• Brief Report •

“Pocket” technique: a multiple-layer amniotic membrane transplant approach for managing graft-versus-host disease-associated corneal perforations

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Abstract

● **AIM:** To describe a modified technique using multiple-layer amniotic membrane (AM) to manage corneal perforations in graft-versus-host disease (GVHD).

● **METHODS:** This case series included three eyes from 3 patients with GVHD and corneal perforation who underwent AM transplantation between 2019 and 2020. AM was collected from human placenta shortly after the cesarean section. During surgery, the AM was folded and sutured into the wound to create an “AM pocket” into which small AM debris was placed. The pocket was then closed using a 10-0 nylon suture and covered with an AM seal. After re-epithelization and AM scarring, sutures were removed.

● **RESULTS:** All 3 patients achieved successful wound closure with a single surgery, and corneal thickness remained >300 μm at the 6-month follow-up. Visual acuity improved in patients with peripheral perforation.

● **CONCLUSION:** The “Pocket” AM transplantation technique is an effective option and recommend for treating acute corneal perforations in patients with GVHD. Our modified AM transplantation method provided definitive wound closure and successfully repaired wounds of various sizes without recurrent corneal melting or perforations.

● **KEYWORDS:** amniotic membrane; graft-versus-host disease; corneal perforation

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the standard treatment for various hematologic malignancies[1]. Graft-versus-host disease (GVHD) is one of the major complications of allo-HSCT, occurring in 30%–70% of patients[2]. At the onset of GVHD, many patients present with inflammatory skin rash, oral sensitivity, dryness, or dry eyes. Other manifestations include skin sclerosis or fasciitis, bronchiolitis obliterans syndrome, oral ulcers unresponsive to local therapies, serositis, and gastrointestinal involvement, which are less common but significantly more challenging to manage[3].

Ocular manifestations affect a substantial number of patients following allogeneic stem cell transplantation, with reported incidences as high as 60%–90% in patients with systemic manifestation[4-5]. Dry eye disease is the most common manifestation of chronic ocular GVHD. In severe cases, persistent epithelial defects can lead to corneal stromal melting and subsequent perforation.

Several approaches exist for treating corneal perforations, including bandage soft contact lenses (SCL), tissue adhesive glue, amniotic membrane transplantation (AMT), and corneal transplantation[6-7]. However, bandage contact lenses and tissue adhesive glue are often ineffective in treating GVHD-associated corneal perforations. Peris-Martínez *et al*[8] successfully treated small perforations using multilayer AMT in severe ocular GVHD. However, multilayer AMT may only provide short-term effectiveness due to the low graft thickness and severe dry eye disease of GVHD[9]. Corneal transplantation is considered a definitive treatment for corneal perforation; however, it is classified as high-risk in ocular GVHD because of intense ocular surface inflammation and dryness, which can result in persistent epithelial defects and recurrent perforations[7].

Table 1 Patients' characteristics

Characteristics	Case 1	Case 2	Case 3
Age/sex, y	63/male	57/male	55/male
Underlying disease	AML	MDS	AML
Time of HSCT	Jun. 2016	Aug. 2018	Mar. 2001
Transplant type	PBSCT	PBSCT	BMT
GVHD manifestations	Skin, eyes	Skin, oral, eyes	Skin, eyes
Months from HSCT to ocular GVHD diagnosis	36	5	12
Months from HSCT to perforation	42	24	234
Site of perforation	Center	Periphery	Center
Major axis of wound, mm	1.0	0.5	1.0
Visual acuity at presentation	OD: CF	OD: 20/2000	OD: CF
Visual acuity at last follow-up visit	OD: CF	OD: 20/32	OD: CF
Stromal thickness at last follow-up visit, μm	345	465	330

HSCT: Hematopoietic stem cell transplantation; GVHD: Graft-versus-host disease; AML: Acute myeloid leukemia; MDS: Myelodysplastic syndromes; PBSCT: Peripheral blood stem cell transplantation; BMT: Bone marrow transplantation; OD: Right eye; CF: Counting fingers.

In recent years, several novel multilayer AMT techniques have been reported, that could restore corneal stromal thickness after corneal perforation, such as Chan *et al*'s[10] "Swiss roll" technique and Namba *et al*'s[11] "Pleats fold" technique. In this case series, we present our experience with AMT for the treatment of GVHD corneal perforations to achieve more corneal stromal thickness and prevent recurrent perforation. All surgeries were performed using our modified multilayer amniotic membrane (AM) "Pocket" technique.

