Clinical Ophthalmology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Self-Retained Cryopreserved Amniotic Membrane for the Management of Corneal Ulcers

This article was published in the following Dove Press journal:
Clinical Ophthalmology

Daniel Brocks[1]
Olivia G Mead[2]
Sean Tighe[3,4]
Scheffer CG Tseng[2]

[1]BostonSight, Needham, MA, USA; [2]Ocular Surface Center and TissueTech Inc, Miami, FL, USA; [3]Department of Ophthalmology, Florida International University, Miami, FL, USA; [4]Department of Biochemistry and Molecular Biology, University of Miami, Miami, FL, USA

Purpose: To evaluate the clinical outcomes of self-retained cryopreserved amniotic membrane (cAM) for the treatment of corneal ulcers.

Methods: This was a single-center, retrospective review of consecutive patients with non-healing corneal ulcers that underwent treatment with self-retained cAM (PROKERA® Slim). The primary outcome measure was time to complete corneal epithelialization. Ocular discomfort, corneal staining, corneal signs, and visual acuity were assessed at 1 week, 1 month, 3 months, and 6 months. Complications, adverse events, and ulcer recurrence were also recorded.

Results: A total of 13 eyes (13 patients) with recalcitrant corneal ulcers were included for analysis, 9 (69%) of which progressed from neurotrophic keratitis (NK). Prior to cAM application, patients used conventional treatments such as artificial tears (n = 11), antibiotics (n = 11), ointment (n = 11), steroids (n = 6), and antivirals (n = 3). Self-retained cAMs (n = 1.5 ± 0.8) were placed for 6.8 ± 3.4 days, during which time antibiotics were continued. Four cases (31%) were subsequently treated with bandage contact lens (n = 3) and tarsorrhaphy (n = 1). All corneal ulcers healed in a median of 14 days (range: 4–43). This was accompanied by a significant improvement in ocular discomfort, corneal staining, and corneal signs at 1 week, 1 month, 3 months, and 6 months ($P<.05$). Recurrence was noted in one case. No adverse events were observed.

Conclusion: Self-retained cAM may be a valuable, in-office treatment option for healing recalcitrant corneal ulcers of various etiologies, especially those with underlying NK. Further prospective, controlled studies are warranted.

Keywords: amniotic membrane, corneal ulcer, neurotrophic keratitis, ocular surface disease

Introduction

Like other epithelial barriers of the human body, the corneal epithelium is continuously self-renewing with a distinct stem cell niche in the limbal basal region.[1] When this protective barrier is subjected to physical, chemical, or biological insult, corneal epithelial cells briskly undergo proliferation and migration to restore corneal integrity and preserve the ocular surface.[2] While most epithelial defects heal without complication, co-existing ocular surface conditions can compromise this regenerative healing process and impair the cornea's ability to restore the epithelium, leading to persistent corneal epithelial breakdown and ulceration. One such condition, neurotrophic keratitis (NK), involves damage to the corneal nerves that can diminish corneal epithelial viability and dampen the blinking and tearing reflexes that maintain ocular surface health.[3,4] This is especially devastating as corneal nerves and epithelial cells mutually support one another via neuropeptides

Correspondence: Daniel Brocks
BostonSight, 464 Hillside Ave Suite 205, Needham, MA 02494, USA
Tel +1 781 726 7337
Email danielbrocks@yahoo.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/OPTH.S253750

and growth factors to ensure corneal wound healing[5–8] and nerve repair and survival.[9] Thus, early diagnosis and treatment of underlying disease are essential in restoring the ocular surface and preventing progressive ulceration, imminent perforation, and irreversible vision loss.

Current therapies aim to promote healing of corneal ulcers by maintaining a stable tear film and providing mechanical protection of the cornea. Conservative treatments include preservative-free artificial tears, ointments, autologous serum eyedrops, and bandage contact lens (BCL). When corneal ulcers respond poorly to conservative methods, adjunctive procedures and surgical interventions are commonly implemented including tarsorrhaphy, punctal occlusion, conjunctival flap, and amniotic membrane transplantation (AMT). Unfortunately, the cosmetic impact of some surgical interventions such as tarsorrhaphy and conjunctival flap may be undesirable to patients; in addition, punctal occlusion with an underlying inflammatory disease can lead to further damage by retention of pro-inflammatory components in the tear film. In contrast, amniotic membrane (AM) contains unique anti-inflammatory, anti-scarring, and pro-regenerative properties[10,11] and has been shown to successfully treat epithelial defects and corneal ulcers of various etiologies, including NK.[12–14] While AMT with sutures has been widely reported for the treatment of corneal ulcers, few studies have assessed the use of self-retained cryopreserved AM (cAM).[15–18] Therefore, the purpose of this study was to assess the clinical outcomes following the treatment of corneal ulcers with self-retained cAM.

