryotherapy</td>
<td>—</td>
<td>AMT + bandage CL</td>
<td>—</td>
<td>NLP</td>
<td>NLP</td>
<td>NVG, optic nerve atrophy</td>
<td>20</td>
<td>3.7</td>
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<tr>
<td>7</td>
<td>51</td>
<td>M</td>
<td>DM</td>
<td>RE</td>
<td>Keratoconjunctivitis, 50% thinning, both eyes/3.5 40% thinning/3.6</td>
<td>Glaucome surgery with Seton ECCE, PKP</td>
<td>—</td>
<td>AMT + AMT as patch</td>
<td>—</td>
<td>LP</td>
<td>HM</td>
<td>NVG</td>
<td>34</td>
<td>8</td>
<td>—</td>
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<td>71</td>
<td>F</td>
<td>Radiation, PKP</td>
<td>RE</td>
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<td>Keratoconjunctivitis: 7/5 thinning/3.6</td>
<td>Punctal occlusion, Partial tarsorrhaphy</td>
<td>AMT (1 layer)+AMT as patch</td>
<td>Partial tarsorrhaphy and removal of gold weight (7 days later)</td>
<td>20/200</td>
<td>20/200</td>
<td>—</td>
<td>6</td>
<td>19</td>
<td>—</td>
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<tr>
<td>11</td>
<td>62</td>
<td>M</td>
<td>Removal of Acoustic Neuroma</td>
<td>RE</td>
<td>Facial palsy, KCS 75% thinning/4.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>6</td>
<td>8.5</td>
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<tr>
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<td>70</td>
<td>M</td>
<td>PKP, DM</td>
<td>RE</td>
<td>Failed graft, limbal deficiency, sterile ulcer with infiltration/2.4</td>
<td>PKP (3x)</td>
<td>—</td>
<td>AMT + Sectorial KLAL</td>
<td>—</td>
<td>HM</td>
<td>HM</td>
<td>—</td>
<td>16</td>
<td>6</td>
<td>—</td>
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<tr>
<td>13</td>
<td>61</td>
<td>F</td>
<td>PKP</td>
<td>LE</td>
<td>Band keratopathy, failed graft, cicatricial entropion, trichiasis, KCS: 4/5</td>
<td>PKP, ECCE</td>
<td>Punctal occlusion, Boton proxis, lid margin eversion</td>
<td>AMT+AMT as patch</td>
<td>Punctual occlusion</td>
<td>HM</td>
<td>20/400</td>
<td>Glaucoma</td>
<td>16</td>
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<tr>
<td>14</td>
<td>86</td>
<td>F</td>
<td>PKP</td>
<td>LE</td>
<td>Band keratopathy, failed graft/3.6</td>
<td>PKP, vitrectomy scleral bucking</td>
<td>—</td>
<td>AMT + AMT as patch</td>
<td>—</td>
<td>NLP</td>
<td>NLP</td>
<td>RD, glaucoma, rheumatoid arthritis</td>
<td>8</td>
<td>6</td>
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</tbody>
</table>

AIDS = acquired immune deficiency syndrome; AMT = amniotic membrane transplantation; CF = counting fingers; CL = contact lens; DM = diabetes mellitus; ECCE = extracapsular cataract extraction; HM = hand movement; HSV = herpes simplex virus; HZO = herpes zoster ophthalmica; KCS = keratoconjunctivitis sicca; KLAL = keratolimbal allograft; LP = light perception; NLP = no light perception; NVG = neovascular glaucoma; PDR = proliferative diabetic retinopathy; PKP = penetrating keratoplasty; RD = retinal detachment; VA = visual acuity.
PREPARATION OF PRESERVED HUMAN AMNIOTIC MEMBRANE
In this study, all except for four patients from a previously approved study used amniotic membranes obtained from Bio-Tissue (South Miami, FL, USA), where procurement includes screening against HIV types 1 and 2, human T lymphoma virus type 1, hepatitis B and C viruses, and syphilis at the time of caesarean delivery and 6 months post partum, and the methods of preparation follow our previous methods.

