Outcomes of Cultivated Oral Mucosal Epithelial Transplantation in Eyes With Chronic Stevens-Johnson Syndrome Sequelae

RENU VENUGOPAL, RITU NAGPAL, SUJATA MOHANTY, SEEMA SEN, SEEMA KASHYAP, TUSHAR AGARWAL, PRAFULLA K. MAHARANA, RASIK B. VAJPAYEE, AND NAMRATA SHARMA

• PURPOSE: To study the outcomes of cultivated oral mucosal epithelial transplantation (COMET) in eyes with chronic Stevens-Johnson syndrome (SJS) sequelae.
• DESIGN: Prospective interventional case series.
• METHODS: Forty-five eyes of 41 patients with chronic SJS sequelae were recruited and evaluated from 2013 to 2017 in an institutional setting. All patients underwent COMET, with an aim of fornix reconstruction and visual rehabilitation. Change in corrected distance visual acuity (CDVA), severity scores of various ocular surface parameters, and the occurrence of complications were documented during a follow up period of 2 years. Attainment and maintenance of a stable ocular surface, as assessed by change in the ocular surface severity scores was the primary outcome measure, while change in CDVA was the secondary outcome measure.
• RESULTS: The mean preoperative CDVA was 2.7± 0.5 logMAR, which improved to 1.5± 0.7 logMAR and 1.49± 0.98 postoperatively, at 1- and 2-year follow-up visit. Overall, 82.2% eyes (37/45) had improvement in visual acuity, 13.3% (6/45) experienced no change, whereas 2 eyes (4.4%) had worsening of visual acuity. The total ocular surface severity scores improved from a mean preoperative value of 29.1± 9.7 to 18.7± 7.2 postoperatively, at 2-year follow-up. Two eyes developed persistent epithelial defects, with progression to corneal melting requiring keratoplasty.
• CONCLUSIONS: COMET allows successful and sustained restoration of ocular surface anatomy with functional improvement, in eyes with chronic sequelae of SJS. (Am J Ophthalmol 2021;222:82–91. © 2021 Elsevier Inc. All rights reserved.)

AJO.com Supplemental Material available at AJO.com.
Accepted for publication Aug 10, 2020.

From the Cornea, Cataract & Refractive Surgery Services (R.V., R.N., T.A., P.K.M., N.S.), Stem cell facility (S.M.), and Department of Ocular Pathology (S.S., S.K.), Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India; Vision Eye Institute, Royal Victorian Eye and Ear Hospital, North West Academic Centre (R.B.V.), University of Melbourne, Melbourne, Australia.

Inquiries to Namrata Sharma, Cornea, Cataract & Refractive Surgery Services, Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India; e-mail: namrata.sharma@gmail.com

VISUAL REHABILITATION IN PATIENTS WITH BILATERAL total limbal stem cell deficiency is a challenging task. Limbal stem cell deficiency accompanied with associated corneal vascularization and opacification, conjunctival fibrosis, symblepharon formation, and severe dry eye alter the normal ocular surface homeostasis and visual functioning. Restoration of normal ocular surface anatomy and functional improvement in such eyes, requires a suitable source of stem cells and an appropriate substrate, helping in corneal re-epithelialization.

Use of amniotic membrane transplantation alone[1,2] or in combination with limbal transplantation[3,4] has been seen to successfully promote epithelialization and forniceal reconstruction, allowing restoration of ocular surface anatomy in patients with ocular surface disease. For patients with bilateral ocular surface disease, the available options include cultivation and transplantation of either allogeneic limbal stem cells or autologous epithelial cells on a suitable substrate. Transplantation of stem cells from an allograft comes with a high risk of rejection, requiring the patient to be put on long-term immunosuppressive therapy. Among the available autografts, oral mucosa has been seen to serve as an ideal substitute for corneal epithelial cells. The short turnover time of oral mucosal epithelial cells requires shorter cultures that can be maintained for prolonged periods.[5] Being an autologous tissue, their transplantation obviates the need for long-term immunosuppression. Other advantages include easy availability of these cells and the regenerated cells having a phenotype similar to that of the corneal epithelial cells.[6,7]

Various studies have shown favorable[8–12] and sustained visual results of cultivated oral mucosal epithelial transplantation (COMET) performed in eyes with severe ocular surface disease, even in the presence of corneal scarring.[13] This study presents the results of COMET, performed in 45 eyes with chronic sequelae of Stevens-Johnson syndrome (SJS). To our knowledge, this is the largest single-center study reporting the outcomes of COMET, performed exclusively in eyes with chronic sequelae of SJS.

METHODS

FORTY-FIVE EYES OF 41 PATIENTS WITH SEQUELAE OF chronic SJS, presenting to the cornea clinic of Dr Rajendra

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.08.022

Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi, India, were recruited between 2013 and 2017, evaluated and planned for COMET. A written and informed consent was obtained from all patients after explaining the nature and consequences of surgical procedure. Ethical clearance was obtained from the ethics committee of AIIMS (IESC/T-275/15.06.2013). The study adhered well to the tenets of Declaration of Helsinki.

The COMET was aimed to restore the ocular surface anatomy by reconstructing the conjunctival fornices and cause improvement in visual axis. Parameters that were evaluated pre- and postoperatively included uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), Schirmer test, tear film breakup time (TBUT), ocular surface staining pattern using 1% sodium fluorescein dye, and evaluation of ocular surface severity scores.

A modified grading system was used for calculation of ocular surface severity scores.[14] The ocular features were divided into 3 main components: corneal, conjunctival, and eyelid. The corneal features included conjunctivalization, neovascularization, loss of palisades of Vogt (POV), keratinization, epithelial defect, and opacification. Conjunctival features included hyperemia, keratinization, and symblepharon formation. Eyelid features included mucocutaneous junction involvement, meibomian gland involvement, and punctal involvement. Each feature was assigned a score from 0 to 5. The total severity score was calculated as the sum total of scores of all the features present in that patient. Besides calculating and comparing the total score for each parameter preoperatively and postoperatively, each of the feature was divided into categories based on the score value as less than or equal to ≤3 and >3, except for superficial punctate keratitis/corneal epithelial defect, meibomian gland, and punctal involvement. The number of eyes with a score >3 was evaluated and compared pre-as well as postoperatively. For superficial punctate keratitis/corneal epithelial defect and meibomian gland involvement, a cutoff score of 2 was taken whereas for punctal involvement a cutoff score of 1 was taken.