Materials and Methods

Following approval and waiver of consent by the Institutional Review Board at Vassar Brothers Medical Center, a retrospective chart review was performed in accordance with the tenets of the Declaration of Helsinki on consecutive patients that were treated between 2016 and 2017 by the study author. Appropriate measures were undertaken and maintained to protect the confidentiality of study participants. Patients were included for analysis if they (1) presented with a recalcitrant corneal ulcer, (2) were treated with self-retained cAM (PROKERA® Slim [PKS], Bio-Tissue, Miami, FL), and (3) had at least 6 months of follow-up data. Recalcitrant corneal ulcers were defined as those that failed to display any tendency of epithelial healing despite conventional treatments. Medical histories and results from comprehensive ophthalmic examinations were obtained. Collected data included demographic variables, corneal ulcer etiology, associated signs and symptoms, concomitant therapies, time to complete healing, and recurrence and/or complications.

Intervention

After PKS was thawed at room temperature for several minutes, it was rinsed with saline and inserted under topical anesthesia with 0.5% proparacaine hydrochloride eye drops in the office. PKS was first placed into the superior fornix while the patient looked down and was then slid under the lower eyelid. Patients returned to the office at 1 week (± 5 days) for PKS removal following the dissolution of the AM and continued to follow-up at 1 month, 3 months, and 6 to 12 months. Slit-lamp examination with fluorescein staining was performed at baseline and all follow-up visits.

Outcome Measures

The primary outcome measure was time to complete corneal epithelialization. Ocular discomfort, corneal surface integrity, and corneal signs were also assessed at 1 week, 1 month, 3 months, and 6 months following the application of cAM via PKS. Corneal symptoms of discomfort, severity, and frequency were graded as none (0), mild discomfort with intermittent frequency (1), moderate discomfort with variable frequency (2), and severe discomfort with constant frequency (3). Corneal surface integrity was assessed using fluorescein staining and was scored as clear (0), mild (1), moderate (2), or severe (3). Corneal signs were graded as absent (0), mild debris and reduced tear meniscus (1), filamentary keratitis with mucus clumping and increased tear debris (2), superficial punctate keratitis (SPK) (3), and defect/ulceration (4). Snellen visual acuity (VA) was also assessed at 1 week, 1 month, 3 months, and 6 months. Change in the number of Snellen lines was calculated by logMAR transformation, and a significant improvement in VA was considered 3 or more lines. “Counting Fingers” (CF) and “hand motion” (HM) were quantified as 0.014 and 0.005, respectively.[19] “Light perception” (LP) was quantified as 0.002.[20]

Statistical Analyses

All statistical analyses were carried out using SPSS Software version 20.0 (IBM; Armonk, NY, USA). Continuous data were reported as mean ± standard deviation or median and range, and categorical data were described using frequency and percentage. Ordinal variables including ocular discomfort, corneal staining, and corneal signs were compared between timepoints using the Wilcoxon Signed-Rank test. Correlations between

parameters were assessed by the Spearman's rank-order correlation. A P value less than 0.05 was considered statistically significant.

Results

Thirteen eyes of 13 consecutive patients (6 females, 7 males) with a mean age of 67.3 ± 11.8 were included in this study. All patients presented with corneal ulcers despite prior conservative treatment with artificial tears (11/13, [85%]), antibiotics (11/13 [85%]), ointment (11/13 [85%]), steroids (6/13 [46%]), antivirals (3/13 [23%]), NSAIDs (3/13 [23%]), BCL (1/13 [8%]), and mydriatics (1/13 [8%]). Ulcers were located centrally (7/13 [54%]), peripherally (3/13 [23%]), or both centrally and peripherally (3/13 [23%]) as evidenced by corneal staining. The majority of corneal ulcers (9/13 [69%]) resulted from underlying neurotrophic keratitis (NK) caused by acoustic neuroma removal (n = 1), lagophthalmos (n = 3), herpetic infection (simplex or zoster) (n = 3), and advanced diabetes mellitus (DM) (n = 2). The etiologies of the remaining ulcers were autoimmune disease (n = 2), chemical burn (n = 1), and dry eye (n = 1). Relevant clinical data for each patient are summarized in Table 1.

