Heliyon 9 (2023) e22693

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Amniotic membrane transplantation combined with conjunctival flap covering surgery for the treatment of corneal perforations in fungal keratitis

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ARTICLE INFO

Keywords:
Fungal keratitis
Amniotic Membrane
Conjunctival flap
Corneal perforation

ABSTRACT

Purpose: To investigate the efficiency of amniotic membrane transplantation (AMT) combined with conjunctival flap covering surgery (CFCS) for patients with corneal perforations in fungal keratitis (FK).

Methods: In this non-comparative, retrospective case series, 16 participants of corneal perforation in FK were successfully treated by a combination of multilayer AMT and bipedicle conjunctival flap with partial tenon's capsule. Corneal healing, recurrence of FK, visual acuity, and relevant complications were reported as outcome measures.

Results: Sixteen patients (13 male, 3 female) had a mean age of 58.8 ± 10.3 (range 29–72) years. The mean diameter of corneal perforation was 1.9 ± 0.7 (range 0.5–2.8) mm. Corneal perforations healed and all the patients preserved their eyeballs. During the 11.0 ± 4.4 (range 6–18) months of follow-up, there was no recurrence of FK in any of these cases. Visual acuity improved in 15 eyes (93.8 %) and remained unchanged in 1 patient (6.3 %) who had no light perception when first admitted. All 6 patients who accepted secondary keratoplasty showed improved best corrected visual acuity of more than 4 lines. The most frequently found fungi were *Aspergillus species* (6 of 16, 37.5 %) and *Fusarium species* (4 of 16, 25.0 %), followed by 1 *Scedosporium apiospermum* (1 of 16, 6.3 %).

Conclusions: Combination AMT with CFCS is a safe and effective surgery for patients with corneal perforations in FK, particularly where eye banks and fresh corneas are not available. This surgery could preserve the integrity of the eyeball and avoid the recurrence of FK. Besides, it provides a greater opportunity for further optical keratoplasty.

1. Introduction

Fungal keratitis (FK) is one of the primary causes of corneal blindness and eyeball loss in developing countries [1–3]. Effective diagnosis and treatment are usually delayed by a limitation of medical care [4]. Up to 50 % of severe fungal infection cases might result

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https://doi.org/10.1016/j.heliyon.2023.e22693
Received 10 May 2023; Received in revised form 14 November 2023; Accepted 16 November 2023
Available online 21 November 2023
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in corneal perforation [5] and need therapeutic penetrating keratoplasty (PKP) [6]. The scarcity of fresh donor corneas remains a major problem, especially during emergencies [7,8]. Besides, in comparison with corneal transplantation done for visual purposes, the graft survival rate is lower and surgical complications are more frequent during acute infection [9–12].

Amniotic membrane (AM) and umbilical cord patch (also called ultra-thick AM, UTAM) serve as alternative treatments for patients with corneal perforations [13–15]. In addition to repairing the damaged corneal stroma, AM has an anti-inflammatory effect as well [16,17]. However, the possibility of AM melting and fungal recurrence should not be neglected [16,17].

Conjunctival flap covering surgery (CFCS) was introduced by Gundersen in 1958 [18]. The abundance of blood and lymphatic vessels in conjunctival flap increases the resistance of cornea against fungal infection and collagenolysis [19,20]. The application of CFCS to the treatment of refractory FK has been proved to be successful [21–23]. However, due to its tectonic instability, CFCS alone used in perforated FK might result in conjunctival flap dissolution and corneal perforation recurrence. We hypothesize that combination of AMT and CFCS enhances both tectonic support and anti-infectious effect. Herein, we report the outcome of AMT combined with CFCS for the surgical management of corneal perforations in FK.

2. Materials and methods

2.1. Subjects

The present study was a non-comparative, retrospective case series. Consecutive 16 eyes of 16 patients with corneal perforations in FK between September 2020 and December 2022 were reviewed. This study was approved by the Ethics Committee of Union Hospital, Tongji Medical College, and Huazhong University of Science and Technology according to the tenets of the Declaration of Helsinki (UHCT21881). Informed consent was obtained from each participant for the publication of their images. All patients were assessed with slit-lamp microscopic examination, confocal microscopy, corneal scraping, and microbiological culture. The study included patients who meet the following criteria: i) hyphae were detected by confocal microscopy (Fig. 1A) or corneal scraping (Fig. 1B); ii) a lesion involving the Descemet membrane with positive Seidel test, and collapse of the anterior chamber; iii) without endophthalmitis. Individuals were excluded based on the following criteria: i) keratitis of other forms; ii) FK without perforation.