The surgical procedure was carried out in 2 stages: a first stage involving harvesting of an oral mucosal epithelial biopsy specimen and culturing it over a de-epithelized human amniotic membrane (HAM) and the second stage involving transplantation of cultured oral mucosal epithelial cells over the ocular surface.

The first stage included harvesting of oral mucosal epithelial cells and then cultivating them over a de-epithelialized HAM. A preoperative dental evaluation was scheduled for all patients to evaluate the health of oral mucosa. A 2 × 2-mm sheet of oral mucosal epithelial tissue was harvested under local anesthesia with adequate asepsis. The oral biopsy specimen was transferred to the stem cell laboratory in a sterile vial containing DMEM medium with antibiotics (penicillin, streptomycin, and amphotericin B) but without fetal calf serum. Epithelial cell cultures were prepared using the explant culture technique using a standard protocol, followed at the Stem Cell Facility, AIIMS, New Delhi, India.[15] The specimen was washed with normal saline (5 cycles, each of 5 minutes) containing antibiotics (penicillin, streptomycin, metronidazole, and amphotericin B). The epithelial cell sheet was cut into 1-mm^2 pieces, which were placed on a de-epithelialized HAM and incubated at 37°C with 95% air and 5% carbon dioxide. Growth media containing DMEM and Ham's F12 nutrient media (1:1) along with antibiotics (penicillin, streptomycin, metronidazole, and amphotericin B), EGF (10 ng/mL), insulin (5 μg/mL), and FCS (10% vol/vol) was added after a gap of 1 to 2 hours to allow adherence of explants with the HAM. The oral epithelial cells were allowed to grow over the HAM for a period of 2 weeks, till confluent growth was obtained, as confirmed by phase contrast inverted microscope. Photographs of growth and expansion of epithelial cells were taken under appropriate magnification. For each eye, cultures were prepared in duplicate, to assess for immunohistochemical phenotype.

Cultures obtained were formalin fixed and paraffin embedded to prepare tissue blocks, which were subjected to histopathology and immunohistochemistry. Immunohistochemistry staining was performed using avidin-biotin-peroxidase kit (UltraVision Quanto Detection System HRP DAB kit; Thermo Fisher Scientific, Waltham, Massachusetts, USA) to look for the expression of various markers including cytokeratin 3/12 (CK3/12) and CK19.

The second stage involved transplantation of cultivated oral mucosal epithelial cells over the ocular surface. The surgeries were performed either under a peribulbar block or under general anesthesia. Eyelid sutures were applied in case adequate exposure was not obtained with an eyelid speculum or the presence of extensive symblepharon

TABLE 1. Demographic Features of Patients With Chronic Stevens-Johnson Syndrome Who Underwent Cultivated Oral Mucosal Epithelial Transplantation.

Age at presentation, y, mean (SD)	25.4 ± 11.6
Duration from onset to presentation, y, mean (SD)	10.36 ± 7.77
Male/female	1.6:1
Kuppuswamy Socioeconomic Status Scale: Middle and upper lower class, %	89
Inciting agent, %	
Drugs	64.4
Febrile illness	36.6
Causative drugs, %	
NSAIDs[a]	48.8
Sulpha drugs	15.5

[a]NSAIDs = nonsteroidal anti-inflammatory drugs.