Chief complaints at baseline visit included ocular pain (3/13 [23%]), discomfort (4/13 [31%]), light sensitivity (2/13 [15%]), blurred vision (11/13 [85%]), and redness (8/13 [62%]). While slit-lamp examination revealed severe corneal staining in all patients, the median ocular discomfort score was 0 out of 3 (mean score of 0.9 ± 1.1), which further indicates that the majority of the study sample had underlying NK.

Each patient received a median of 1 PKS application (range: 1–3); eight eyes (62%) received a single application, whereas a second and third PKS was placed in 3 (23%) and 2 (15%) eyes, respectively. Placement and removal of PKS were uneventful in all patients, and PKS was well tolerated by all patients. PKS was placed for a duration of 6.8 ± 3.4 days (range: 2–15), during which time antibiotics were continued and steroids discontinued. The cAM fell out spontaneously in one eye prior to dissolution on day 4. Aside from this case, the cAM was completely dissolved in all eyes upon removal of PKS.

Corneal ulcers healed in all eyes in a median of 14 days (range: 4–43); only one neurotrophic corneal ulcer in a blind eye secondary to angle-closure glaucoma failed to heal within the first month and required full-sutured tarsorrhaphy to complete epithelialization (Figure 1). Following PKS removal, BCL was placed in 3 eyes (23%) to complete corneal epithelialization.

Complete corneal epithelialization was accompanied by significant improvements in clinical signs and symptoms at all follow-up timepoints (Table 2). Ocular discomfort significantly improved from 0.9 ± 1.1 at baseline to 0.2 ± 0.6 at 1 week ($P = 0.04$), 0.2 ± 0.4 at 1 month ($P = 0.03$), 0.1 ± 0.4 at 3 months ($P = 0.02$), and 0.1 ± 0.4 at 6 months ($P = 0.04$). In addition, corneal staining improved from 3.0 ± 0.0 at baseline to 1.5 ± 1.3 at 1 week ($P = 0.01$), 0.5 ± 0.9 at 1 month ($P = 0.001$), 0.2 ± 0.6 at 3 months ($P < 0.001$), and 0.3 ± 0.5 at 6 months ($P = 0.001$). This was accompanied by a significant improvement in corneal signs from 4.0 ± 0.0 at baseline to 2.7 ± 1.9 at 1 week ($P = 0.03$), 1.1 ± 1.7 at 1 month ($P = 0.002$), 0.3 ± 1.1 at 3 months ($P = 0.001$), and 0.8 ± 1.5 at 6 months ($P = 0.001$). When excluding 4 eyes with poor visual potential due to underlying conditions or treatment such as tarsorrhaphy or PKS, VA improved from a median of 0.70 logMAR (20/100) at baseline to 0.48 logMAR (20/60) at 1 month, 0.48 logMAR (20/60) at 3 months, and 0.30 logMAR (20/40) at 6 months. None of these improvements were statistically significant. At 1 month, 7 eyes (54%) demonstrated clinically significant improvement in VA (≥3 lines) compared to baseline. This improvement in VA remained relatively stable in 5 (38%) and 6 eyes (46%) at 3 and 6 months, respectively.

No adverse events were reported throughout the study period. Corneal ulcer recurrence was noted in one severely diabetic patient with a neurotrophic cornea. The ulcer initially healed in 14 days with PKS but recurred 3 months later and was treated with tarsorrhaphy to complete healing. Furthermore, 2 patients with autoimmune diseases presented with mild SPK at 6 months, and 6 patients displayed signs of minimal stromal opacity.

