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Novel technique for amniotic membrane transplantation for acute Stevens-Johnson syndrome/toxic epidermal necrolysis patients

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ABSTRACT

Purpose: To report a novel technique to facilitate amniotic membrane transplantation (AMT) for acute stage Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).
Design: Laboratory investigation and retrospective, single-center case series.
Methods: The polylactic acid (PLA) amniotic fornical ring (AFR) have been successfully manufactured by three-dimensional (3D) printing technology for AMT. This study retrospectively analyzed the medical records of 5 SJS/TEN patients at the acute stage between 2019 and 2023. Patients were surgically treated with AFR or sutured amniotic membrane transplant (SAMT). Epidemiology, best-corrected visual acuity (BCVA), acute ocular severity score, operative duration, epithelial healing time, amniotic dissolution and follow-up time were evaluated.
Results: Of all five patients, three patients (6 eyes) received AFR/AMT (Group A), and 2 patients (4 eyes) received SAMT (Group B). There were no significant differences between two groups in the mean preoperative days and vision changes. The mean operation duration was 11.7 ± 3.8 mins in group A. Compared with the SAMT (48.8 ± 5.3 mins), the operation duration was reduced by 76.02%. The mean times for epithelial healing were 32.5 ± 29.2 days in group A and 12.0 ± 0.0 days in group B. In addition, there were no significant side effects of 3D-printed sterile AFR on the eyes.
Conclusions: 3D-printed PLA scaffolds could be used as an AFR device for acute SJS/TEN. In addition, personalized 3D-printed AFR is superior to conventional SAMT in operation duration.

1. Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) can cause severe scarring of the ocular surface, dry eye and visual impairment [1]. The use of amniotic membranes (AM) in the acute phase of SJS/TEN can minimize the development of late complications [2]. AMT is becoming the gold standard for the management of acute SJS and TEN [3]. To facilitate the repair of ocular surface and eyelid damage, both the entire ocular surface and the palpebral margin must be covered by AM. However, acute tissue edema leads to difficult exposure of the conjunctival fornix, which increases the difficulty and duration of AMT. In addition, the

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ProKera device only covers the corneolimbal region. ProKera cannot prevent cicatricial formation in the fornix, tarsus, and eyelid margin [4]. Therefore, we need to discover new methods to individually, rapidly and effectively cover the entire ocular surface and the palpebral margin using the AM.

At present, 3D printing technology has shown great potential in fabricating personalized scaffolds [5]. Fused deposition modeling has become one of the most popular 3D printing technologies and has several advantages, including wide material sources, low cost and customized high precision. As a biomedical material, polylactic acid (PLA) has been approved by the FDA because of its excellent biocompatibility [6]. In this study, for acute SJS/TEN patients who need ocular surface reconstruction, we used 3D printing technology to fabricate individualized amniotic fornical rings (AFRs) according to the size of the conjunctival sac measured.

This is a new technique compared with previously described techniques. We present a detailed description of this novel technique using a cryopreserved AM and a personalized 3D printing AFR in AMT for patients with acute SJS and TEN. Then, we report a clinical evaluation of the use of AFR in patients with SJS and TEN.

2. Patients and methods

This work was conducted in accordance with the Declaration of Helsinki. The research consisted of a laboratory investigation, retrospective and single-center case series. The study was approved by the Ethics Committee of Henan Eye Hospital (ID: HNEEC-2023 (01)). The clinical and imaging data presented in this work were obtained with the informed consent of patients or guardians.

