A Case of Recurrent Fungal Keratitis Post-Amniotic Membrane Transplantation for Corneal Perforation

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Abstract
Here, we report a rare case of recurrent fungal keratitis (FK) post-amniotic membrane transplantation (AMT) for corneal perforation. A 75-year-old female who had undergone systemic 15 mg prednisolone administration for interstitial pneumonia developed FK in her left eye following treatment for herpetic epithelial keratitis at another clinic. The FK was effectively cured via an oral and local antifungal treatment. However, 1 year later, FK recurred in her left eye, and she was subsequently referred to our hospital since fungal infection had penetrated deep into the cornea. Upon examination, her best-corrected visual acuity was 20/20 OD and hand motion OS. Slit-lamp examination revealed infiltration of corneal ulcers and posterior corneal deposits in her left eye, so she was treated with 0.1% miconazole eye drops in addition to oral miconazole and 1% pimaricin ointment. However, corneal perforation occurred 1 week later, so debridement and AMT were performed, which resulted in a successful outcome. At the 4-month postoperative period, the antifungal eye-drop treatment was discontinued due to no clinical signs of infection with scar formation. However, at the 6-month postoperative period, increased white deposits and the emergence of keratic precipitates were observed around the AMT graft. Recurrent FK was suspected, and anterior-chamber irrigation was performed. Immunostaining revealed a yeast-type fungus, and a cultivation test revealed Candida sp. Thus, penetrating keratoplasty was performed, and there has been no recurrence of FK for 1.5 years. In FK cases, AMT should be carefully considered for surgical indications, with strict follow-up in order to detect any possibility of FK recurrence.
Introduction

The amniotic membrane (AM) is a thin semitransparent membrane covering the uterus and the innermost placenta and has a five-layer structure consisting of the amniotic epithelial tissue and the underlying basement membrane, compact layer, fibroblast layer, and spongy layer. In the field of ophthalmology, AM transplantation (AMT) was first reported in 1940 by de Röth [1], a Hungarian physician who used the novel surgical procedure for conjunctiva reconstruction in patients with symblepharon due to chemical injury. AMT is now widely performed for ocular-surface reconstruction in cases with severe ocular-surface disorders such as Stevens-Johnson syndrome and toxic epidermal necrolysis, ocular cicatricial pemphigoid, chemical burn, recurrent pterygium, corneal perforation, and persistent epithelial defects [2–4].

Previous clinical studies have reported that the AM is useful for the treatment of infectious corneal disease as the amnion can promote wound healing [2] and inhibit inflammatory cell infiltration [5] due to it containing several growth factors. Moreover, it induces the differentiation and proliferation of the corneal and conjunctival epithelium by supplying normal substrate [6].

As an alternative treatment to corneal transplantation for severe ocular-surface disorders, including corneal perforations (i.e., more advanced-stage corneal ulcers), it has been reported that multilayer AMT is effective for reconstruction of the cornea, conjunctiva, and anterior chamber with no recurrence of infection [3, 4]. However, there have been few reports of AMT for corneal perforation caused by fungal infection. Here, we report a case in which recurrent fungal keratitis (FK) was observed after AMT was performed for corneal perforation due to fungal infection.

Case Report/Case Presentation

This study involved a 75-year-old female who was being treated with prednisolone 15 mg daily for interstitial pneumonia and rheumatoid arthritis. She had a history of herpetic and FK in her left eye that was successfully treated with acyclovir eye ointment, natamycin eye ointment, and oral itraconazole. However, she was referred to our hospital due to the fungal corneal infection in her left eye recurring with a deep corneal infiltrate.

At initial presentation, the best-corrected visual acuity in her infected left eye was 30-cm hand motion, and corneal ulcers and infiltration on the temporal side, as well as posterior corneal plaque, were observed. For daily treatment, she was prescribed miconazole 0.1% (every 2 h), gatifloxacin (6 times daily), 1% natamycin ophthalmic ointment (6 times daily), and oral voriconazole (300 mg). Although a reduction in the size and density of the deep corneal infiltration was initially obtained (Fig. 1a), corneal perforation and iris herniation occurred 3 weeks after the initial examination. Moreover, fibrin formation was found around the pupil area and along to the corneal infiltration (Fig. 1b). Since it appeared that a conservative treatment would be difficult, AMT with debridement around the perforated area was performed (Fig. 2). At the 5-month postoperative period, there were no signs of fungal infection, so the natamycin ophthalmic ointment and miconazole were discontinued, and treatment was switched to only a 4-times daily administration of gatifloxacin. However, at the 6-month postoperative period, an increase of keratic precipitates was observed, and adhesion of a pigmentary and white substance was observed on the amnion endothelial surface (Fig. 3b).
We suspected the possibility of recurrent fungal infection, so anterior-chamber irrigation was performed in the 9-month postoperative period. Yeast-like fungi were detected (Fig. 3c) via Fungiflora Y staining of the aqueous humor, and *Candida species* were identified from culture. At the 11-month postoperative period, anterior-segment optical coherence tomography imaging revealed a high intensity of endothelial plaque material beneath the AMT region (Fig. 3e). Intravenous voriconazole and multiple injections of 1 μg/mL voriconazole into the anterior chamber (Fig. 3d) were ineffective, so at 1 year post-AMT, penetrating keratoplasty was performed. Postsurgery, polymerase chain reaction testing showed a positive finding for fungal deposition in the posterior cornea, and immunohistochemistry of a specimen using Fungiflora Y staining revealed fungal deposition throughout the posterior plaque between the transplanted AM and the residual stroma (Fig. 4).