Discussion

Over the last two decades, many studies have reported the palliative benefits of AM in treating various ocular surface diseases.[21,22] AM contains many growth factors such as nerve growth factor (NGF), keratinocyte growth factor (KGF), and hepatocyte growth factor (HGF), all of which have been shown to promote healing of the corneal epithelium.[23–25] The abundance of NGF in the AM stroma[24,26-29] is especially important because NGF supports and repairs corneal nerves,[9] which are essential in eliciting protective reflexes that respond to corneal injury and help to maintain a healthy epithelium.[30] Furthermore, mononuclear cells such as lymphocytes and macrophages have been shown to adhere to the cAM stroma and undergo rapid apoptosis, significantly reducing corneal inflammation and

Table 1 Demographic and Clinical Characteristics

Case	Age	Sex	Eye	Ulcer Etiology	Previous Surgery	Prior Management	Comorbidities	Additional Treatment	PKS Placement (Days)	Epithelial Healing (Days)
1	52	F	L	Acoustic neuroma removal		Antibiotics, Steroid, BCL	NK		8 (x3)	24
2	58	F	L	Lagophthalmos		Antibiotics, Ointment	NK, Bell's Palsy		7, 5	12
3	56	M	R	Chemical Burn		Tears, Antibiotics, Steroid, Mydriatic			15	15
4	70	M	R	Dry Eye		Tears, Antibiotics, Steroid, Ointment	MGD		3, 2	5
5	47	F	L	Autoimmune	PRK	Tears, Antibiotics, Ointment, NSAIDs	Thyroid disease		3	4
6	86	M	L	DM	Cataract	Tears, Antibiotics, Ointment, NSAIDs	NK, Advanced DM	Tarsorrhaphy[a]	14	14
7	67	F	L	DM	Cataract, PI	Tears, Antibiotics, Ointment	NK, Severe advanced glaucoma	Tarsorrhaphy	10, 7	43
8	75	F	L	Autoimmune, HZV		Tears, Antibiotics, Ointments	Autoimmune disease (Unspecified)	BCL	8, 5, 3	29
9	68	M	L	HSV		Tears, Antiviral, Steroid, Ointment, NSAIDs	NK, severe advanced glaucoma, MGD		4	10
10	68	M	R	Lagophthalmos	Cataract, Tarsorrhaphy	Antibiotics, Ointment, Tears	NK, MGD	BCL	7	20
11	80	M	L	HZV	Cataract	Tears, Steroid, Ointment, Antiviral, Antibiotics	NK, Ptosis		7	7
12	82	F	L	Lagophthalmos	Cataract	Tears, Ointment	NK, DED, Bell's Palsy		6	6
13	66	M	R	HSV		Antibiotics, Antiviral, Ointment, Steroid, Tears	NK, Leukemia, MGD	BCL	6	24

Note: [a]Tarsorrhaphy was performed at month 3 following ulcer recurrence.

Abbreviations: BCL, bandage contact lens; DED, dry eye disease; DM, diabetes mellitus; F, female; HSV, herpes simplex virus; HZV, herpes zoster virus; L, left; M, male; MGD, meibomian gland dysfunction; NK, neurotrophic keratitis; NSAIDs, nonsteroidal anti-inflammatory drugs; PI, peripheral iridotomy; PKS, PROKERA Slim; PRK, photorefractive keratectomy; R, right.