2.2. AM and UTAM preparation

AM and UTAM were prepared by professional ophthalmologists under sterile environment in the operating room. Following cesarean sections, healthy human placentas with umbilical cords were removed under sterile settings with signed informed permission. Individuals who tested positive for syphilis, hepatitis B and C viruses, and the human immunodeficiency virus were not included. AM and UTAM were manually isolated from the chorion by blunt dissection following a saline wash. After careful removal of umbilical vessels and loose jelly tissues, AM and UTAM were cut into 2 × 2 cm pieces. Following three rounds of washing with saline containing 50 μg/ml penicillin, 50 μg/ml streptomycin, and 2.5 μg/ml amphotericin B, AM and UTAM were stored at −20 °C in sterilized pure glycerin for a maximum of three months. AM and UTAM were thawed, cleaned of the glycerin, and then submerged in saline for 10 min before use [15].

2.3. Surgical technique

All surgeries were performed under local infiltration anesthesia with 2 % lidocaine. Using a blade to remove the necrotic tissue and epithelial cells around the corneal perforation (Fig. 2A). Then, a small amount of sodium hyaluronate was used to separate posterior synechiae of the iris. Multilayered AM or UTAM with epithelium side facing up was trimmed to fill the cavity of the ulcer depending on the shape of the ulcer, using 10–0 nylon to suture onto the cornea interruptedly (Fig. 2B). Subsequently, 100 μg/0.1 ml voriconazole

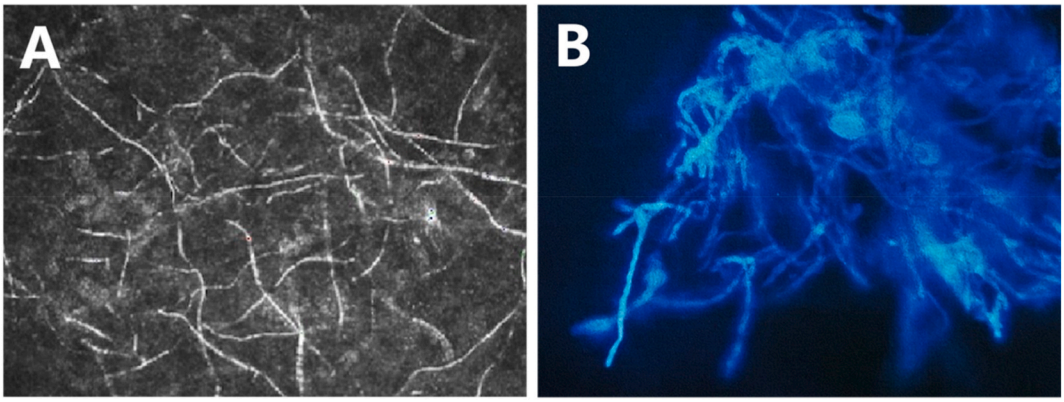

Fig. 1. Diagnosis of FK. Hyphae detected by confocal microscope (A) or corneal scraping (B).

solution was injected to form the anterior chamber. Eventually, a bipedicle conjunctival flap with partial tenon's capsule was divided depending on the position and size of the ulcer, and then it was sutured to the cornea over the AM using 10–0 nylon (Fig. 2C). Suture knots were embedded if possible.

2.4. Postoperative management and follow-up

Following surgery, all patients were prescribed antifungal agents including 0.15 % amphotericin B and 1 % voriconazole eye drops every hour daily for 2 weeks, every 2 h for at least 6 weeks, combined with 200 mg of voriconazole tablets orally twice daily for 4 weeks. Hepatic and renal functions were regularly evaluated. Nonsteroidal anti-inflammatory 0.1 % bromfenac sodium eye drops and mydriatic 1 % atropine were routinely used postoperatively to prevent posterior synechiae. If intraocular pressure was found to be high after surgery, effective therapies such as oral methazolamide tablets or topical hypotensive medications were given promptly. Sutures were removed 3–4 weeks postoperatively. The primary outcome measures were ulcer and perforation healing, FK recurrence, acuity of vision and related complications. A follow-up was performed daily for a week, biweekly for 1 month, monthly for 3 months, and then at different intervals for each patient post-operative.