The postoperative course was good, and pimaricin and miconazole were gradually decreased. At 1.5 years post-penetrating keratoplasty, no recurrence of fungal infection was found, and the best-corrected visual acuity in her left eye was 6/60 (Fig. 5).

**Discussion/Conclusion**

Fungi that cause corneal mycoses are roughly classified into 2 groups, filamentous fungi and yeasts [7]. The filamentous fungus is mainly a genus *Fusarium* including *Fusarium solani*, and the onset is often triggered by an eye injury [8]. Infiltrates are characterized by white to off-white
boundary unclear lesions. Exacerbation of infection sometimes leads to pus formation and corneal perforation after substantial melting. On the other hand, most yeast are *Candida* genus, including *Candida albicans*, and they often develop as opportunistic infections in patients with a history of steroids, immunosuppressants, anticancer drugs, malignant tumors, and acquired immunodeficiency syndrome [9, 10].

Infiltration is exhibited as a clear round boundary, often confined to the superficial corneal stroma. In routine clinical practice, FK is only occasionally encountered, so a proper diagnosis is sometimes delayed. In addition, therapeutic response to pharmaceutical intervention can be slow in comparison to bacterial infections, and treatment is often difficult due to the fact that the number of effective drugs is limited. Thus, early diagnosis and treatment is necessary to prevent progression, with proper diagnosis via
fungal staining of a smear (i.e., Fungi Y staining) or an isolated fungal culture using lesion scrapes.

In the field of ophthalmology, commonly used antifungal agents include polyenes, azoles, pyrimidines, and CANDINs, which all must be used properly depending on the pathogen. In addition to the topical and systemic administration of these drugs, focal scraping sometimes needs to be performed in order to reduce the amount of total fungus load, increase the level of eye-drop penetration into the tissue, and improve the overall effectiveness of the treatment. When the focus is localized at the central region of the cornea, corneal transplantation surgery may be considered after antifungal treatment, if necessary [11].

It should be noted that there were several reasons for the recurrence of fungal infection in the case presented in this study. First, transplanted AM tissue is not as transparent as corneal tissue, thus impairing the ability to observe the lesion and delaying detection of the recurrent fungal infection. Moreover, since no signs of infection were observed posttransplantation, the dose of the antifungal drug was tapered from the 4-month postoperative period. However, and as stated above, beginning at the 6-month postoperative period, an increase in the posterior corneal deposition was observed. Thus, we theorize that fungal growth continued in the areas that could not be observed. Although corneal endothelial plaque can be observed in the advanced stage, anterior-segment optical coherence tomography may possibly provide one effective method to detect early recurrence of fungal infection in areas that cannot be observed.
Second, the patient had become immunosuppressed due to long-term oral administration of steroids for chronic interstitial pneumonia and rheumatoid arthritis, and that administration could not be reduced or discontinued throughout the entire period that the fungal infection was being treated. Thus, it could be theorized that the immunosuppression was possibly related to fungal infection. However, our findings suggest that the recurrent fungal deposition observed was not a reinfection as Fungiflora Y staining showed that the cells that stained positive for fungi were mainly found only in the middle and deep layer of the cornea and the posterior corneal plaque, while the anterior side of the cornea was completely intact.

Third, it is possible that the transplanted AM had become a scaffold for fungal infection. According to Fungiflora Y staining of samples obtained in therapeutic corneal transplantation, cells staining positive for fungi are continuously found from the middle to deep layer of the cornea and the posterior corneal plaque at the boundary between the AM graft and the host cornea. However, detailed imaging of the boundary region has suggested that fungal growth may have spread from only the host cornea and not from the AM graft as no cells staining positive for fungi are observed in the AM area.

Previous studies have reported that multilayer AMT combined with antibiotics and antiviral agents is a successful treatment for corneal ulcers and corneal perforation caused by bacterial infection or herpes virus infection [3]. However, the failure of AMT in this present case can possibly be attributed to the characteristics of fungal infection which, inherently, is difficult to cure. In conclusion, the findings in this study indicate that AMT for FK should be carefully considered in relation to surgical indications and that strict follow-up is necessary to avoid the possibility of recurrence.

**Statement of Ethics**

The protocols of this study were approved by the Ethics Committee of Kyoto Prefectural University of Medicine, Kyoto, Japan. In accordance with the tenets set forth in the Declaration of Helsinki, prior written informed consent was obtained from the patient for publication of this case report and any accompanying images.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

Rina Soda combined the data and designed, drafted, and finalized the manuscript. Hideki Fukuoka performed the surgery and conducted the follow-up of the patient. Chie Sotozono critically reviewed the manuscript. All the authors approved the final version of the manuscript and are accountable for all aspects of the work. All the authors attest that they meet the current ICMJE criteria for authorship.
Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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