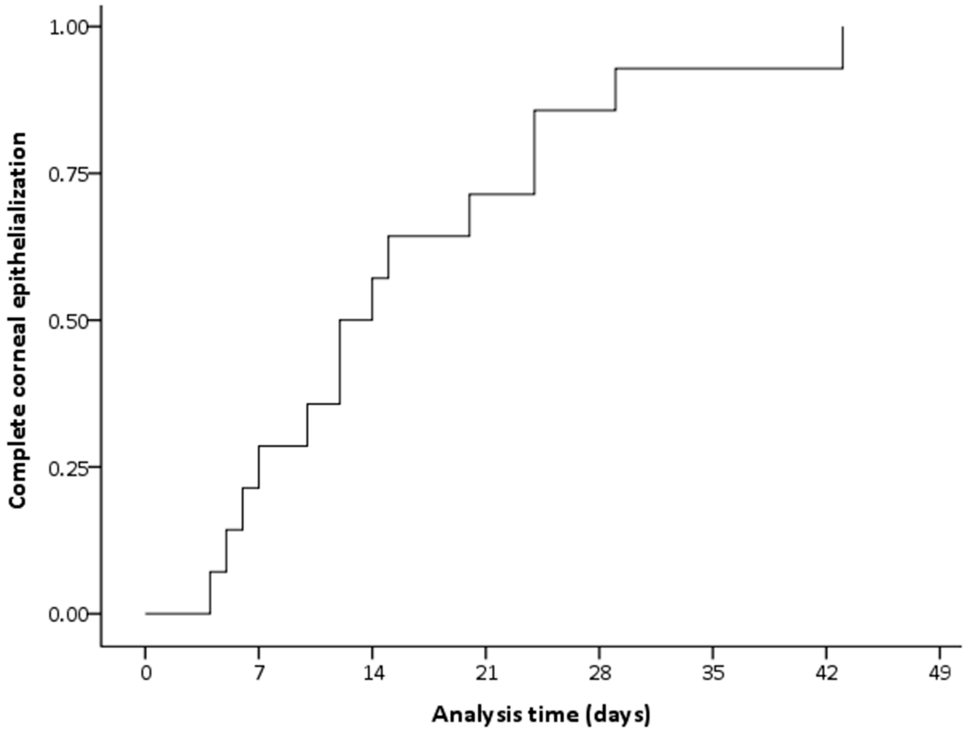

Figure 1 Time to complete corneal epithelialization. Kaplan–Meier survival analysis shows the days to complete corneal epithelialization in patients treated with self-retained cryopreserved amniotic membrane (cAM) via PROKERA Slim for corneal ulcers.

promoting healing.[31–36] While a number of studies have reported the benefits of AMT in treating corneal ulcers, there is increased interest in utilizing a sutureless approach to eliminate suture-induced inflammation and circumvent surgical complications such as infection. Sutureless application is highly beneficial as the cAM can be placed in-office via PKS, facilitating timely intervention and evading delays inherent to scheduling surgery.

Following placement of self-retained cAM, all corneal ulcers in the present study exhibited rapid corneal

Table 2 Clinical Outcomes

	Visual Acuity					Discomfort Severity & Frequency					Corneal Staining					Corneal Signs				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
1	CF	20/60	20/60	20/200	20/150	0	0	0	0	0	3	1	0	0	0	4	3	0	0	0
2	20/300	20/400	N/A[a]	20/400	20/300	0	0	0	0	0	3	3	3	0	0	4	4	4	0	0
3	20/60	20/150[a]	20/25	20/25	20/25	3	1	1	1	0	3	1	1	0	0	4	4	3	0	0
4	20/50	20/50	20/25	20/25	20/25	1	0	1	0	1	3	0	0	0	0	4	0	0	0	0
5	20/100	20/70	20/20	20/20	20/20	3	0	1	0	0	3	0	0	0	1	4	0	0	0	3
6	CF	HM	20/400	CF	CF	2	0	0	1	1	3	3	0	2	1	4	4	0	4	4
7	HM[b]	HM[b]	HM[b]	LP[b]	LP[b]	1	0	0	0	0	3	3	1	0	0	4	4	4	0	0
8	20/60	CF[a]	20/400	20/200	20/200	2	2	0	0	0	3	3	0	0	1	4	4	0	0	3
9	HM[b]	HM[b]	HM[b]	HM[b]	HM[b]	0	0	0	0	0	3	1	0	0	0	4	4	0	0	0
10	LP	LP	HM	20/400	20/400	0	0	0	0	0	3	3	0	0	0	4	4	0	0	0
11	20/100	20/400[a]	20/100	20/100	20/200	0	0	0	0	0	3	0	0	0	0	4	0	0	0	0
12	20/50	20/100[a]	CF[a]	20/60	20/40	0	0	0	0	0	3	0	1	0	1	4	0	3	0	0
13	20/100	20/400[a]	20/50	20/60	20/20	0	0	0	0	0	3	2	0	0	0	4	4	0	0	0

Notes: [a]Vision was obstructed from PKS, tarsorrhaphy, or thick ointment covering eye. [b]Poor visual potential due to severe advanced glaucoma.
Abbreviations: CF, counting fingers; HM, hand motion; LP, light perception; T_0, Baseline; T_1, Week 1; T_2, Month 1; T_3, Month 3; T_4, Month 6.