The patients in this study developed clinical symptoms after exposure to drugs or infection. Diagnosis of SJS/TEN based on typical clinical symptoms after durg use or infection. The causal relationship between the above factors and SJS/TEN was probable according to the objective causality assessment by the Naranjo probability scale and 'probable/likely' as per the WHO-Uppsala Monitoring Center criteria [7]. Patients were classified as having SJS, SJS-TEN overlap, or TEN based on the percentage of total body surface area (TBSA) with epidermal detachment (SJS: <10% TBSA; SJS-TEN overlap: 10–30% TBSA; TEN: >30% TBSA) [8,9]. According to the previous description, the acute phase of SJS/TEN was within 2 weeks of onset of symptoms [10]. Acute ocular involvement was retrospectively graded according to clinical evaluation for each eye (Table 1) [11]. Patients with acute SJS or TEN who underwent AMT with AFR (group A) or sutured AMT (SAMT, group B) between January 2019 and January 2023 in Henan Eye Hospital were enrolled. Every patient signed an informed consent form. The evaluation parameters included patient age, sex, symptoms and etiology, corneal and conjunctival epithelial healing, operation duration, amniotic dissolution and follow-up time. Visual acuity (VA) and ocular complications were also assessed. The percentage healed corneal surface area (PHCA = (final area - initial area)/initial area × 100%) was calculated at the time of amniotic dissolution [12]. For all patients, levofloxacin eye drops (0.5%), Tacrolimus eye drop (0.1%) and Oxybuprocaine hydrochloride eye drops (0.4%) from Japanese company Santen were used to prevent infection, alleviate inflammation and perform intraoperative local anesthesia, respectively. Both TobraDex eye drops and TobraDex eye ointment from the American company Alcon also used to alleviate inflammation [13]. Eye gel of deproteinized calf blood extract (20%) from Shenyang Xingqi Pharmaceutical Co. and preservative-free sodium hyaluronate eye drops (0.1%) from Germanywere used to promote epithelial repair and to lubricate the ocular surface, respectively.

2.1. Description of the new technology

For this study, we used Autodesk 123D design software to design 3D printing AFR. And the STL files were coverted into gcode format by CURA software (Fig. 1A). PLA filaments (diameter: 1.75 mm) were added to the HORI Z300 PLUS 3D printer (Huitianwei Technology Co., LTD, Beijing, China), melted and extruded through a nozzle for printing. The printing speed and the layer height were 60 mm/s and 0.2 mm, respectively. AFR's thickness is 0.8 mm. And the type of AFR is determined by measuring the size of patients' conjunctiva sac. The AFR was printed and then sterilized by ethylene oxide prior to surgical use (Fig. 1B and C). Please refer to the website for 3D printing video (Video 1). The cut off part of the AFR is used to discharge the large amount of secretion form the conjunctiva sac in acute stage of SJS/TEN (Fig. 1D). The intact AFR had no obvious deformation under 500 g force. When a notch was cut off, the ring had better elasticity, which made it easier to insert into the conjunctival sac. Different weights can lead to a certain shape change of the ring with a notch, especially at 500 g (Fig. 1E).

First, the size of the conjunctival sac is determined by the distance between the superior and inferior orbital rims and select the appropriate diameter of the AFR as described previously [14]. Next, the operation was performed under local anesthesia with Oxybuprocaine hydrochloride eye drops. Depending on the patient's degree of inflammatory exudation, the AFR was trimmed into an incision during the operation to facilitate the expulsion of exudation in the acute phase (Fig. 2A, yellow arrow). The cryopreserved AM (40 × 60 mm) was unfolded (Fig. 2A). Then, the AFR was enclosed in the AM to form the AFR-AM complex. The upper and lower eyelids were gently pulled, the AFR-AM complex was inserted into the conjunctival fornix, and the entire ocular surface and the

Table 1
Acute ocular severity score.

Ocular manifestations	Severity	Scoring
No involvement	None	0
Conjunctival hyperemia	Mild	1
Ocular surface epithelial defect or pseudomembranous formation	Severe	2
Ocular surface epithelial defect and pseudomembranous formation	Very severe	3