2.5. Statistical analysis

Microsoft Excel (Microsoft, Redmont, WA) was used to conduct the statistical analysis. Means and standard deviations were calculated for demographics and clinical variables.

3. Results

3.1. Demographics and clinical features

A total of 16 patients were included in this study, including 13 males (81.3 %) and 3 females (18.7 %). The average age was 58.8 ± 10.3 years, ranging from 29 to 72 years. Ten patients (62.5 %) had a definite history of vegetative trauma. Corneal ulcers were 6.9 ± 0.7 mm in diameter on average, ranging from 5.2 to 8.2 mm. The mean diameter of corneal perforation was 1.9 ± 0.7 mm, ranging from 0.5 to 2.8 mm. Eleven patients (68.8 %) were detected with fungal hyphae through corneal scraping and 14 (87.5 %) through confocal microscopy. As for basal visual acuity, 4 individuals (25 %) presented of light perception (LP). Hand movement (HM) was observed in 7 patients (43.8 %) and count fingers (CF) was tested in 4 individuals (25 %). One patient had no light perception (NLP) when first admitted due to diabetic retinopathy and neovascular glaucoma. The demographic and preoperative characteristics of the patients are summarized in Table 1.

3.2. Postoperative results

The mean follow-up time was 11.0 ± 4.4 months, ranging from 6 to 18 months. There was no recurrence in any of the cases during the whole follow-up period. At the perforation site, multilayered AM or UTAM was incorporated into the corneal stroma. All corneal ulcers (Fig. 3A) healed, and the anterior chamber formed well without aqueous leakage or hypopyon at 3-week follow-up (Fig. 3B). A stable corneal leucoma with superficial blood vessels regression was noted at 3-month postoperatively (Fig. 3C).

Two typical patients were referred to our hospital for recurrence of corneal perforation after simple CFCS (Fig. 4A). The anterior chamber formed without presence of hypopyon at 1 week after AMT and CFCS (Fig. 4B) and the ocular surfaces were stable without fungal recurrence at 2-month follow-up (Fig. 4C).

Secondary keratoplasty for optical indications was performed for 6 patients to further improve their vision. Two of them with eccentric perforations accepted deep anterior lamellar keratoplasty (DALK) (Fig. 5A). Four patients with central perforations underwent PKP (Fig. 5B). Six patients were unwilling to accept further corneal transplantation surgical treatment. The rest 4 patients were waiting for fresh donor corneas.

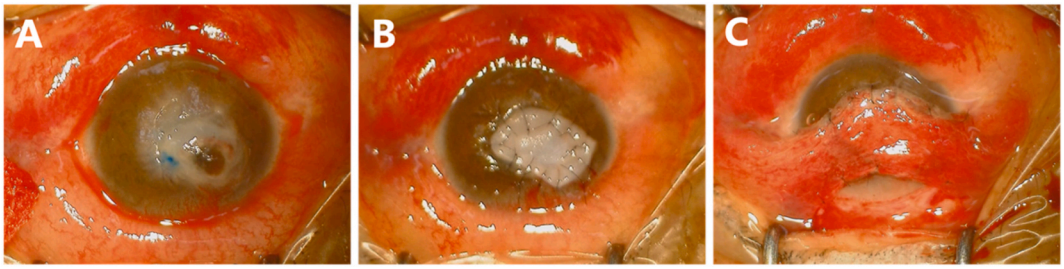

Fig. 2. Video images of surgical procedures. The necrotic tissue was removed (A). Multilayer AM or UTAM with epithelium side facing up was trimmed to fill in the corneal ulcer (B). The bipedicle conjunctival flap with partial tenon's capsule was sutured with a 10–0 nylon to the cornea over the AM (C).

Table 1
Demographic and clinical features of patients: pretreatment.