epithelialization without complication in a median of 14 days. Four (31%) severe cases underwent subsequent interventional measures to complete epithelialization via BCL or tarsorrhaphy. Tarsorrhaphy was performed in 2 cases (of which one case after recurrence), in which both cases had underlying advanced diabetes, and one case also had advanced glaucoma. The need for tarsorrhaphy may be related to the severe nerve impairment in these patients. Furthermore, 6 eyes (46%) with visual potential improved by 3 or more Snellen lines by 6 months in the present study. These findings are similar to what was reported in a meta-analysis by Liu et al, which found a 2.3% (9/358) complication rate and pooled vision improvement rate of 53%.[12] Schuerch et al recently published the largest retrospective analysis to date of patients who underwent AMT for the treatment of corneal ulcers.[13] In that study, Schuerch et al reported a 47% (7/15) healing rate of neurotrophic corneal ulcers at 1 month following double-layered AMT with adjunctive therapeutic contact lens[13] in comparison to a 92% (12/13) healing rate in the present study. These findings suggest sutureless application of cAM via PKS may accelerate healing when compared to traditional AMT with sutures. However, it is important to note that their healing rate improved to 87% (13/15) at 3 months and 93% (14/15) at 6 months;[13] thus, it is possible that double-layered AMT with sutures may take longer to resorb, rendering it more difficult to monitor complete epithelization at 1 month. Overall, these findings support the use of AM in promoting complete epithelialization of corneal ulcers caused by underlying NK.

Three retrospective case series[15–17] have reported findings regarding the use of self-retained cAM for the treatment of corneal ulcers. Cheng and Tseng found 4 herpetic corneal ulcers underwent rapid corneal epithelization within 5 ± 4 days of PKS placement along with reduced ocular surface inflammation and improved VA during 3–51 months of follow-up.[16] In another short-term study, Suri et al described how 73% (8/11) of corneal ulcers healed following PROKERA® placement despite failure to heal with prior use of punctal plugs (n = 5), BCL (n = 4), and tarsorrhaphy (n = 2);[15] remarkably, one eye with descemetocele re-epithelialized and formed an anterior chamber following treatment with PROKERA® despite failure to heal with BCL.[15] In a case series involving corneal ulcers with bacterial keratitis, Sheha et al found 2 of 3 corneal ulcers healed in 14 and 23 days.[17] Our similar results, with a larger sample size and various etiologies, further support the current literature of using self-retained cAM to promote corneal epithelialization. Furthermore, use of cAM for NK is further supported by two recent studies, in which PKS was shown to promote corneal nerve regeneration in patients with severe dry eye disease and concurrently reduce corneal neuropathic pain.[37,38] These findings may shed light on the successful outcomes observed in this retrospective series, which predominantly comprised corneal ulcers with underlying NK. Further prospective studies are warranted to confirm our findings.

Disclosure

The development of PROKERA was supported in part with Grant no. EY014768 from the National Institute of Health (NIH) and National Eye Institute (NEI). The content is solely the responsibility of the authors and does not necessarily represent the opinion of the NIH or the NEI. OM, ST, and SCGT are employees of TissueTech. Dr. Tseng has obtained a patent for the method of preparation and clinical uses of amniotic membrane and has licensed the rights to TissueTech, Inc, which procures and processes, and to BioTissue, Inc, which is a subsidiary of TissueTech, Inc, to distribute cryopreserved amniotic membrane for clinical and research uses. Dr Daniel Brocks reports Consultant to Biotissue (manufacturer of Prokera) from Aug 2015 to Aug 2017. The authors report no other conflicts of interest in this work.