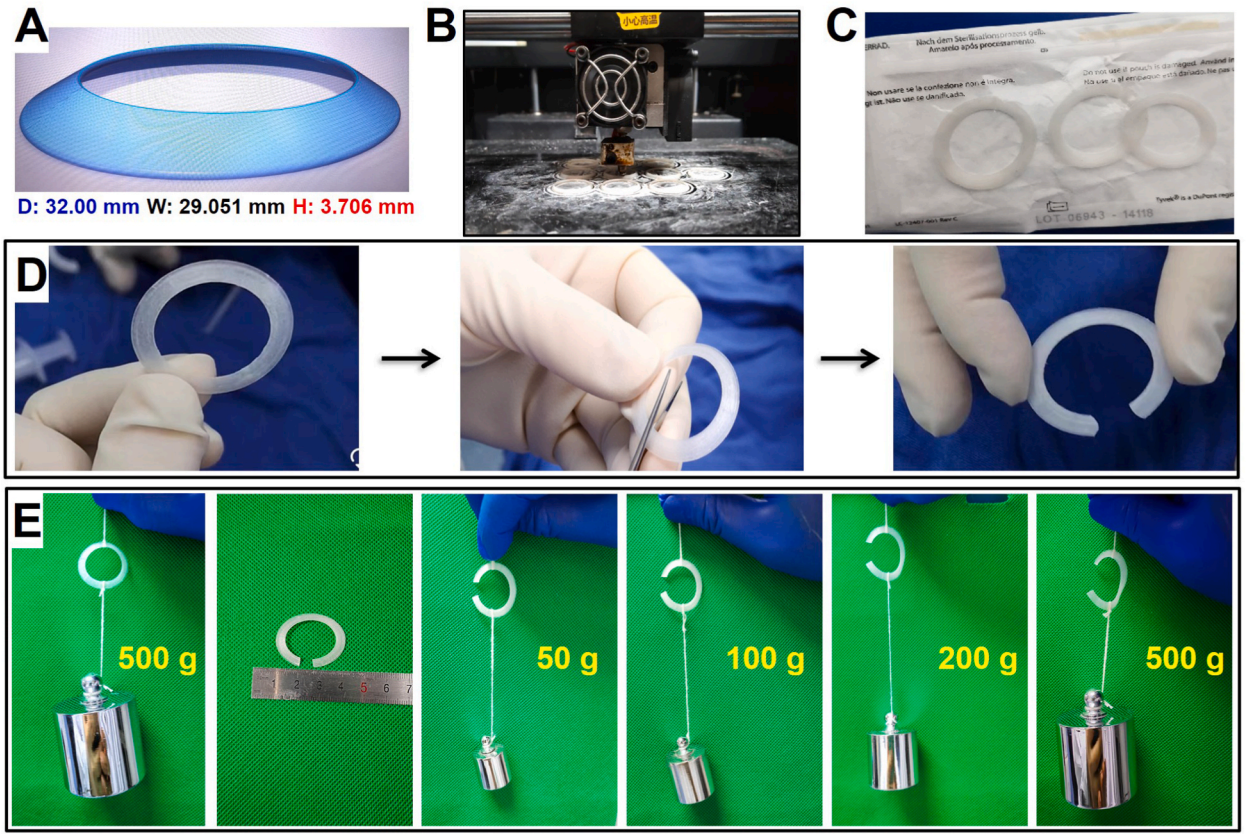

Fig. 1. Design, fabrication and mechanical properties of 3D-printed amniotic fornical ring (AFR). (A) Computer design drawing. (B) Printing process. (C) Ethylene oxide-sterilized AFR. (D) Manufacturing process of the lower notch of AFR during operation. (E) Mechanical properties of AFR.

palpebral margin is completely covered by the AM or secure it to the anterior eyelid when necessary. Finally, gently pull down the lower eyelid to remove the AFR when the AM dissolved. Video 2 (Supplemental Material, available at Heliyon.com) shows the method of the AFR application.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18853

2.2. Statistical analysis

All statistical analyses were performed using SPSS version 25.0 statistics software. The statistically results of group A and B are compared by the mean ± SD or median with interquartile range. The chi-square test or Fisher's exact test was used for qualitative data. Independent t tests or Mann-Whitney U tests were used for quantitative data. A P value of <0.05 was considered statistically significant.

3. Results

From January 2019 to January 2023, 10 eyes of 5 patients (9–48 years old) were enrolled in our retrospective study, and the eye conditions included 3 SJS and 2 TEN. A summary of the enrolled patient data is shown in Table 2. Three patients (2 SJS and 1 TEN) underwent AFR-assisted AMT as group A, and two patients (1 SJS and 1 TEN) received sutured AMT as group B (Table 3). Data from 3 patients' (2 female and 1 male) 6 eyes were collected in group A. 2 patients' (1 female and 1 male) 4 eyes were analyzed in group B. The average age was 23.0 ± 21.7 years in group A and 16.5 ± 0.71 years in group B ($p = 0.564$). The patients' ages of 2 group had no significant difference. The mean follow-up times were 13.0 ± 4.6 months and 2.0 ± 0.0 months. The sample size was relatively small. The mean initial VA, final VA and VA improvement (LogMAR) were 1.50 ± 0.77, 0.10 ± 0.13 and 1.40 ± 0.82 in group A, respectively. The mean initial VA, final VA and VA improvement (LogMAR) were 1.60 ± 0.81 ($p = 0.374$), 0.00 ± 0.00 ($p = 0.248$) in group A and 0.90 ± 0.00 ($p = 0.492$) in group B with no significant difference. The mean preoperative days of group A and group B were 5.7 ± 2.1 days and 7.0 ± 1.4 days, respectively. The mean operation duration was 11.7 ± 3.8 mins in group A and 48.8 ± 5.3 mins in group B. Fig. 2A shows that the AM still covered the ocular surface and eyelid margin 3 weeks after surgery in patients with SJS. The red arrow shows the 3D-printed AFR in the conjunctival sac. We found that the mean times for AM dissolution and epithelial healing were 14.7 ± 7.3 days and 32.5 ± 29.2 days in group A and 12.0 ± 0.0 days and 12.0 ± 0.0 days in group B, respectively. The average PHCA was