Demographic and Clinical Features	Data
Age (years),	
Mean (SD)	58.8 (10.3)
Range	29–72
Male gender	13 (81.3 %)
Vegetative trauma	10 (62.5 %)
Involved right eye	9 (56.3 %)
Diameter of corneal ulcer	
Mean (SD)	6.9 (0.7)
Range	5.2–8.2
Diameter of corneal perforation	
Mean (SD)	1.9 (0.7)
Range	0.5–2.8
Hyphae detection	
scraping	11 (68.8 %)
confocal	14 (87.5 %)
Basal BCVA	
NLP	1 (6.3 %)
LP	4 (25.0 %)
HM	7 (43.8 %)
CF	4 (25.0 %)

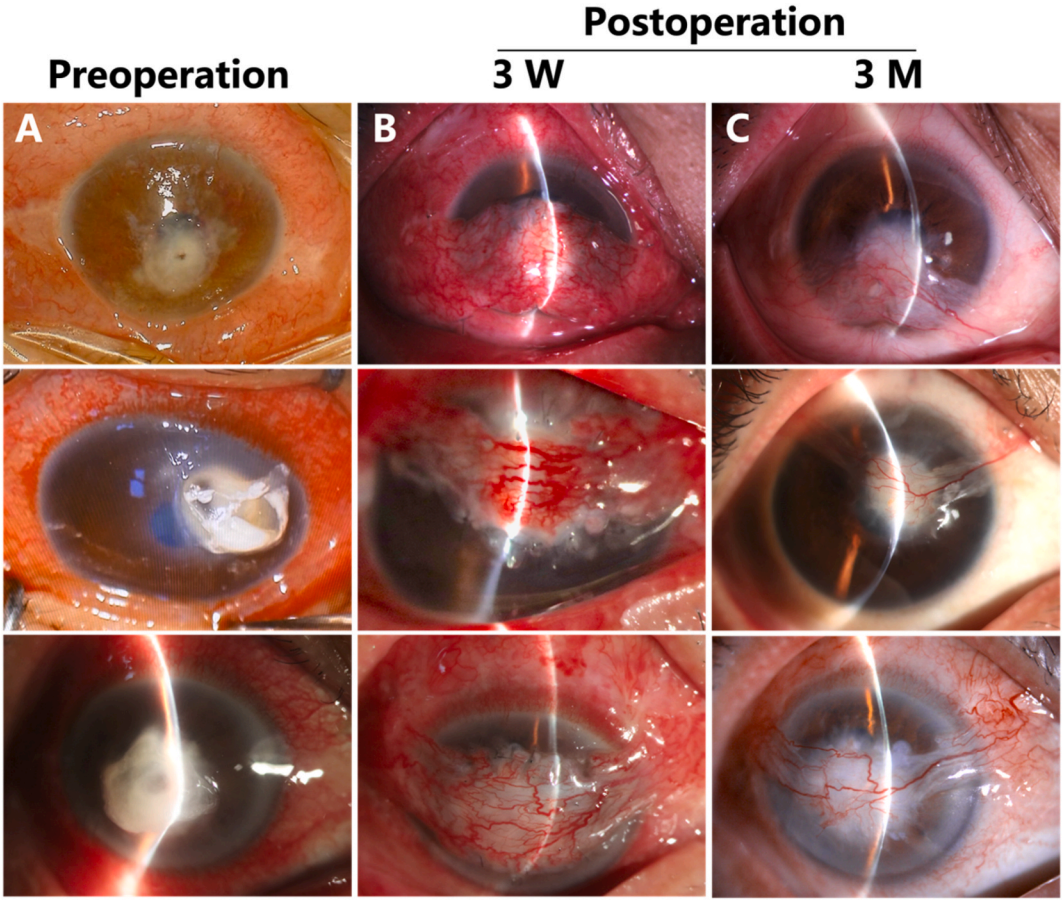

Fig. 3. Slit-lamp biomicroscopic photographs of pre- and post-operative corneas. The corneas perforated preoperatively (A). The anterior chamber formed well without aqueous leakage or hypopyon at 3-week follow-up (B). A stable corneal leucoma with superficial blood vessels regression was noted at 3-month postoperatively (C).

