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Accelerated corneal collagen cross-linking in clinical management of infectious keratitis

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Abstract

Objective: To evaluate the clinical efficacy of corneal collagen cross-linking (CXL) in the treatment of infectious corneal diseases.

Methods: This study retrospectively analyzed the clinical efficacy of CXL in 65 eyes with infectious keratitis in Jinan Second People's Hospital from December 2016 to June 2018. During 6 months of follow-up after CXL treatment, the results of confocal microscopy and anterior segment optical coherence tomography, as well as visual acuity and corneal biomechanical parameters, were recorded in detail.

Results: In general, the overall cure rate was 93.85%; no corneal endothelial dysfunction was encountered in any patients. After 6 months of follow-up, the visual acuity of cured patients was significantly enhanced, while corneal thickness was significantly reduced. Hyphae growth of patients with fungal keratitis was completely inhibited at 1 month postoperatively. Furthermore, corneal biomechanical parameters (i.e., central corneal thickness, deformation amplitude, and pachymetry intraocular pressure) were significantly improved after surgery, compared with baseline measurements.

Conclusion: Accelerated CXL may be an effective adjuvant treatment for infectious keratitis.

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Introduction

Infectious keratitis is an inflammatory disease caused by pathogen infection after corneal injury, which is associated with a high rate of blindness.[1] The common pathogens are bacteria, fungi, and viruses; the primary form of disease is bacterial keratitis.[2,3] Thus far, drug therapy remains the main clinical treatment;[1] however, nonstandard use of antibiotics causes emergence of drug-resistant strains.[4,5] Moreover, anti-fungal and anti-amoebic drugs are relatively limited and exhibit poor efficacy, which leads to recurrent disease and difficulty in achieving cure.[6,7] Therapeutic keratoplasty can only be performed for patients with advanced disease; the postoperative rejection and recurrence rates are high.[8] Hence, new technologies are urgently needed to solve this problem.

Corneal collagen cross-linking (CXL) has become a research hotspot in the past 20 to 30 years. In the 1990s, Spoerl et al.[9] found that the reaction of photosensitizer riboflavin and ultraviolet A could cause corneal fiber to produce covalent bonds, which would enhance the cornea and its biomechanical stability; this was the basis for development of CXL. CXL was originally proposed for the treatment of keratectactic diseases, such as keratoconus.[10,11] In 2000, Schnitzler et al.[12] were the first to report the use of CXL for treatment of corneal ulcer, with a remarkable outcome. Subsequently, the efficacy of CXL was studied for use in the treatment of corneal ulcers; a large number of clinical reports regarding its efficacy have since emerged.[13–16]

Traditional CXL has been reported for the treatment of infectious keratitis;[17,18] however, the long duration of surgery and radiation in this method cause discomfort for patients.[17,19] Raiskup et al.[19] proposed the use of accelerated CXL, which can greatly shorten the duration of surgery and reduce damage to the eyes. Thus far, there have been few clinical studies regarding accelerated CXL in the treatment of infectious keratopathy; most of the published studies have been animal experiments, and biomechanical analyses of infectious keratitis have not been reported.

This study examined the clinical efficacy of accelerated CXL in the treatment of infectious keratitis; it also analyzed corneal biomechanics after CXL, with the aim of providing reference data for clinicians.

Patients and methods

Patients

This retrospective study included patients who had been diagnosed with infectious keratitis and treated with CXL in the Department of Ophthalmology in Jinan Second People's Hospital from December 2016 to July 2018,. All patients were informed of the surgical process and risks associated with surgery; they provided written informed consent to undergo surgery. The study protocol was approved by the Ethics Review Committee of Jinan Second People's Hospital. Because of the retrospective nature of the study, the committee waived the requirement for informed consent to participate in this study.