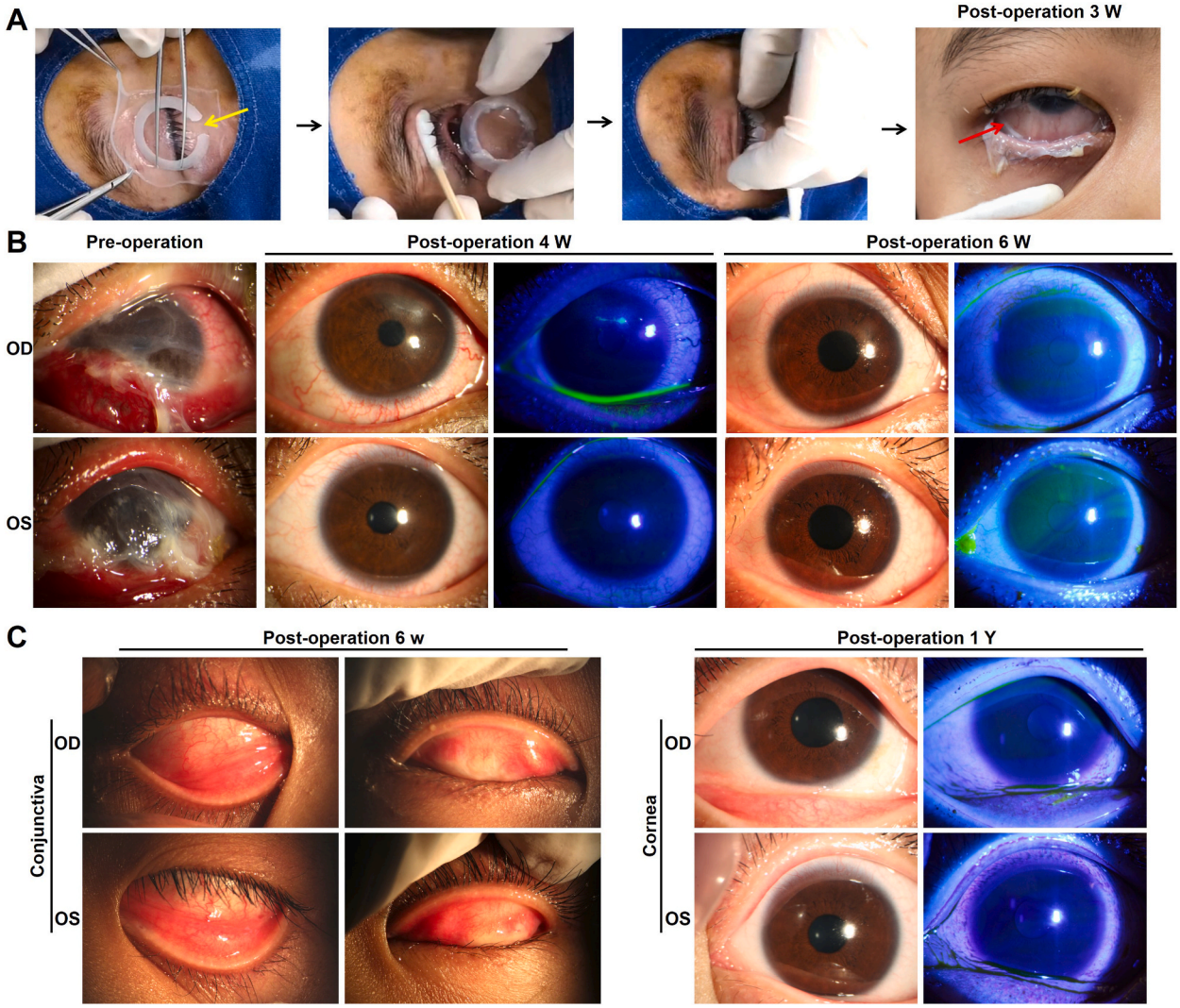

Fig. 2. (A) A procedure of amniotic membrane transplantation (AMT) with a 3D-printed amniotic fornical ring (AFR). (B, C) The long-term follow-up of an SJS patient with an AFR.