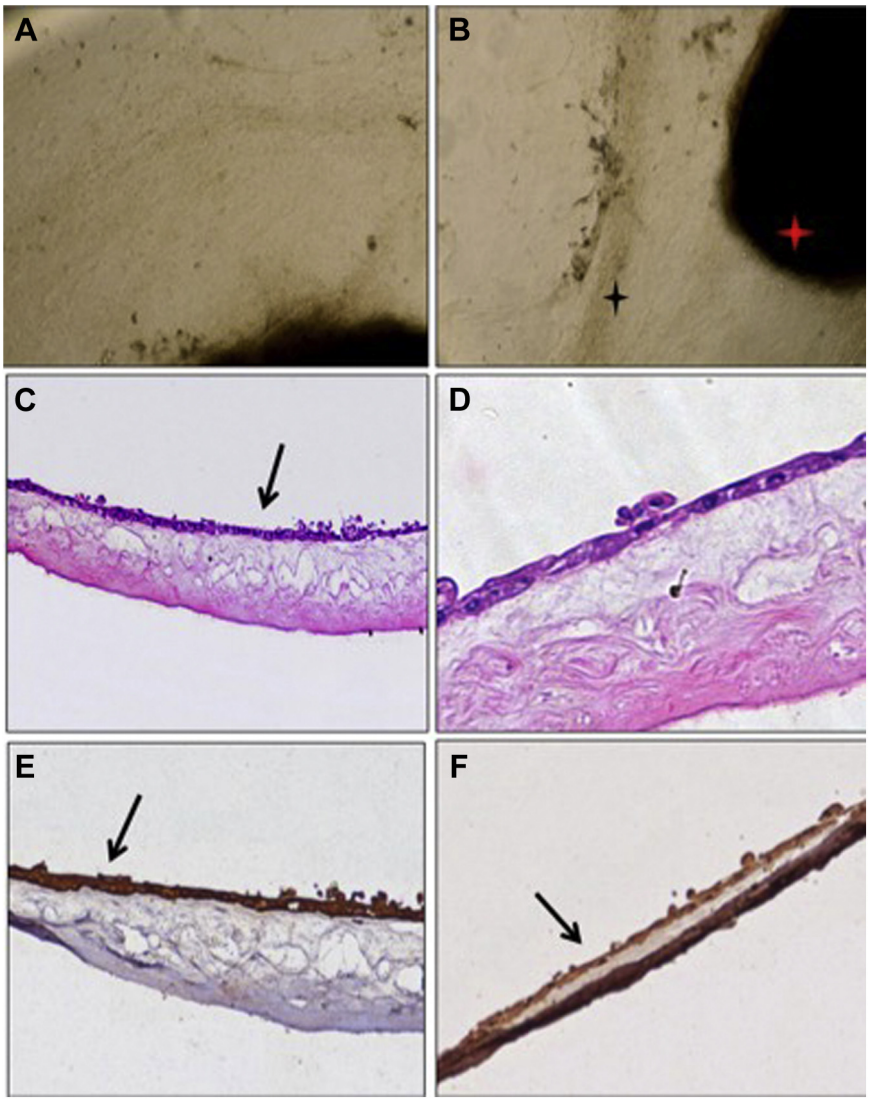

FIGURE 1. Collage showing photographs of cultivated oral mucosal epithelial cell sheet over a de-epithelized human amniotic membrane at day 15, from a patient with chronic Stevens-Johnson syndrome. (A, B). Images showing explant* and the outgrown epithelial layer.* (C, D) Hematoxylin and eosin–stained images of the duplicate culture showing a monolayer of cells over a denuded amniotic membrane at ×100 and at ×200 magnification respectively. (E, F) Immunohistochemistry images suggestive of cytokeratin 3/12 and cytokeratin 19 positivity at ×100 magnification.

precluded the insertion of an eyelid speculum. A 360° peritomy was performed followed by removal of fibrovascular pannus, overlying the corneal surface using blunt dissection. The Tenon capsule underlying the retracted edges of conjunctiva was cut to remove the entire excessive tissue. The oral mucosal epithelial sheet cultivated over the HAM was transferred to the corneal surface and using fibrin glue (TISSEEL kit; Baxter, Deerfield, Illinois, USA) with its edges tucked inside the cut edge of the conjunctiva. A bandage contact lens was then placed over it. Preservative-free 0.5% moxifloxacin hydrochloride ophthalmic solution (Vigamox; Alcon, Fort Worth, Texas, USA) was instilled at the end of surgery in all cases. Postoperatively all patients were advised topical 0.5% moxifloxacin hydrochloride (Vigamox, Alcon, Fort Worth, Texas, USA) 4 times daily, 1% prednisolone phosphate (Predforte; Allergan, Dublin, Ireland) every 2 hours in the immediate postoperative period followed by slow tapering and 0.5% carboxymethyl cellulose drops (Refresh Tears; Allergan) 10 times daily. All patients also received oral prednisolone (Wysolone, Pfizer Limited, New York, United States) starting at a dose of 1 mg/kg body weight followed by tapering.

Statistical analysis was done using Stata software, version 9.0. McNemar test, to evaluate improvement in visual acuity grades and severity scores of ocular surface features. Change in postoperative CDVA and various corneal features was evaluated at various follow up time points

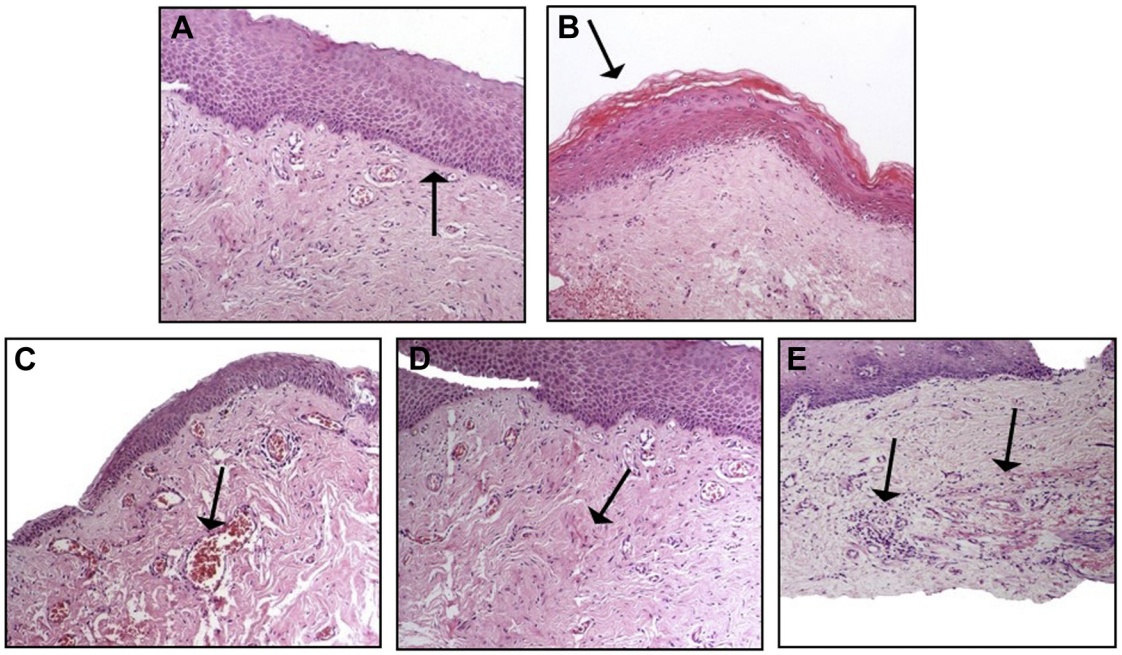

FIGURE 2. Light microscopic images. (A) epidermal hyperproliferation (hyperplasia) (hematoxylin-eosin stain, ×200 magnification). (B) Keratinization of outer pannus epithelium (hematoxylin-eosin stain, ×200 magnification). (C) Stromal vascularization (hematoxylin-eosin stain, ×200 magnification). (D) Stromal fibrosis (hematoxylin-eosin stain, ×40 magnification). (E) Lymphocytic infiltration (hematoxylin-eosin stain, ×200 magnification).

using Friedman's test (skewed data) followed by Wilcoxon's signed value.

RESULTS

• **DEMOGRAPHIC FEATURES:** The study evaluated 45 eyes of 41 patients with chronic ocular SJS, which were planned for COMET (Table 1). Of these, 3 patients (6.7%) underwent bilateral surgery. Mean age of patients was 25.4 ± 11.6 years. There was a male preponderance—28 patients being male and 17 being female. The primary inciting etiology for SJS was found to be drugs (77% eyes). With respect to drugs, the majority had a history of intake of nonsteroidal anti-inflammatory drugs (44.4%), followed by intake of sulfa drugs (13.3%). Mean time from onset of SJS to first presentation was 10.36 ± 7.77 years. Mean preoperative CDVA was 2.68 ± 0.47 logMAR, and the mean preoperative baseline ocular surface severity score was 29.1 ± 9.7. Patients were followed up for a mean duration of 23.1 ± 13.4 months.