References

1. Nowell CS, Radtke F. Corneal epithelial stem cells and their niche at a glance. *J Cell Sci*. 2017;130(6):1021–1025.
2. Bukowiecki A, Hos D, Cursiefen C, Eming SA. Wound-healing studies in cornea and skin: parallels, differences and opportunities. *Int J Mol Sci*. 2017;18(6):1257. doi:10.3390/ijms18061257
3. Dua HS, Said DG, Messmer EM, et al. Neurotrophic keratopathy. *Prog Retin Eye Res*. 2018;66:107–131. doi:10.1016/j.preteyeres.2018.04.003
4. Mead OG, Tighe S, Tseng SCG. Amniotic membrane transplantation for managing dry eye and neurotrophic keratitis. *Taiwan J Ophthalmol*. 2020;10(1):13–21. doi:10.4103/tjo.tjo_5_20
5. Okada Y, Sumioka T, Ichikawa K, et al. Sensory nerve supports epithelial stem cell function in healing of corneal epithelium in mice: the role of trigeminal nerve transient receptor potential vanilloid 4. *Lab Invest*. 2019;99(2):210–230. doi:10.1038/s41374-018-0118-4
6. Shi X, Wang L, Clark JD, Kingery WS. Keratinocytes express cytokines and nerve growth factor in response to neuropeptide activation of the ERK1/2 and JNK MAPK transcription pathways. *Regul Pept*. 2013;186:92–103. doi:10.1016/j.regpep.2013.08.001
7. Yang L, Di G, Qi X, et al. Substance P promotes diabetic corneal epithelial wound healing through molecular mechanisms mediated via the neurokinin-1 receptor. *Diabetes*. 2014;63(12):4262–4274. doi:10.2337/db14-0163