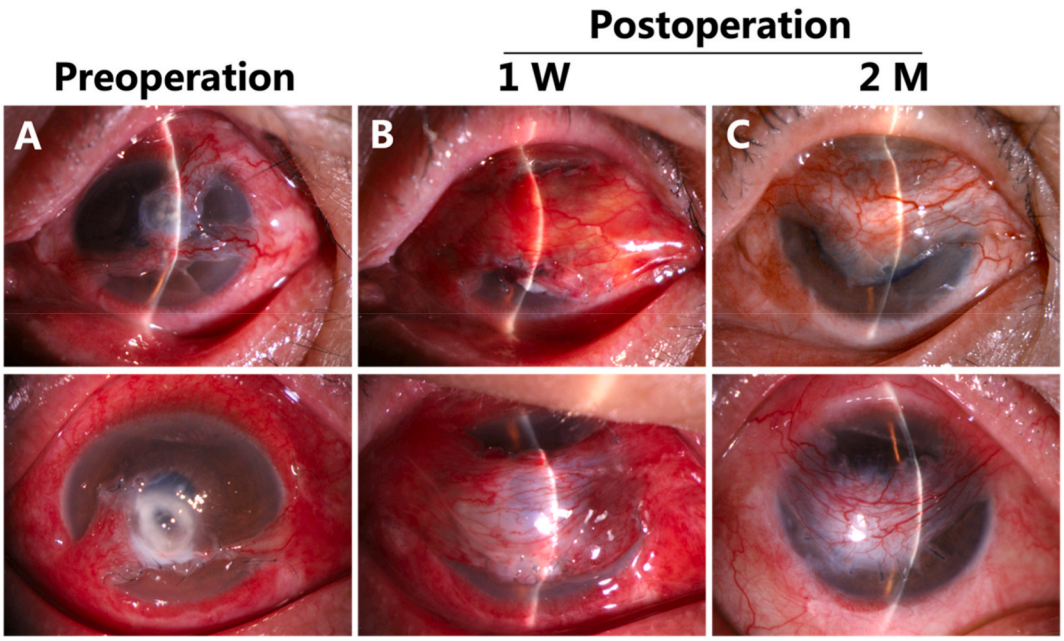

Fig. 4. Slit-lamp biomicroscopic photographs from 2 recurrent patients after CFCS. Perforations were noted preoperatively (A). The anterior chamber formed at 1 week after AMT and CFCS (B). The ocular surfaces were stable without fungal recurrence at 2-month follow-up (C).