• **ORAL MUCOSAL EPITHELIAL CELL CULTURE:** A 90% culture confluency was achieved in 2 weeks' time for all cases. Duplicate cultures of oral mucosal epithelium showed multilayered cells attached to a de-epithelialized amniotic membrane (Figure 1). Immunohistochemistry confirmed the expression of both CK3/12 and CK19 proteins, specific to both corneal and oral mucosal epithelial phenotype (Figure 1).

• **PATHOLOGICAL RESULTS:** The key histologic findings of SJS pannus tissues were found to be epithelial hyperplasia (hyperproliferation of epithelium), epithelial keratinization, and squamous metaplasia in the pannus epithelium. Stromal findings were documented to be chronic neovascularization, inflammation marked by lymphomononuclear infiltration, and fibrosis (Figure 2).

• **VISUAL OUTCOMES:** There was a significant improvement in mean CDVA, from a preoperative value of 2.5 ± 0.5 to 1.54 ± 0.74 logMAR at 12 months and 1.49 ± 0.98 logMAR at the 24-month follow-up (Figure 3). Overall, 82.2% eyes (37/45) had improvement in visual acuity, 13.3% (6/45) experienced no change, whereas 2 eyes (4.4%) had worsening of visual acuity. About 55% eyes showed CDVA improvement to at least 6/60 postoperatively (Figure 3).

The CDVA curve showed gradual improvement postoperatively, with stabilization at 6-month follow-up. A significant change compared to the preoperative value was not observed till the 1-month follow-up, following which the values continued to improve till the 6-month follow-up (Figure 4).

The 6 eyes that stayed same after the surgery continued to develop corneal vascularization and opacification with persistent dryness. Two eyes where the visual acuity

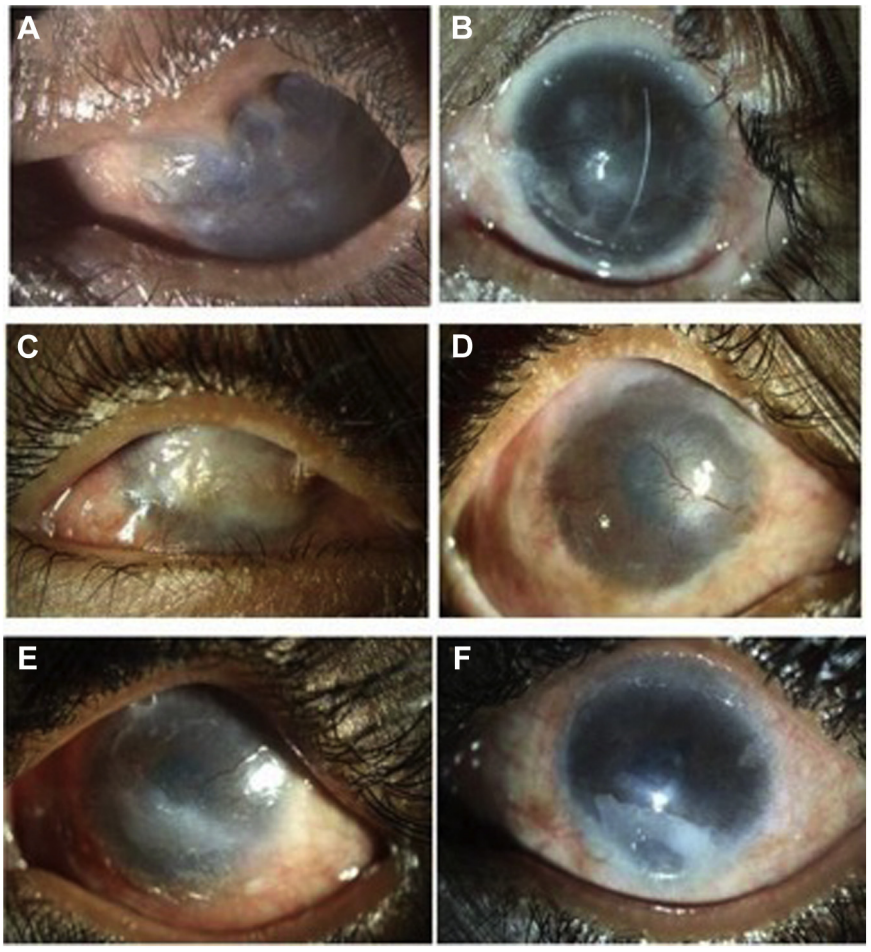

FIGURE 3. Pre- and postoperative slit-lamp photographs of 3 patients who attained a stable ocular surface with accompanied improvement in visual acuity, 2 years following cultivated oral mucosal epithelial transplantation (COMET). (A) Preoperative slit-lamp photograph of the left eye of a 32-year-old man with total limbal stem cell deficiency and conjunctivalization of cornea with superior symblepharon formation. (B) Two-year postoperative photograph of the left eye of the same patient, following COMET showing significant improvement in corneal clarity with restoration of normal ocular surface anatomy. (C) Preoperative slit-lamp photograph of the right eye of a 36-year-old woman with total limbal stem cell deficiency and total corneal opacification with localized symblepharon formation. (D) Two-year postoperative photograph of the right eye of the same patient, following COMET showing significant clearing of the visual axis and widening of the palpebral aperture. (E) Preoperative slit-lamp photograph of the left eye of a 26-year-old woman with total limbal stem cell deficiency with inferior corneal opacification and deep vascularization involving the superior cornea. (F) Two-year postoperative photograph of the left eye of the same patient, following COMET showing significant reduction in corneal haze and vascularization.

worsened developed corneal persistent epithelial defects, progressing to corneal melting in one eye and microbial keratitis in the second one, at around 2 weeks postoperatively, requiring tectonic and therapeutic grafts, respectively (Figure 5).