8. Mikulec AA, Tanelian DL. CGRP increases the rate of corneal re-epithelialization in an in vitro whole mount preparation. *J Ocul Pharmacol Ther*. 1996;12(4):417–423. doi:10.1089/jop.1996.12.417
9. Di G, Qi X, Zhao X, Zhang S, Danielson P, Zhou Q. Corneal epithelium-derived neurotrophic factors promote nerve regeneration. *Invest Ophthalmol Vis Sci*. 2017;58(11):4695–4702. doi:10.1167/iovs.16-21372
10. Tseng SC. HC-HA/PTX3 purified from amniotic membrane as novel regenerative matrix: insight into relationship between inflammation and regeneration. *Invest Ophthalmol Vis Sci*. 2016;57(5):ORSFh1–8. doi:10.1167/iovs.15-17637
11. Tseng SC, Espana EM, Kawakita T, et al. How does amniotic membrane work? *Ocul Surf*. 2004;2(3):177–187. doi:10.1016/S1542-0124(12)70059-9
12. Liu J, Li L, Li X. Effectiveness of cryopreserved amniotic membrane transplantation in corneal ulceration: a meta-analysis. *Cornea*. 2019;38(4):454–462. doi:10.1097/ICO.0000000000001866
13. Schuerch K, Baeriswyl A, Frueh BE, Tappeiner C. Efficacy of amniotic membrane transplantation for the treatment of corneal ulcers. *Cornea*. 2019.
14. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol*. 1997;123(3):303–312. doi:10.1016/S0002-9394(14)70125-4
15. Suri K, Kosker M, Raber IM, et al. Sutureless amniotic membrane ProKera for ocular surface disorders: short-term results. *Eye Contact Lens*. 2013;39(5):341–347. doi:10.1097/ICL.0b013e3182a2f8fa
16. Cheng AMS, Tseng SCG. Self-retained amniotic membrane combined with antiviral therapy for herpetic epithelial keratitis. *Cornea*. 2017;36(11):1383–1386. doi:10.1097/ICO.0000000000001316
17. Sheha H, Liang L, Li J, Tseng SC. Sutureless amniotic membrane transplantation for severe bacterial keratitis. *Cornea*. 2009;28(10):1118–1123. doi:10.1097/ICO.0b013e3181a2abad
18. Sheha H, Tighe S, Cheng AMS, Tseng SCG. A stepping stone in treating dendritic keratitis. *Am J Ophthalmol Case Rep*. 2017;7:55–58. doi:10.1016/j.ajoc.2017.06.002
19. Schulze-Bonsel K, Feltgen N, Burau H, Hansen L, Bach M. Visual acuities "Hand motion" and "Counting fingers" can be quantified with the freiburg visual acuity test. *Invest Ophthalmol Vis Sci*. 2006;47(3):1236–1240. doi:10.1167/iovs.05-0981
20. Bach M, Schulze-Bonsel K, Feltgen N, Burau H, Hansen L. Author response: numerical imputation for low vision states. (eLetter). *Invest Ophthalmol Vis Sci*. 2007;26.
21. Jirsova K, Jones GLA. Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review. *Cell Tissue Bank*. 2017;18(2):193–204. doi:10.1007/s10561-017-9618-5
22. Tighe S, Mead OG, Lee AP, Tseng SC. Basic science review of birth tissue uses in ophthalmology. *Taiwan J Ophthalmol*. 2020;10(1):3.
23. He M, Han T, Wang Y, et al. Effects of HGF and KGF gene silencing on vascular endothelial growth factor and its receptors in rat ultraviolet radiation-induced corneal neovascularization. *Int J Mol Med*. 2019;43(4):1888–1899. doi:10.3892/ijmm.2019.4114
24. Touhami A, Grueterich M, Tseng SC. The role of NGF signaling in human limbal epithelium expanded by amniotic membrane culture. *Invest Ophthalmol Vis Sci*. 2002;43(4):987–994.
25. Wang L, Wu X, Shi T, Lu L. Epidermal growth factor (EGF)-induced corneal epithelial wound healing through nuclear factor kappaB subtype-regulated CCCTC binding factor (CTCF) activation. *J Biol Chem*. 2013;288(34):24363–24371. doi:10.1074/jbc.M113.458141
26. Uchida S, Inanaga Y, Kobayashi M, et al. Neurotrophic function of conditioned medium from human amniotic epithelial cells. *J Neurosci Res*. 2000;62:585–590. doi:10.1002/1097-4547(20001115)62:4<585::AID-JNR13>3.0.CO;2-U
27. Koizumi N, Inatomi T, Sotozono C, et al. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res*. 2000;20:173–177. doi:10.1076/0271-3683(200003)2031-9FT173
28. Davis GE, Engvall E, Varon S, Manthorpe M. Human amnion membrane as a substratum for cultured peripheral and central nervous system neurons. *Dev Brain Res*. 1987;33:1–10. doi:10.1016/0165-3806(87)90170-2
29. Davis GE, Blaker SN, Engvall E, Varon S, Manthorpe M, Gage FH. Human amnion membrane serves as a substratum for growing axons in vitro and in vivo. *Science*. 1987;236(4805):1106–1109. doi:10.1126/science.3576223
30. Tseng SC. A practical treatment algorithm for managing ocular surface and tear disorders. *Cornea*. 2011;30(Suppl 1):S8–S14. doi:10.1097/ICO.0b013e318228218c
31. Heiligenhaus A, Bauer D, Meller D, Steuhl KP, Tseng SC. Improvement of HSV-1 necrotizing keratitis with amniotic membrane transplantation. *Invest Ophthalmol Vis Sci*. 2001;42(9):1969–1974.
32. Heiligenhaus A, Li H, Hernandez Galindo EE, Koch JM, Steuhl KP, Meller D. Management of acute ulcerative and necrotising herpes simplex and zoster keratitis with amniotic membrane transplantation. *Br J Ophthalmol*. 2003;87(10):1215–1219. doi:10.1136/bjo.87.10.1215
33. Shimmura S, Shimazaki J, Ohashi Y, Tsubota K. Antiinflammatory effects of amniotic membrane transplantation in ocular surface disorders. *Cornea*. 2001;20(4):408–413. doi:10.1097/00003226-200105000-00015
34. Park WC, Tseng SC. Modulation of acute inflammation and keratocyte death by suturing, blood, and amniotic membrane in PRK. *Invest Ophthalmol Vis Sci*. 2000;41(10):2906–2914.
35. Bauer D, Wasmuth S, Hennig M, Baehler H, Steuhl KP, Heiligenhaus A. Amniotic membrane transplantation induces apoptosis in T lymphocytes in murine corneas with experimental herpetic stromal keratitis. *Invest Ophthalmol Vis Sci*. 2009;50(7):3188–3198. doi:10.1167/iovs.08-3041
36. Bauer D, Wasmuth S, Hermans P, et al. On the influence of neutrophils in corneas with necrotizing HSV-1 keratitis following amniotic membrane transplantation. *Exp Eye Res*. 2007;85(3):335–345. doi:10.1016/j.exer.2007.05.009
37. Morkin MI, Hamrah P. Efficacy of self-retained cryopreserved amniotic membrane for treatment of neuropathic corneal pain. *Ocul Surf*. 2018;16(1):132–138. doi:10.1016/j.jtos.2017.10.003
38. John T, Tighe S, Sheha H, et al. Corneal nerve regeneration after self-retained cryopreserved amniotic membrane in dry eye disease. *J Ophthalmol*. 2017;2017:6404918. doi:10.1155/2017/6404918