PARTICIPANTS AND METHODS

Ethical Approval This study were approved by the Medical Ethics Committee of Peking University People's Hospital (2026PHB186-001). All studies adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients before surgery.

Participants This case series included consecutive patients with corneal perforation who underwent AMT at the Department of Ophthalmology, Peking University People's Hospital, between 2019 and 2020. All patients were diagnosed with ocular GVHD, had corneal perforation, and were unable to maintain anterior chamber depth. Patients with infectious corneal ulcers were excluded.

Preparation of Human AM AM tissue was obtained from human placenta following cesarean section. The donor mother tested negative for hepatitis B and C, human immunodeficiency virus, and syphilis. After extraction, the AM was separated from the placenta using blunt dissection and thoroughly rinsed with 500 mL of sterile physiological saline. The tissue was then immersed in a solution containing 100 U/mL penicillin and 100 μg/mL streptomycin for 5min, followed by rinsing with 500 mL of sterile physiological saline. The AM was then cut into 3 cm×3 cm pieces, which were individually placed in sterile vials containing glycerol in Dulbecco's modified Eagle's medium at a 1:1 ratio[12]. The vials were then frozen at -80°C. Before surgery, the membrane was thawed at room temperature.

Surgical Procedure All surgeries were performed by a single surgeon (Zhang Q) under retrobulbar anesthesia. At the site of the corneal perforation, a 10-0 nylon needle was inserted from the outside to the inside at 90% depth. The suture was then threaded through the AM to create pleats and stitched from the inside to outside as per Namba *et al*'s[11] "Pleats fold" technique (Figure 1A, 1B). The second stitch was inserted at a 70% depth perpendicular to the first one and threaded through the AM to form a second "pleats fold" above the first one (Figure 1C). As a result, the two layers of folded AM form a small pocket-like structure.

Four interrupted 10-0 nylon sutures were placed across the base at 90% depth to seal the "Pocket". Small pieces of AM were packed between the two-layer pocket to increase the thickness of the AM patch, and the sutures were ligated (Figure 1D). Finally, the AM patch was transplanted and stitched with 10-0 nylon sutures to serve as a basement membrane on the surface of the patched AM. The "Pocket" technique is inspired by Namba *et al*'s[11] "Pleat fold" technique; it not only seals the perforation but also increases the thickness of the AM tissue plugged into the perforation, which would restore more corneal stromal thickness. After corneal re-epithelization, the 4 interrupted sutures were removed. Approximately 1mo after surgery, the 2 AM plug sutures were removed.

RESULTS

Three eyes from 3 patients underwent the "Pocket" AMT technique, achieving a 100% success rate over a two-year period. The clinical presentations, treatments, and outcomes of the 3 patients are summarized in Table 1.

Patient 1 A 63-year-old male was referred to our clinic for decreased vision and ocular dryness in the right eye. The