• **CHANGE IN OCULAR SURFACE PARAMETER SEVERITY SCORES:** The mean total severity scores improved from a preoperative value of 29.1 ± 9.7 to 18.7 ± 7.2 at 2-year follow-up visit (Table 2). All corneal parameters showed decrease in mean severity scores, indicating an overall improvement (Table 2). This was statistically significant for all components except keratinization ($P = .204$) (Table 2). Among eyelid features, only mucocutaneous junction involvement scores showed significant improvement from 2.1 ±1.1 to 1.4 ± 0.8 ($P = .010$) (Table 2). There was an increase in mean scores for meibomian gland involvement from 1.4 ± 0.8 to 1.5 ± 0.8 ($P = .012$), suggestive of worsening. The scores for punctal involvement showed reduction but did not reach a significance level ($P = .503$) (Table 2). Among the conjunctival features, all parameters showed reduction although the values did not significantly reduce for conjunctival hyperemia (1.9 ± 1.7 to 0.4 ± 0.9; $P = .136$) (Table 2).

The number of eyes in the higher score category (cutoff value of 2 for superficial punctate keratitis/corneal

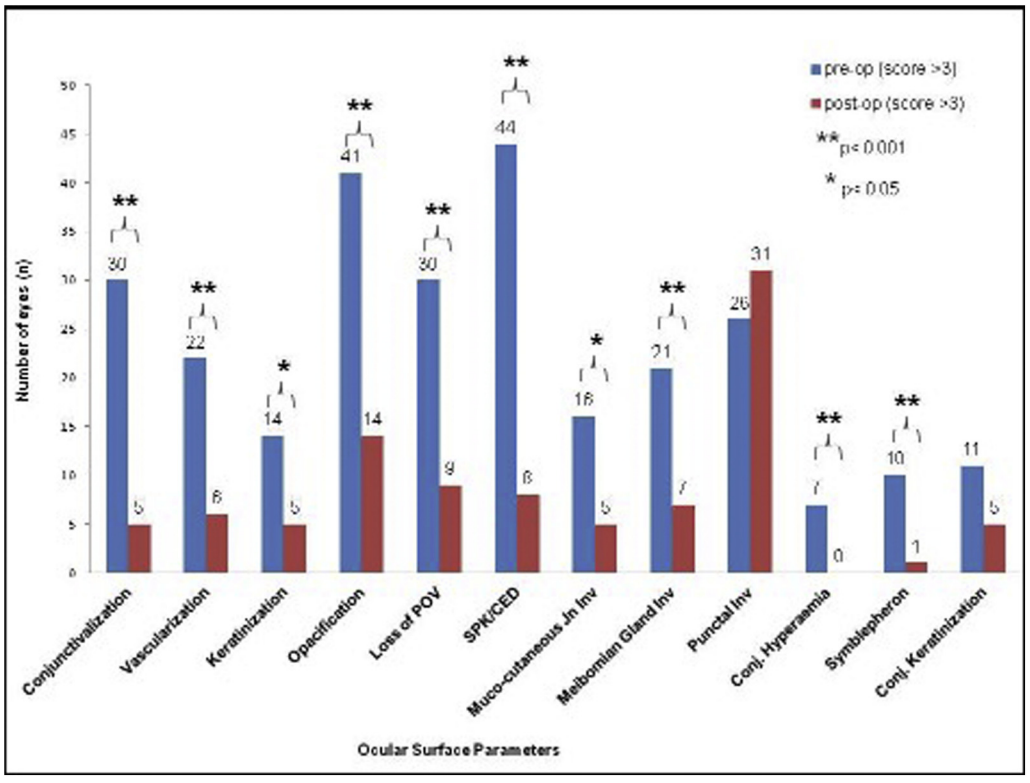

FIGURE 4. Bar diagram showing comparison of number of eyes in the score > 3 category for each of the ocular feature (except for superficial punctate keratitis/corneal epithelial defect, meibomian gland and punctal involvement), preoperatively and at a 2-year follow-up visit after cultivated oral mucosal epithelial transplantation. (For superficial punctate keratitis/corneal epithelial defect and meibomian gland involvement a cutoff score of 2 was taken while for punctal involvement a cutoff score of 1 was taken.)

epithelial defect and meibomian gland involvement, 1 for punctal involvement, and 3 for rest of the parameters) was calculated and compared preoperatively as well as postoperatively at 2-year follow-up. The numbers fell for all the parameters except for punctal involvement (Figure 4).

More than 50% of all corneal parameters were in the score >3 category, with 100% eyes belonging to the grade 4 category, suggestive of severe chronic ocular surface disease (Table 3). Postoperatively, there was a redistribution of eyes, with a shift of more than 50% eyes to lower grades; 62.2% eyes belonged to grades 1 to 3, and 37% continued to stay in grade 4.

Ocular surface parameter severity scores were also followed up at various time points, following COMET to look for any trend or pattern (Figure 6). The graphs for postoperative conjunctivalization, keratinization, and opacification scores showed a significant drop at the 1-week follow-up, compared to the preoperative scores (Figure 6). Corneal conjunctivalization scores then increased till the 6-month follow-up from 3.9 ± 0.8 to 2.3± 1.0 followed by stabilization. The scores for keratinization also increased after the 1-week follow-up and became stable by the 1-year follow-up. On the other hand, the scores for corneal opacification continued to drop significantly till 1 month (from 4.1 ± 0.6 to 3.8± 0.2) followed by stabilization by 6 months. The graph for corneal vascularization scores showed gradual drop till the 6-month follow-up, followed by stabilization at the 6-month visit.

At the 2-year postoperative follow-up, all parameters were found to have the similar mean scores with marginal variations as those at the 1-year time point (Figure 6). Mean postoperative CDVA at 24 months was 1.49 ± 0.98 logMAR. The mean score for conjunctivalization, vascularization, keratinization, and corneal opacification was also found to be similar to that of the 12-month follow-up as 2.35 ± 1.93, 2.53 ± 1.7, 1.54 ± 1.68, and 2.87 ± 1.83, respectively. This similarity pattern was observed for all other parameters for the cornea, conjunctiva, and eyelid.