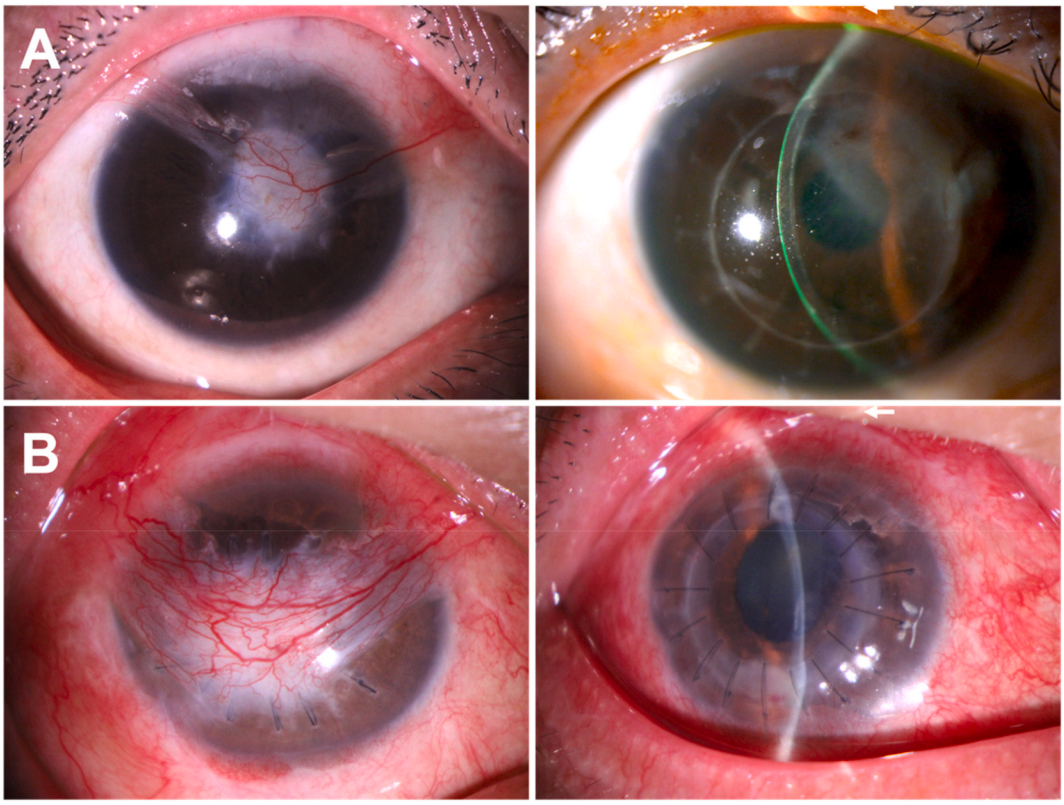

Fig. 5. Secondary keratoplasty for optical indications. DALK and PKP were performed for patients with eccentric (A) and central (B) corneal perforations respectively.

The best corrected visual acuity (BCVA) improved in 15 eyes (93.8 %) and remained unchanged in 1 patient (6.3 %) with eyeball atrophy after cyclophotocoagulation. He was tested for NLP when first admitted to the hospital. All 6 patients who accepted secondary keratoplasty showed improved BCVA of more than 4 lines. No relevant complications were observed after keratoplasty. The post-operative outcomes of the patients are summarized in Table 2.

3.3. Fungal etiology

All patients underwent microbiological culture, and the positive rate was 68.7 % (11 of 16). The most frequently found fungi were *Aspergillus species* (6 of 16, 37.5 %) and *Fusarium species* (4 of 16, 25.0 %), followed by 1 *Scedosporium apiospermum* (1 of 16, 6.3 %). There was no growth of microorganism in 31.3 % of the cases (5 of 16). No bacteria or multiple fungi were detected in all cases.

4. Discussion

FK is a worldwide challenging problem because of various fungal pathogens, complex clinical manifestations, and the limitation of effective antifungal therapies [3]. It has a poor prognosis compared to other forms of infectious keratitis [3]. About 25.0 %–35 % of patients with FK need surgical intervention to prevent infection spreading or corneal perforation [24–26]. It is estimated that up to 50 % of severe FK cases result in corneal perforation and require therapeutic keratoplasty [5]. However, there is a large discrepancy between the supply and demand of fresh donors, especially in developing countries such as China [7,8]. It has been well accepted that outcomes of PKP during acute infection fare worse than those for optical indications [9–12]. The success rate may be promoted by postponing such definitive surgical procedures until the infection and inflammation have subsided. Therefore, transitional surgeries, such as AMT and CFCS, have a crucial role in inhibiting the progression of the disease.

Multilayered AM or UTAM is widely used for corneal reconstruction by filling in new collagen to the ulcer bed as well as suppressing inflammation [13–15]. In addition, it is popular in ocular surface surgery because of its nonimmunogenic and lack of human leukocyte antigens [27]. In this study, AMT was adopted as an emergency remedy to preserve the integrity of the globe and avoid evisceration. However, multilayered AM or UTAM alone tends to dissolve in an early stage after surgery and had a risk of persistent or recurrent infection in FK [16,17].

Conjunctival flap forms a protective layer over the lesional tissue, preventing tears, proteolytic enzymes, and proinflammatory mediators from reaching the corneal ulcer [20]. The abundant blood and lymphatic vessels supply nutrients to the cornea, and enhance its resistance against infection and collagenolysis [20]. Besides, conjunctival flap with Tenon's capsule could improve mechanical support for the cornea of FK with deep stromal infiltration [23,28]. However, CFCS alone is not able to counteract the intraocular pressure. In the case series, 2 patients experienced recurrent perforations after previous CFCS, and both recovered following AMT and CFCS. In addition, suture skill is also an important factor affecting the success rate of the surgery. Complications of CFCS that are previously reported are flap retraction, conjunctival buttonholes [29]. In our experience, an oversized conjunctival flap with a partial Tenon's capsule was sewed to the cornea loosely to ensure abundant blood supply and prevent conjunctiva necrosis.

Aspergillus species was the dominating pathogen in this study. There is a tendency for the hyphae of the *Aspergillus* to grow perpendicularly [30,31], and the deep stroma and Descemet's membrane are prone to be invaded. Therefore, *Aspergillus* keratitis might be a predisposition for corneal perforation.