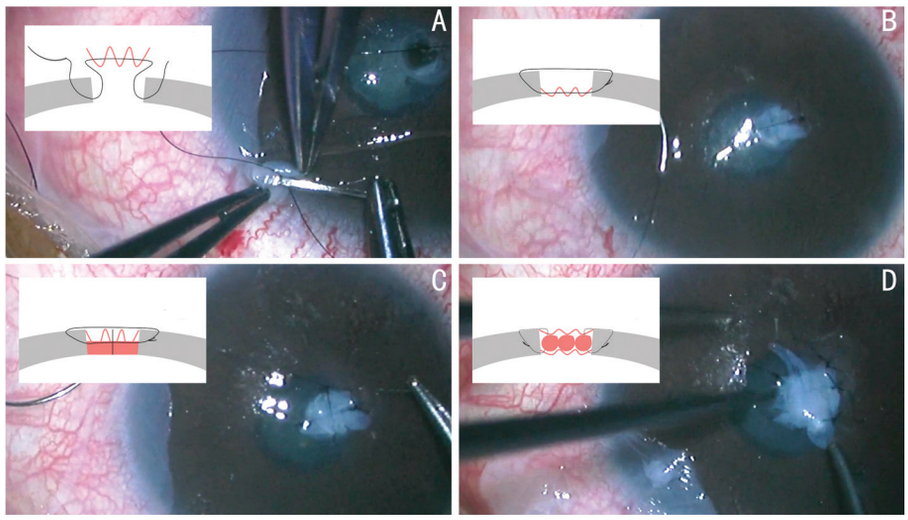

Figure 1 Intraoperative photographs with cross-sectional schematic insets A: The needle entered to 90% depth using an outside-to-inside 10-0 nylon needle suture, then threaded through amniotic membrane (AM) to make pleats and stitched from the inside to outside as Namba *et al*'s[11] “Pleats fold” technique; B: The suture was tied; C: The second stitch entered to 70% depth the perpendicular to the first one and threaded through AM to form a second “Pleats fold” above the first one; D: Four interrupted 10-0 nylon sutures were placed across the base at 90% depth to seal the “Pocket”, small pieces of AM were packed between the two-layer pocket.

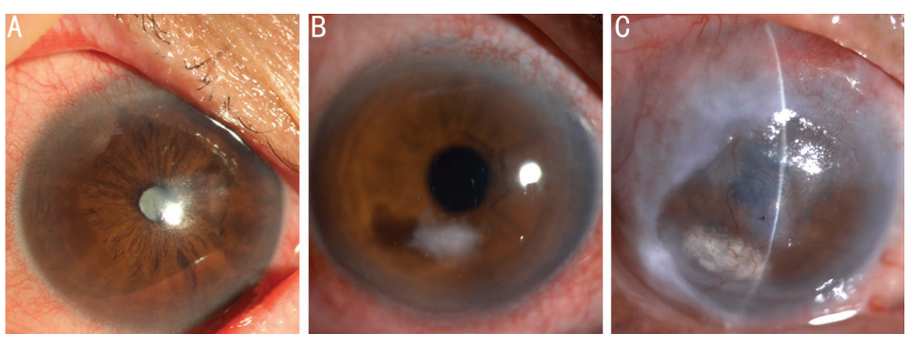

Figure 2 Slit-lamp photographs of follow-up visit A: Patient 1 at 12-month follow-up visit; B: Patient 2 at last follow-up visit; C: Patient 3 at 6-month follow-up visit.

patient’s medical history was significant for acute myelocytic leukemia, which necessitated allogeneic HSCT and resulted in ocular GVHD. Ocular examination revealed a 4.0 mm×2.0 mm central corneal ulcer with stromal thinning in the right eye. The affected eye was treated with preservative-free artificial tear drops, prophylactic antibiotics, corticosteroids, and AM. However, the corneal ulceration perforated 10d later, and ocular examination revealed a micro 1.0 mm×1.0 mm central corneal perforation. Consequently, a “Pocket” AMT was performed on the right eye. The patient received preservative-free artificial tear drops, topical corticosteroids, and topical tacrolimus after the surgery. On examination 24h later, we found that the cornea had sealed, and the anterior chamber had deepened, as indicated by a negative Seidel test. At the 12-month follow-up visit, his best corrected visual acuity (BCVA) was counting fingers (CF) in his right eye with an intumescent cataract, and the stromal thickness of the perforation area was 345 μm (Figure 2A).

Patient 2 A 57-year-old male patient with GVHD presented with corneal perforation in the right eye. The patient had been diagnosed with myelodysplastic syndrome 24mo prior and subsequently underwent allogeneic peripheral blood stem cell transplantation. Ten months after the transplant, the patient developed a burning sensation and a foreign body sensation in the right eye and was diagnosed with ocular GVHD. The patient was treated with prednisone to control GVHD and artificial tear drops to alleviate the ocular symptoms. Three days before the referral to our clinic, the patient developed a corneal perforation in the right eye. Ocular examination revealed an inferocentral corneal ulcer and perforation plugged by iris tissue in the patient’s right eye. The anterior chamber was shallow, and the Seidel test result was positive. The patient received “Pocket” AMT in the right eye. The patient’s right eye underwent cataract surgery four months later. The corneal perforation healed successfully with stable stromal integrity and visual improvement during follow-up (Table 1, Figure 2B).