• **SCHIRMER TEST AND TBUT OUTCOMES:** Mean preoperative Schirmer test reading was 1.4 ± 3.5 mm, with a median reading of 0 mm and a range of 0 to 19 mm, which improved to 1.5 ± 0.4 mm. Improvement in Schirmer test reading was seen in 20 eyes (44.4%), whereas it remained the same in 16 eyes (35.5%) and worsened in 9 (20%). The mean preoperative TBUT of 0.1 ± 0.6 seconds with a range of 0-4 seconds

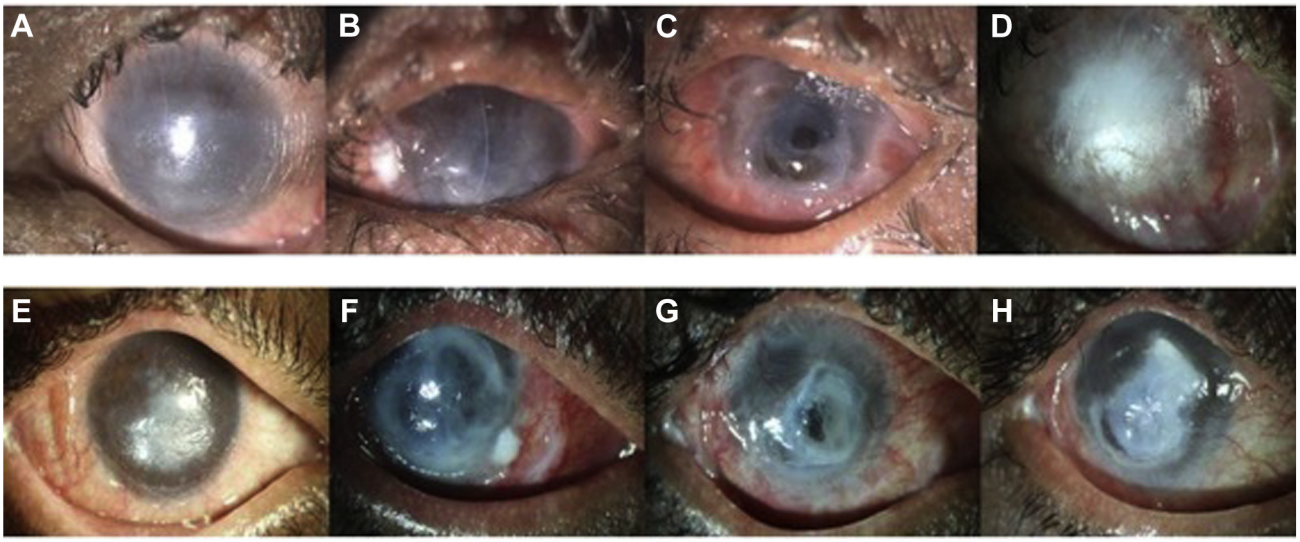

FIGURE 5. Pre- and postoperative slit-lamp photographs of 2 patients who developed ocular surface breakdown following cultivated oral mucosal epithelial transplantation. (A) Preoperative slit-lamp photograph of the left eye of a 28-year-old man with total limbal stem cell deficiency and conjunctivalization of cornea with opacification. (B) Two-week postoperative photograph of the same eye showing central corneal melt. (C) Four-week postoperative photograph of the same eye showing corneal melting with perforation. (D) Two-year postoperative photograph of the same eye following tectonic keratoplasty with opaque graft. (E) Preoperative slit-lamp photograph of the right eye of a 30-year-old woman with total limbal stem cell deficiency with central dense corneal opacification. (F) Two-week postoperative photograph of the same eye showing dense peripheral corneal stromal ring infiltrate with adjoining scleral involvement. (G) Four-week postoperative photograph of the same eye showing corneal melting with central perforation. (H) Two-year postoperative photograph of the same eye following therapeutic keratoplasty with recurrence of infection in the graft.

and median TBUT of 0 seconds improved to 0.8 ± 0.5 seconds. Improvement in TBUT was seen in 19 eyes (42.2%), whereas it remained the same in 26 (57.7%).

Univariate Cox regression analysis was used to assess the overall survival of SJS chronic ocular sequelae cases undergoing COMET. It revealed that none of the preoperative clinical parameters were associated with poor surgical prognosis with respect to post-COMET logMAR CDVA, total severity score, Schirmer test, and TBUT. However, a trend was seen between conjunctival keratinization and poor Schirmer test outcomes post COMET with a hazard ratio of 0.287 (95% CI 0.067-1.23) and $P = .047$ (Supplemental Material at AJO.com).

All the preoperative clinical parameters were correlated with COMET graft survival for improvement in logMAR CDVA using Kaplan-Meier survival analysis. None of the preoperative clinical parameters were found to have significant association with improvement in logMAR CDVA, total severity score, Schirmer test, and TBUT outcomes of COMET (Supplemental Material at AJO.com).

DISCUSSION

COMPLICATIONS OCCURRING IN THE CHRONIC PHASE OF SJS disrupt the ocular surface both anatomically and functionally. Retainment of normal structural anatomy allows further surgical procedures to be taken up for visual rehabilitation. We performed COMET in 45 eyes with chronic sequelae of SJS with an aim to restore the ocular surface anatomy, as close as possible to the normal architecture.

The mean age of patients in our series was 25.4 ± 11.7 years, with a male preponderance (62%). This is lower than that reported in other reported studies including patients with SJS.[8–10,13,16] The mean time duration from the onset of SJS to presentation was 124.4 ± 93.3 months, which was again longer than that in previous studies. Our study therefore had a comparatively younger cohort of patients with a longer interval from disease onset to the time of presentation. The time interval from onset to presentation is known to play an important role in deciding the overall success or failure of treatment.[9,10] The main causative agent for the onset of SJS in our study was intake of drugs, the most common being nonsteroidal anti-inflammatory drugs. Drugs have been implicated as the primary inciting factors in prior published studies, with acetaminophen being the most common cause of disease onset.[17–19]

All 45 cultivated oral mucosal epithelial sheets were successfully used for transplantation. Expression of CK3/12 and CK19 proteins on immunohistochemistry was suggestive of corneal as well as oral mucosal epithelial phenotype.[20,21] In an experimental study by Kim and

TABLE 2. Comparison of Median and Mean Preoperative and 2-Year Postoperative Ocular Surface Severity Scores of 45 Eyes With Chronic Stevens-Johnson Syndrome That Underwent Cultivated Oral Mucosal Epithelial Transplantation