The present finding suggests that AMT and CFCS might be an effective treatment for patients of corneal perforations in FK. In this study, all eyeballs were preserved, and the cornea demonstrated conjunctival re-epithelization without fungal recurrence after surgery. Moreover, the improvement of patients' eyesight could be achieved through secondary surgeries such as corneal transplantation. DALK was performed on patients with eccentric perforations. The retention of recipient endothelium could reduce the possibility of endothelial immunological rejection as well as surgical complications [9,32–34]. Furthermore, using glycerol cryopreserved corneas for DALK also alleviated the problem of insufficient fresh corneal donors [7,8,35].

The limitations of the present technique include the small sample size. In addition, the rich blood supply of conjunctiva may accelerate the dissolution of AM and UTAM. The interaction of AM and conjunctival flap also deserves further research and discussion.

5. Conclusions

Combination AMT with CFCS is a safe and effective surgery for patients with corneal perforations in FK, particularly in areas where there are not any fresh corneal donors or eye banks. This procedure is critical in maintaining global integrity as well as providing metabolic and mechanical support for corneal healing. It also serves as a transitional surgery for subsequent optical keratoplasty.

8. Ethics declarations

This retrospective study was approved by the Ethics Committee of Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science and Technology according to the tenets of the Declaration of Helsinki (UHCT21881).

Funding statement

This study was supported by the National Natural Science Foundation of China (82171025, 82070934), the Fundamental Research Funds for the Central Universities, China (HUST: 2019kfyXMBZ065), the Clinical Research Foundation of Wuhan Union Hospital,

Table 2
Postoperative results of AMT and CFCS.

Outcomes	Data
Follow-up (months)	
Mean (SD)	11.0 (4.4)
Range	6–18
Fungal recurrence	0 (0)
Best corrected visual acuity	
Improved	15 (93.8 %)
Unchanged	1 (6.3 %)
Decreased	0 (0)
Secondary Keratoplasty	
DALK	2
PKP	4