Patient 3 A 55-year-old male patient with a history of ocular GVHD was referred to our clinic for decreased vision and

ocular discomfort. The patient had developed acute myeloid leukemia and had undergone allogeneic bone marrow transplantation 19y earlier. One year after the procedure, the patient was diagnosed with ocular GVHD in both eyes and presented with corneal perforation in the left eye due to ocular GVHD and enucleation of the left eye 18y prior. The patient visited our clinic due to progressive ocular discomfort for a month. On initial examination, the vision acuity of the right eye was CF. The right eye showed severe symblepharon, conjunctivalization of the superior cornea, stromal neovascularization, and lipid keratopathy of temporal cornea. The central cornea was markedly inflamed, with a large epithelial defect. AM patching and tarsorrhaphy were performed on the right eye, and topical corticosteroids, artificial tear drops, and ointment were applied; however, the corneal epithelial defect persisted. Despite repeated tarsorrhaphy and AM patching, the cornea continued to melt, and corneal perforation occurred 2wk later. An emergency "Pocket" AMT was performed. The patient was treated postoperatively with topical corticosteroids, tacrolimus, and artificial tear drops. The corneal perforation and ulcer healed with 330 μm stromal thickness. At the 6-month follow-up visit, the patient's BCVA was CF in the right eye (Figure 2C).

DISCUSSION

GVHD results from a highly complex immune process involving donor T-cell responses to host antigens and dysregulation of pro-inflammatory cytokines. This process leads to immune-mediated inflammation and fibrosis of target tissues and organs[13]. A previous study showed that half of the patients developed ocular GVHD 5y after transplantation[14]. Corneal perforation occurs in 0.7%–4.9% of patient with GVHD[1,14]. The surgical management of corneal perforations includes corneal gluing, conjunctival flaps, AMT, Tenon's patch graft, and corneal transplantation[15].

Peris-Martínez *et al*[8] reported the use of multilayer AMT to seal corneal perforations in severe GVHD. However, corneal perforations may recur following AM resorption and require tectonic keratoplasty[7]. Nevertheless, corneal transplantation in patients with ocular GVHD is considered high-risk because of the dryness and intense inflammation of the ocular surface[5].

Successful treatment of GVHD corneal perforation requires not only secure wound closure but also sufficient stromal thickness to prevent further recurrence. Multilayered AMT is a widely used technique to seal corneal perforation[16-18]. AM contains growth factors, including hepatocyte growth factor, keratocyte growth factor, and epidermal growth factor, which promote epithelial differentiation and migration across the basement membrane and provide a scaffold for stromal repair[15]. AMs have anti-inflammatory and antifibrotic properties[10]. It is non-immunogenic and devoid of human leukocyte antigens, making it a suitable material for ocular surgery with GVHD. Successful AMT wound closure for GVHD perforations requires a secure and tight donor plug fit and sufficient plug thickness to achieve a relatively smooth surface for epithelization. To achieve this, we modified Namba *et al*'s[11] "Pleats fold" AMT technique by adding perpendicular second "Pleats fold" suture to achieve a "Pocket" and packing small pieces of AM debris between the two-layer AM "Pocket", achieving a 100% success rate for GVHD corneal perforation. Compared to Chan *et al*'s[10] "Swiss roll" technique and Namba *et al*'s[11] "Pleats fold" technique, we use cross-pleats folds or "X" pleats folds to form a "Pocket", similar to corneal transplantation suturing. Additional pleat fold suturing can effectively close the perforation. Compared to Namba *et al*'s[11] "Pleats fold" technique, "Pocket" technique can increase the thickness of the AM plug by adjusting the amount of AM debris packed between the two-layer AM "Pocket" resulting in multiple AM layers, which provides more corneal thickness after AM absorption. This technique can also be used to obtain smooth corneal surfaces for epithelial differentiation and migration. Additionally, the downward pressure applied by the multidirectional sutures makes the amniotic tissue denser and firmer. In this case series, no patient required additional surgery during the follow-up period. We believe that our "Pocket" technique is sufficient to heal the perforation and provide tectonic integrity. It is also effective against other non-infectious diseases, such as rheumatoid arthritis and Steven-Johnson syndrome. The sample size was relatively small because GVHD-associated corneal perforation is a rare but severe clinical condition. However, as a preliminary case series introducing a novel surgical technique, the presented cases were sufficient to demonstrate the feasibility and potential clinical value of the "Pocket" technique.

Therefore, this technique is suitable for any circular or irregular corneal perforations smaller than 3 mm, with the best indication being peripheral corneal perforations caused by non-infectious diseases. If the central cornea is affected, AMT leaves corneal nebula along the optical axis; however, AMT can temporally seal the perforation and provide sufficient stromal thickness until a proper ocular surface condition for keratoplasty is achieved.

In conclusion, we recommend the "Pocket" technique for AMT for the management of non-infectious corneal perforation, especially in GVHD. This technique ensures wound closure and provides greater stromal thickness.

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