AMNIOTIC MEMBRANE TRANSPLANTATION
All surgical procedures were performed by the same surgeon (SCGT). Except for case 1, in whom AMT was performed twice in the same eye, all others received AMT once in the eye with ulcers. After retrobulbar anaesthetic injection in eyes with neurotrophic ulcer, the base of the ulcer was debrided with a microsponge and fine forceps, and the poorly adherent epithelium adjacent to the edge of the ulcer was removed up to the area where the epithelium became adherent. The amniotic membrane was then removed from the storage medium, peeled from the nitrocellulose filter paper, transferred to the recipient eye, and fitted to fill up the ulcer and cover the defect by trimming off the excess edges. This fashioned membrane with stroma-side down was then secured to the edge of the defect by interrupted 10-0 nylon sutures (if on the cornea or extending it beyond the limbus with the basement membrane side facing the surface of the eye, and fitted to fill up the ulcer and cover the defect by trimming off the excess edges. These procedures could have contributed to the development of the neurotrophic state. In this series, the neurotrophic state of these 16 eyes was established by the lack of corneal sensation using a Charcot–Bonnet aesthetiometer. As a result, there was markedly reduced and infrequent blinking resulting in exposure keratopathy, and varying degrees of aqueous tear deficiency leading to keratoconjunctivitis sicca (see Table 1). For exposure problems, patches of Amo Softflex (Alcon, Miami, FL, USA), where procurement in-...
Figure 1  Case 3 had previously undergone a penetrating keratoplasty with tarsorrhaphy for a progressive ulcer and descemetocele caused by herpes zoster ophthalmicus on the left eye. He developed a recurrent herpes zoster with epithelial dendrites and ulcers stained with rose bengal (A) and fluorescein (B), and progressed into disciform and necrotising stromal keratitis. This was complicated by bacterial keratitis, and a large inferior one third hypopyon (C). Following appropriate antibiotics and aciclovir, the sterilised ulcer became thin in several locations of the cornea. Amniotic membrane transplantation was performed and resulted in total healing of the ulcer with a quiet ocular surface in 13 days (D and E). After the corneal surface had been stable for 15 months (F), a repeat PKP was performed and covered with an amniotic membrane as a patch, which was dissolved in 2 weeks, and the graft showed a smooth surface and clear stroma 8 months later (G and H).
refractory glaucoma in five eyes and partial limbal stem cell deficiency in three eyes. Systemically, six patients had diabetes mellitus, three patients had hypertension, one patient had AIDS, and one patient had Parkinson’s disease.

The neurotrophic ulcer was located in the central cornea in 14 eyes, in the nasal corneal periphery in one eye and at the corneal graft host junction in one eye. According to the respective histories, all these ulcers had been persistent for more than 2 months, and 10 eyes had shown progressive thinning of the ulcer bed. Five eyes (31.2%) developed hypopyon. All corneas had significant stromal oedema and inflamed limbus and conjunctiva.

AFTER AMNIOTIC MEMBRANE TRANSPLANTATION
Depending on the depth of stromal ulceration, the ulcer was covered by one or more than one layer of amniotic membrane, and the basement membrane side of the last layer was congruent with the patient’s exposed surface. These denuded surfaces healed rapidly after AMT. Except for four eyes, all others (13/17, 76.4%) healed within 16.6 (SD 9.0) days. In the two eyes of case 7 with severe diabetes mellitus, the ulcers were not healed on day 14 and partial tarsorrhaphy was added, and the healing was completed on day 34. In the one eye of case 5 with herpes zoster ophthalmicus (HZO) and diabetes mellitus and status post-penetrating keratoplasty, the ulcer did not heal on day 27.
when partial tarsorrhaphy was added and healing was completed on day 74. In the eye of case 1, the ulcer was successfully healed by the first attempt of AMT. After healing, penetrating keratoplasty was performed and vision improved from LP to HM. Unfortunately, the corneal surface broke down with progressive ulcer leading to corneal perforation at the graft-host junction. The second attempt at AMT was performed without success and finally the eye required repeat PKP and partial tarsorrhaphy.