Ocular Surface Parameters	Preoperative Score, Median (Mean ± SD)	Postoperative Score, Median (Mean ± SD)	*P* Value
Corneal parameters			
Conjunctivalization	4 (3.8 ± 0.9)	3 (2.3 ± 1.2)	**.000**
Loss of palisades of Vogt	4 (3.6 ± 0.9)	3 (2.3 ± 1.1)	**.001**
Neo-vascularization	4 (3.4 ± 1.2)	3 (2.3 ± 1.1)	**.009**
Keratinization	3 (2.3 ± 1.9)	3 (1.5 ± 1.5)	.204
Superficial punctate keratitis/corneal epithelial defect	4 (2.7 ±1.4)	3 (1.4 ± 0.7)	**.000**
Opacification	3 (4.1 ± 0.6)	0 (2.8 ± 1.0)	**.000**
Eyelid parameters			
Mucocutaneous junction involvement	3 (2.1 ±1.1)	1 (1.4 ± 0.8)	**.010**
Meibomian gland involvement	2 (1.4 ± 0.8)	1 (1.5 ± 0.8)	**.012**
Punctal involvement	2 (1.6 ± 1.7)	2 (1.2 ± 0.8)	.503
Conjunctival parameters			
Conjunctival hyperemia	1 (1.9 ± 1.7)	1 (0.4 ± 0.9)	.136
Conjunctival keratinization	2 (1.5 ± 1.7)	0 (1.2 ± 1.5)	**.030**
Symblepharon	1 (1.8 ±1.8)	0 (0.4 ± 0.5)	**.010**
Total score	29 (29.1 ± 9.7)	20 (18.7 ± 7.2)	**.003**

Bold used to highlight the significant *P* value which is <.05.

associates,[20] the regenerative potential of cultured oral mucosal epithelial cells sheets from SJS subjects was compared with those from non-SJS subjects. The oral mucosal epithelial cells from SJS patients were found to have delayed initial migratory potential compared with those obtained from non-SJS patients with comparable colony-forming efficiency, proliferation potential, and expression of cytokeratins. The epithelial defects observed early on were larger in eyes treated with SJS cultured oral mucosal epithelial cells compared with non–SJS cultured oral mucosal epithelial cells with no differences later on, by day 7.

The success rate of COMET in the present study was 82.2% (37/45) in terms of improvement in CDVA and 88.8% (40/45) in terms of reduction in severity scores. Two eyes in our series developed persistent epithelial defects with progression to corneal melting and perforation. Nakamura and associates[13] reported occurrence of persistent epithelial defects in 7 of their 19 eyes. Five of these 7 eyes had ocular sequelae of SJS. The authors attributed this to the altered biological ability of oral epithelial cells in patients with SJS, despite attainment of a successful sheet of oral mucosal epithelial cells.

At 1-week follow-up, various corneal parameters such as conjunctivalization, keratinization, and opacification showed significant improvement compared to preoperative scores. However, an increase in conjunctivalization and keratinization was noted at 1-month follow-up likely because of ongoing chronic inflammation. By the 6-month follow-up, these parameters including corneal vascularization stabilized. Overall, a clinical deterioration was observed during the first 6 months after an initial phase of improvement, but later on the ocular surface stabilized, with minimal change after 6 months. Nishida and colleagues[12] reported peripheral vascularization following COMET to peak by the 3-6-month follow-up and then becoming stable. Sotozono and associates[9] reported significant improvement in ocular surface scores at 4, 12, and 24 weeks postoperation following COMET.

Iyer and associates[11] in 2019 reported that the clinical outcomes of COMET performed for ocular surface reconstruction in 25 eyes, including 12 eyes with both acute and chronic SJS sequelae. COMET allowed successful re-epithelialization in eyes with acute-stage epithelial defects

TABLE 3. Preoperative and Postoperative Distribution of Number of Eyes in Various Grades (of a Total of 45 Eyes), Based on Total Ocular Surface Severity Scores

Grade (Total Ocular Surface Severity Score)	Number of Eyes (%)	
	Preoperation	Postoperation
Grade I (0-11)	0	7 (15.5)
Grade II (12-16)	0	5 (11.1)
Grade III (17-22)	0	16 (35.5)
Grade IV (23-53)	45 (100)	17 (37.7)