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ya-Li Du: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology. **Jia-Song Wang:** Software, Resources, Methodology. **Wen Geng:** Software, Methodology. **Chao-Ye Duan:** Software, Methodology. **Xing-Hua Wang:** Software, Methodology. **Hua-Tao Xie:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ming-Chang Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.P. Whitcher, M. Srinivasan, Corneal ulceration in the developing world–a silent epidemic, Br. J. Ophthalmol. 81 (8) (1997) 622–623.
[2] J.P. Whitcher, M. Srinivasan, M.P. Upadhyay, Corneal blindness: a global perspective, Bull. World Health Organ. 79 (3) (2001) 214–221.
[3] N. Sharma, et al., Fungal keratitis: a review of clinical presentations, treatment strategies and outcomes, Ocul. Surf. 24 (2022) 22–30.
[4] N.V. FlorCruz, J.R. Evans, Medical interventions for fungal keratitis, Cochrane Database Syst. Rev. (4) (2015) Cd004241.
[5] N.V. Prajna, et al., Effect of oral voriconazole on fungal keratitis in the mycotic ulcer treatment trial II (mutt II): a randomized clinical trial, JAMA Ophthalmol 134 (12) (2016) 1365–1372.
[6] N.V. Prajna, et al., Predictors of corneal perforation or need for therapeutic keratoplasty in severe fungal keratitis: a secondary analysis of the mycotic ulcer treatment trial II, JAMA Ophthalmol 135 (9) (2017) 987–991.
[7] X. Shang, M. Zhang, Body and organ donation in Wuhan, China, Lancet 376 (9746) (2010) 1033–1034.
[8] D.T. Tan, et al., Corneal transplantation, Lancet 379 (9827) (2012) 1749–1761.
[9] L. Xie, H. Zhai, W. Shi, Penetrating keratoplasty for corneal perforations in fungal keratitis, Cornea 26 (2) (2007) 158–162.
[10] D.T. Tan, et al., Penetrating keratoplasty in asian eyes: the Singapore corneal transplant study, Ophthalmology 115 (6) (2008) 975–982 e1.
[11] M. Ayalew, et al., Penetrating keratoplasty at a tertiary referral center in Ethiopia: indications and outcomes, Cornea 36 (6) (2017) 665–668.
[12] J. Mundra, et al., Outcomes of therapeutic penetrating keratoplasty in 198 eyes with fungal keratitis, Indian J. Ophthalmol. 67 (10) (2019) 1599–1605.
[13] A. Solomon, et al., Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers, Ophthalmology 109 (4) (2002) 694–703.
[14] M.T. Rodríguez-Ares, et al., Multilayer amniotic membrane transplantation in the treatment of corneal perforations, Cornea 23 (6) (2004) 577–583.
[15] H.T. Xie, et al., Umbilical cord patch transplantation for corneal perforations and descemetoceles, J Ophthalmol 2017 (2017), 2767053.
[16] H.C. Chen, et al., Amniotic membrane transplantation for persistent corneal ulcers and perforations in acute fungal keratitis, Cornea 25 (5) (2006) 564–572.
[17] D.S.J. Ting, et al., Amniotic membrane transplantation for infectious keratitis: a systematic review and meta-analysis, Sci. Rep. 11 (1) (2021), 13007.
[18] T. Gundersen, Conjunctival flaps in the treatment of corneal disease with reference to a new technique of application, AMA Arch Ophthalmol 60 (5) (1958) 880–888.
[19] L.S. Lim, et al., Gundersen flaps in the management of ocular surface disease in an Asian population, Cornea 28 (7) (2009) 747–751.
[20] M. Zemba, et al., Conjunctival flap surgery in the management of ocular surface disease, Exp. Ther. Med. 20 (4) (2020) 3412–3416.
[21] G.H. Sun, et al., Clinical observation of removal of the necrotic corneal tissue combined with conjunctival flap covering surgery under the guidance of the AS-OCT in treatment of fungal keratitis, Int. J. Ophthalmol. 5 (1) (2012) 88–91.
[22] H. Nizeyimana, et al., Clinical efficacy of conjunctival flap surgery in the treatment of refractory fungal keratitis, Exp. Ther. Med. 14 (2) (2017) 1109–1113.
[23] Y.C. Wang, et al., Two-step strategy-conjunctival flap covering surgery combined with secondary deep anterior lamellar keratoplasty for the treatment of high-risk fungal keratitis, Int. J. Ophthalmol. 16 (7) (2023) 1065–1070.
[24] R.H. Rosa Jr., D. Miller, E.C. Alfonso, The changing spectrum of fungal keratitis in south Florida, Ophthalmology 101 (6) (1994) 1005–1013.
[25] M.A. Tanure, et al., Spectrum of fungal keratitis at wills eye hospital, Philadelphia, Pennsylvania, Cornea 19 (3) (2000) 307–312.
[26] X. Jin, et al., A 5-year retrospective analysis of the risk factors, treatment, and prognosis of patients with fungal keratitis in heilongjiang, China, Am. J. Ophthalmol. 244 (2022) 48–57.
[27] K. Jirsova, G.L.A. Jones, Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review, Cell Tissue Bank. 18 (2) (2017) 193–204.

[28] B.E. Abdulhalim, et al., Amniotic membrane graft to conjunctival flap in treatment of non-viral resistant infectious keratitis: a randomised clinical study, The British journal of ophthalmology 99 (1) (2015) 59–63.
[29] A.M. Alino, et al., Conjunctival flaps, Ophthalmology 105 (6) (1998) 1120–1123.
[30] L. Xie, et al., Lamellar keratoplasty for the treatment of fungal keratitis, Cornea 21 (1) (2002) 33–37.
[31] L. Xie, et al., Hyphal growth patterns and recurrence of fungal keratitis after lamellar keratoplasty, Ophthalmology 115 (6) (2008) 983–987.
[32] W.J. Reinhart, et al., Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the american academy of ophthalmology, Ophthalmology 118 (1) (2011) 209–218.
[33] Y.Y. Cheng, et al., Endothelial cell loss and visual outcome of deep anterior lamellar keratoplasty versus penetrating keratoplasty: a randomized multicenter clinical trial, Ophthalmology 118 (2) (2011) 302–309.
[34] D. Hos, et al., Immune reactions after modern lamellar (DALK, DSAEK, DMEK) versus conventional penetrating corneal transplantation, Prog. Retin. Eye Res. 73 (2019), 100768.
[35] J. Li, et al., Deep anterior lamellar keratoplasty using acellular corneal tissue for prevention of allograft rejection in high-risk corneas, Am. J. Ophthalmol. 152 (5) (2011) 762–770 e3.