A remarkable reduction in ocular inflammation was accompanied by rapid epithelialisation. During the follow up period of 18.8 (13.0) months, visual acuity was improved in eight eyes (50%) with two eyes improving in visual acuity to 20/50 (case 3) (Fig 1) following repeat penetrating keratoplasty, and AMT as a patch yielding 20/80 (case 4) (Fig 2). The visual acuity did not show any change in six eyes (37.5%), but was worsened in two eyes (12.5%) owing to progressive glaucoma and cataract. All eyes achieved the objective of maintaining anatomical integrity. All membranes were partially (Fig 3E, F) or completely dissolved (Fig 2E, F), and the remaining stroma showed variable amounts of opacity.

Figure 3  Case 15 had received multiple surgeries for recurrent retinal detachment in the left eye resulting in a persistent neurotrophic corneal ulcer with band keratopathy (A and B). Eight days after amniotic membrane transplantation (AMT), part of the membrane used as a patch started to dissolve (arrow) (C), while epithelial healing had taken place as shown by fluorescein staining (D). One month after AMT the membrane covered surface was totally healed and smooth with a small part of the membrane dissolved (indicated by asterisks) (E and F). The corneal surface continued to be stable and remained uninflamed (F).
we usually laid more than one layer of membrane to build up the corneal thickness in eyes with deep ulcers or descemetocoele. In some eyes, we added a larger layer of membrane on top of this membrane as a temporary patch in a manner proposed by Kim et al.17 This membrane as a patch frequently dissolved upon epithelialisation as shown in case 15 (Fig 3C, D). The rationale of using AMT as a patch (case 4) was when the stromal thinning was minimal and the ulcer bed appeared to be non-necrotic. The rationale of using AMT as a patch in addition to using it as a graft (cases 8, 11, 12, 14, and 15) was to prevent surface exposure and dryness and promote epithelial healing in these cases with poor blinking reflex. Constant protection and wetting of the ulcerated area by the membrane is also beneficial and AM uses as a patch may function like tarsorrhaphy to minimise exposure. When used as a patch, AM is invariably dissolved. When used as a graft, AM promotes epithelialisation over it, and is frequently preserved and may become quite transparent over time.

Also consistent with the report by Kruse et al,13 we noted in this study that ocular surface inflammation was markedly reduced following AMT (Fig 2E, F). This finding may be explained by other recent studies showing that the stromal matrix of the amniotic membrane excludes inflammatory cells,38 39 contains various forms of protease inhibitors,40 and suppresses transforming growth factor β (TGF-β) signalling, and proliferation and myofibroblast differentiation of normal human corneal and limbal fibroblasts.41

Nevertheless, it is important to point out that persistent exposure is one limiting factor for AMT as delayed epithelialisation was noted in four eyes with very poor eyelid blink; one had Parkinson’s disease following intracranial surgery (case 1), two had severe diabetes with amputation of both legs (case 7), and one suffered combined HZO and severe diabetic neuropathy (case 5). To remedy this situation, we advise early partial tarsorrhaphy. Because the neurotrophic state invariably leads to aqueous tear deficiency, we advise that punctal occlusion be performed before tarsorrhaphy. Prolonged exposure also explains why the membrane eventually dissolved partially or totally. Although recurrent breakdowns have been reported,15 for reasons still not clear some ulcers remained healed even if the membrane was completely dissolved.

Another limitation of AMT may occur in patients with limbal (stem cell) deficiency (reviewed by Tseng and Tsubota). Based on the cytological criterion of conjunctivalisation, we have previously reported that limbal stem cell deficiency can develop in patients with neurotrophic keratitis.42 In these 16 eyes we noted three eyes developing partial limbal deficiency (Table 1). It is worth noting that such complications developed in two of three eyes after radiation for ocular tumours. Unlike what was reported by Fujishima et al.,43 neither of our cases reverted to normal limbal function during a prolonged follow up period and their limbal deficiency persisted. With respect to
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Zwiers RTF, Choshki A, Tseng SCG. Amniotic membrane transplantation or limbal conjunctival autograft for limbal stem cell deficiency induced by 5-fluorouracil in glaucoma surgeries. Cornea 2000;in press.


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proprietary interest: SCGT has a financial interest in the preparation and clinical uses of amniotic membrane and Bio-Tissue.