89.92% ± 14.62% in group A and 100.00% ± 0.00% in group B. The average time of dissolution was longer than that of intravenous fornical rings reported in the literature [14]. We found that every patient developed various degrees of meibomian gland dysfunction (MGD) in the early postoperative period. Meibomian gland function gradually recovered in three SJS patients. Temporal symblepharon and trichiasis developed in the patients with TEN in group A. One patient with TEN died of a secondary skin infection in group B. Herein, we provided a dynamic follow-up of the ocular surface in an SJS patient (Fig. 2B and C). There was plenty of exudation in the conjunctival sac before the operation. 4 weeks after the operation, the AM dissolved in two eyes. The corneal epithelium of the left eye was repaired. And as for the right eye, there was still a small patch of corneal epithelial defect. Repair of the corneal epithelium of both eyes was almost complete 6 weeks after the operation. There was mild MGD and conjunctival congestion and a small amount of scar formation in the upper eyelid conjunctiva in both eyes. One year after the operation, ocular surface inflammation was significantly reduced, meibomian gland secretion was clear, and meibomian gland function was recovered. All patients could tolerate the device during therapy. There was no spontaneous shedding of the AFR or presentation of an associated infection.

4. Discussion

We demonstrated a novel AMT technique utilizing a 3D-printed AFR in acute SJS and TEN. We believe this technique has significant advantages over previously reported methods [14]. The 3D-printed AFR was customized for each patient before the operation. The 3D-printed AFR saved much operation time compared with the fornical ring made during the operation [14]. Compared with the fornical ring made from intravenous (IV) tubing for AMT in ocular SJS (84 ± 22 mins), the operation duration was reduced by 86.07%

Table 2
Clinical data of five patients (10 eyes) with acute Stevens-Johnson syndrome or toxic epidermal necrolysis.

	AFR			SAMT	
Case	1	2	3	4	5
Diagnose (Presumed Etiology)	SJS (Lamotrigine)	SJS (Mycoplasma pneumonia)	TEN (Allopurinol)	SJS (Acetaminophen and Ibuprofen)	TEN (Penicillin)
Gender	Female	Female	Male	Male	Female
Age (years)	12	9	48	17	16
Preoperative days	4	8	5	6	8
Acute ocular severity score	3	3	3	3	3
Follow-up time (Mons)	12	18	9	2.0	/
Initial VA (LogMAR) (OD/OS)	0.5/0.5	2.0/2.0	2.0/2.0	0.9/0.9	2.3/2.3
Final VA (LogMAR) (OD/OS)	0.2/0.1	0.0/0.0	0.0/0.3	0.0/0.0	/
VA improvement (LogMAR)	0.3/0.4	2.0/2.0	2.0/1.7	0.9/0.9	/
Operation duration per eye (mins)	12.5	7.5	15	52.5	45
AM dissolution time (days) (OD/OS)	14/14	25/7	9/17	12/12	/
Epithelial healing time (days) (OD/OS)	14/14	35/21	9/90	12/12	/
The percentage of healed corneal area (PHCA) (OD/OS)	100%/100%	93.64%/82.22%	100%/63.64%	100%/100%	/
Ocular findings on presentation	Corneal and conjunctival epithelium defects, moderate exudation on ocular surface, conjunctival congestion	Lid edema and erythema, complete lid margin staining, large exudation on the ocular surface, corneal and conjunctival epithelium defects, conjunctival congestion	Lid edema and exfoliation, lid margin ulcerations, extensive corneal and conjunctival epithelium defects, large exudation on the ocular surface, conjunctival fornix foreshortening, early symblepharon OS temporally, trichiasis	Corneal and conjunctival epithelium defects, large conjunctival epithelium defects, conjunctival congestion	Lid edema and exfoliation, lid margin ulcerations, extensive corneal and conjunctival epithelium defects, large exudation on the ocular surface
Ocular findings in last follow up	MGD	MGD, mild palpebral conjunctival scarring	MGD, palpebral conjunctival scarring, small symblepharon OS	MGD, severe scarring of palpebral conjunctiva OU	AM was not dissolved at the 1-week reexamination, the patient died later due to infection secondary to systemic epidermal exfoliation

SJS: Stevens-Johnson syndrome, TEN: toxic epidermal necrolysis, AFR: amniotic fornical ring, SAMT: sutured amniotic member transplant, VA: visual acuity, PHCA: the percentage of healed corneal area, MGD: meibomian gland dysfunction, AM: amniotic membrane.