Inclusion criteria were one or more of the following: (1) Confocal microscopy or corneal scraping revealed fungal hyphae or *Acanthamoeba* cysts in the lesion; (2) bacterial culture showed bacterial growth; (3) the above examinations revealed negative results, but the patient's history and physical signs were insufficient to exclude infection. Exclusion criteria were one or more of the following: (1) lesion depth was more than two-thirds of the corneal thickness; (2) thickness of non-infiltrated cornea was $<400\,\mu m$; (3) viral keratitis was present; (4) the patient refused surgery.

Clinical evaluation

Visual acuity (VA), anterior segment optical coherence tomography (OCT, CIRROS HD-OCT4000, Carl Zeiss AG, Jena, Germany), and confocal microscopy (HRT3, Heidelberg, Heidelberg, Germany) examinations were performed in all patients at baseline, as well as at 1 week, 2 weeks, 1 month, 3 months, and 6 months postoperatively. In vivo confocal microscopy was used to evaluate whether keratitis comprised fungal or *Acanthamoeba* infection; it was also used to determine the effect of postoperative treatment. OCT was used to examine the depth of corneal lesions and degree of edema. Corvis ST (OCULUS, Wetzlar, Germany) was used to measure cornea biomechanical parameters (i.e., central corneal thickness [CCT] and deformation amplitude [DA]) and intraocular pressure (IOP) in 21 eyes where lesions were not within 5 mm of the central corneal area at baseline and after corneal epithelial healing (i.e., 3 months postoperatively). To eliminate the influence of age and individual differences, healthy fellow eyes were used as the control group.

Curative effects were evaluated as follows: (1) cure comprised disappearance of local irritation, resolution of the ulcer, and disappearance of hypopyon; (2) improvement comprised relief of local irritation symptoms, reduction of the ulcer area, and reduction or disappearance of hypopyon; (3) no effect comprised aggravation of symptoms, enlargement of the corneal ulcer surface, or presence of new corneal perforations.

Surgical procedure

Topical anesthetic was applied in a sterile environment. The infected area was curetted as neatly as possible. The CXL instrument (Avedro Inc., Waltham, MA, USA) was used to irradiate the cornea. Before irradiation, 0.1% riboflavin (Avedro Inc.) was instilled topically on the cornea, five times at 2-minute intervals. After sensitization with riboflavin, the cornea was exposed to ultraviolet-A, then directly irradiated with ultraviolet light at a wavelength of 365 nm. The irradiation intensity was $45\,mW/cm^2$ for 160 s; the total dose was $7.2\,J/cm^2$. After irradiation, the patient wore a soft hydrophilic contact lens (Alcon Laboratories, Beijing, China) and applied erythromycin eye ointment (Cisen Pharmaceutical Co., Ltd., Jining, China) with a sterile dressing. All procedures were performed by a single experienced chief physician. Anti-infective drugs were administered postoperatively; the corneal contact lenses were removed or replaced at 1 month postoperatively. If the condition was not effectively controlled by surgery, penetrating keratoplasty was considered. Cauterization with 2% silver nitrate corneal ulcer was performed for fungal keratitis, when necessary.

Statistical analysis

SPSS Statistics, version 19.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. The chi-squared test was used to analyze differences between the fungal and bacterial groups.

The Kolmogorov–Smirnov test was used to determine whether variables exhibited a normal distribution. The t-test was used to compare differences in normally distributed variables between the two groups; the Wilcoxon rank sum test was used to compare differences in non-normally distributed variables between the two groups. Linear regression was used to analyze the index correlation between non-contact tonometry IOP and pachymetry IOP. Differences were considered statistically significant when $P < 0.05$.