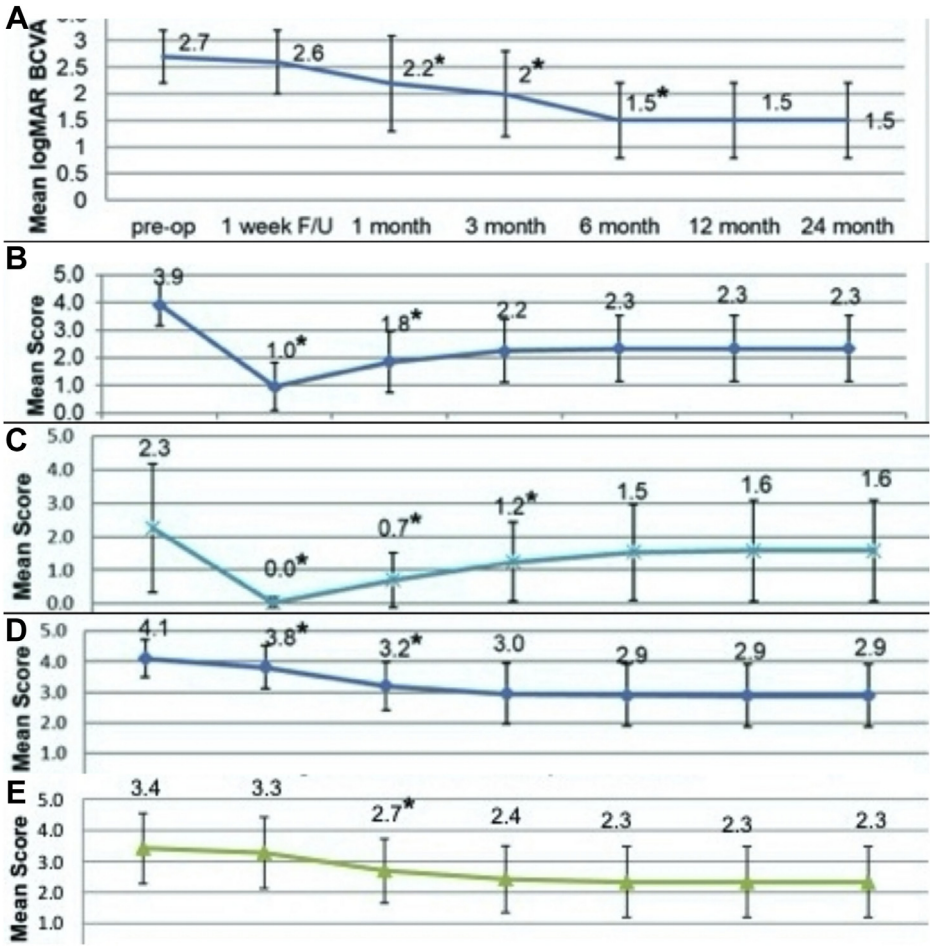

FIGURE 6. Line diagram showing (A) change in corrected distance visual acuity and severity scores of various corneal parameters including (B) conjunctivalization, (C) keratinization, (D) opacification, and (E) vascularization at various time points, following cultivated oral mucosal epithelial transplantation.

and reconstruction of fornices in eyes with chronic sequelae. Nakamura and associates in 2011 reported long-term outcomes of COMET, performed in 19 eyes with severe ocular surface disorders with associated corneal scarring.[13] Postoperative conjunctivalization and symblepharon were found to be significantly inhibited over a follow-up period of 55 months. All eyes manifested various degrees of corneal neovascularization, which gradually abated followed by stabilization at the 6-month follow up. Kaplan-Meier graft survival analyses were unable to reflect the role of preoperative parameters in COMET graft success. A similar ocular severity presentation (all 45 are grade 4) could be one of the contributing factors.

Our visual and surgical outcomes correlate well with those published in the literature earlier. This is the largest study that evaluated the results of COMET, exclusively performed in SJS patients, documenting the trend of change of various ocular surface parameters over a 2-year postoperative period. The long-term stability of visual acuity and various ocular surface parameters suggest that SJS patients can well be considered suitable candidates for oral mucosal epithelial cell transplantation.

FUNDING/SUPPORT: THIS STUDY RECEIVED NO FUNDING. FINANCIAL DISCLOSURES: THE AUTHORS INDICATE NO FINANCIAL support or conflicts of interest. All authors attest that they meet the current ICMJE criteria for authorship.

REFERENCES

1. Tseng SC. Amniotic membrane transplantation for ocular surface reconstruction. *Biosci Rep* 2001;21(4):481–489.
2. Solomon A, Espana EM, Tseng SCG. Amniotic membrane transplantation for reconstruction of the conjunctival fornices. *Ophthalmology* 2003;110(1):93–100.
3. Tsubota K, Satake Y, Ohyama M, et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. *Am J Ophthalmol* 1996;122(1):38–52.
4. Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000;343(2):86–93.
5. Hata K, Kagami H, Ueda M, Torii S, Matsuyama M. The characteristics of cultured mucosal cell sheet as a material for grafting; comparison with cultured epidermal cell sheet. *Ann Plast Surg* 1995;34(5):530–538.
6. Krishnan S, Iyer GK, Krishnakumar S. Culture & characterisation of limbal epithelial cells & oral mucosal cells. *Indian J Med Res* 2010;131(3):422–428.
7. Nakamura T, Inatomi T, Cooper LJ, Rigby H, Fullwood NJ, Kinoshita S. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. *Ophthalmology* 2007;114(6):1080–1088.
8. Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura M, Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol* 2004;88(10):1280–1284.
9. Sotozono C, Inatomi T, Nakamura T, et al. Cultivated oral mucosal epithelial transplantation for persistent epithelial defect in severe ocular surface diseases with acute inflammatory activity. *Acta Ophthalmol* 2014;92(6):e447–e453.
10. Sotozono C, Inatomi T, Nakamura T, et al. Visual improvement after cultivated oral mucosal epithelial transplantation. *Ophthalmology* 2013;120(1):193–200.
11. Gopakumar V, Agarwal S, Srinivasan B, Krishnakumar S, Krishnan UM, Iyer G. Clinical outcome of autologous cultivated oral mucosal epithelial transplantation in ocular surface reconstruction. *Cornea* 2019;38(10):1273–1279.
12. Nishida K, Yamato M, Hayashida Y, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 2004; 351(12):1187–1196.
13. Nakamura T, Takeda K, Inatomi T, Sotozono C, Kinoshita S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. *Br J Ophthalmol* 2011;95(7):942–946.
14. Sharma N, Venugopal R, Maharana PK, et al. Multistep grading system for evaluation of chronic ocular sequelae in patients with Stevens-Johnson syndrome. *Am J Ophthalmol* 2019;203(7):69–77.
15. Sen S, Sharma S, Gupta A, et al. Molecular characterization of explant cultured human oral mucosal epithelial cells. *Invest Ophthalmol Vis Sci* 2011;52(13):9548–9554.
16. Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. *Ophthalmology* 2011;118(8):1524–1530.
17. Sethuraman G, Sharma VK, Pahwa P, Khetan P. Causative drugs and clinical outcome in Stevens Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and SJS-TEN overlap in children. *Indian J Dermatol* 2012;57(3): 199–200.
18. Venugopal R, Satpathy G, Sangwan S, et al. Conjunctival microbial flora in ocular Stevens-Johnson syndrome sequelae patients at a tertiary eye care center. *Cornea* 2016;35(8): 1117–1121.
19. Ueta M, Kaniwa N, Sotozono C, et al. Independent strong association of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement. *Sci Rep* 2014;4:4862.
20. Kim YH, Kim DH, Shin EJ, et al. Comparative analysis of substrate-free cultured oral mucosal epithelial cell sheets from cells of subjects with and without Stevens-Johnson syndrome for use in ocular surface reconstruction. *PLoS One* 2016;11(1):e0147548.
21. Sheth R, Neale MH, Shortt AJ, et al. Culture and characterization of oral mucosal epithelial cells on a fibrin gel for ocular surface reconstruction. *Curr Eye Res* 2015; 40(11):1077–1087.