[14]. In addition, compared with traditional sutured AMT, the new technology also drastically reduced the operation time, decreasing exposure to anesthesia. We believe this is achieved by anchoring the membrane to the conjunctival fornix at the beginning. Anchoring enabled the amniotic membrane to spread better on the ocular surface and eyelid skin and margins.

Topical anesthesia and required minimal suturing is another advantage of the technique described here, which decreased intra-operative bleeding and ocular surface inflammation caused by suture. Placing or removing the AFR was less time-consuming because it was a bedside procedure. For all we know, this is the first study to demonstrate the role of a 3D-printed AFR in AMT. Sutureless Prokera, as cryopreserved amniotic fluid, has been widely used to treat several ocular surface disorders, including dry eye [15], bacterial keratitis [16], and chemical injury of the ocular surface [17]. It has also been reported that Prokera could be available for AMT in acute SJS/TEN, which is a bedside procedure under topical anesthesia [3,18,19]. However, the disadvantage of Prokera was that it could not cover the entire ocular surface. It cannot prevent cicatricial complications of the fornical conjunctival and eyelid

Table 3
Comparison of different outcomes of the patients in the 2 groups.

	AFR (Mean ± SD)	SAMT (Mean ± SD)	P Value* (Intergroup comparison)
Age (years)	23.0 ± 21.7	16.5 ± 0.71	0.564
Preoperative days	5.7 ± 2.1	7.0 ± 1.4	0.374
Follow-up time (Mons)	13.0 ± 4.6	2.0 ± 0.0	0.180
Initial VA (LogMAR)	1.50 ± 0.77	1.60 ± 0.81	0.374
Final VA (LogMAR)	0.10 ± 0.13	0.0 ± 0.0	0.248
VA improvement (LogMAR)	1.40 ± 0.82	0.9 ± 0.0	0.492
Operation duration per eye (mins)	11.7 ± 3.8	48.8 ± 5.3	0.009
AM dissolution time (days)	14.7 ± 7.3	12.0 ± 0.0	0.497
Epithelial healing time (days)	32.5 ± 29.2	12.0 ± 0.0	0.042
The percentage of healed corneal area	89.92% ± 14.62%	100% ± 0.0%	0.252

SD: standard deviation, AFR: amniotic fornical ring, SAMT: sutured amniotic member transplant.
*Based on Mann-Whitney test.

margins [5]. Therefore, ProKera cannot replace conventional AMT as the treatment for acute SJS or TEN [12]. Moreover, ProKera is easily dislodged from the eye [3]. In the present study, we did not find spontaneous slip of the AFR. In addition, 3D-printed AFR can assist the amniotic membrane in covering the entire ocular surface and eyelid margin. Therefore, we believe that AFR-assisted AMT has the potential to replace traditional AMT for acute SJS and TEN. However, the small sample size and retrospective nature are the boundedness in this study.

The average time of dissolution was longer than that of intravenous fornical rings reported in the literature [14]. There were no significant differences in amniotic membrane dissolution time between AFR and traditional suture AMT. This may be related to the small sample size. One patient (2 eyes) died 2 weeks after AMT due to severe skin infection in SAMT. Corneal and conjunctival epithelium defects still existed in two eyes of the patient at that time. In the acute phase of SJS, there is usually a large amount of exudation and secretion in the conjunctival sac. However, we found no accumulation of fluid under the amniotic membrane by cutting a small incision under the ring and amnion for drainage. In early postoperative period, only one patient described mild foreign body sensation in the operative eye. And after two days, this discomfort disappeared. These results suggested that 3D-printed AFR for AMT was time-saving, safe and effective in acute SJS and TEN.

In summary, this study suggests that 3D-printed AFR may be an alternative treatment for ocular surface reconstruction in patients with acute SJS and TEN. This technique was simple to perform, and we hope that the technique can be applied not only in acute SJS/TEN, but also to other ocular surface diseases requiring AMT.

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.

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