Results

Clinical characteristics at baseline

Patient characteristics are shown in Table 1. In total, 65 eyes from 65 patients were included in this study (37 men and 28 women; mean age, 57 ± 13 years; mean visit duration, 15.07 ± 11.16 days). Among these eyes, 51 (78.5%) exhibited fungal keratitis, 12 (18.5%) exhibited bacterial keratitis, one (1.5%) exhibited *Acanthamoeba* keratitis, and one (1.5%) exhibited an undetermined type of keratitis. Eyes were stratified into two groups, according to baseline ulcer size: $\leq 4\,mm \times 4\,mm$ (42, 64.6%) and $4\,mm \times 4\,mm$ to $7\,mm \times 7\,mm$ (23, 35.4%). Additionally, eyes were stratified into two groups according to baseline lesion depth: lesion depth of $<1/3$ (27, 41.5%) and lesion depth between 1/3 and 2/3 (38, 58.5%).

Table 1. Baseline demographic and clinical characteristics of patients with infective keratitis.

Parameter	
Patient number/eye	65/65
Sex (male/female)	37/28
Eyes (left/right)	24/31
Mean age (years)	57.00 ± 13.00
Type of inflammation (fungal keratitis/bacterial keratitis/*Acanthamoeba* keratitis/undetermined)	51/12/1/1
Ulcer size ($\leq 4\,mm \times 4\,mm$ vs. $4\,mm \times 4\,mm$ to $7\,mm \times 7\,mm$)	42/23
Lesion depth ($<1/3$ vs. between 1/3 and 2/3)	27/38
Mean visit duration (days)	15.07 ± 11.16

Cure rate

The overall cure rate was 93.85% (94.12% for eyes with fungal keratitis and 91.67% for eyes with bacterial keratitis); these cure rates did not significantly differ between the two groups (Table 2). One eye with *Acanthamoeba* keratitis and one eye with undetermined type of keratitis were cured. Cure rates were 95.23% in the group with baseline ulcer size $\leq 4\,mm \times 4\,mm$ and 91.30% in the group with baseline ulcer size $4\,mm \times 4\,mm$ to $7\,mm \times 7\,mm$; these cure rates did not significantly differ between the two groups (Table 2). Cure rates were 96.29% in the group with baseline lesion depth $<1/3$ and 92.10% in the group with baseline lesion depth between 1/3 and 2/3; these cure rates did not significantly differ between the two groups (Table 2).

Among the four eyes with poor surgical results, one eye with bacterial keratitis and one eye with fungal keratitis were ultimately treated with penetrating keratoplasty; two eyes with fungal keratitis were treated with 2% silver nitrate cauterization. No corneal endothelial dysfunction was observed in any patients.

Changes in VA and corneal thickness

For eyes that exhibited cure after CXL, the mean VA at each follow-up time point exhibited significant improvement, compared with the previous time point (Table 3,

Table 2. Cure rate.

Classification	Group	Baseline	Cure	Cure rate (%)	P
Type of inflammation	Fungal keratitis	51	48	94.121	0.754
	Bacterial keratitis	12	11	91.67	
	Acanthamoeba keratitis	1	1	100	
	Undetermined	1	1	100	
	Total	65	61	93.85	
Ulcer size	$\leq$4 mm $\times$ 4 mm	42	40	95.23	0.526
	4 mm $\times$ 4 mm to 7 mm $\times$ 7 mm	23	21	91.30	
	Total	65	61	93.85	
Lesion depth	$<$1/3	27	26	96.29	0.866
	Between 1/3 and 2/3	38	35	92.10	
	Total	65	61	93.85	

The χ^2 test was used to compare fungal keratitis and bacterial keratitis.

Table 3. Visual acuity (logMAR) in eyes cured after corneal collagen cross-linking treatment.

	Baseline	1 week	2 week	1 month	3 month	6 month
n = 61	1.00 $\pm$ 0.75	0.80 $\pm$ 0.59	0.63 $\pm$ 1.16	0.52 $\pm$ 0.56	0.31 $\pm$ 0.37	0.22 $\pm$ 0.29
t		2.99	3.16	2.20	4.23	3.86
P	–	$<$0.01	$<$0.01	$<$0.05	$<$0.001	$<$0.001

Data are shown as mean $\pm$ standard deviation. Values were compared by paired t-tests; P values indicate comparison with previous time point.
Abbreviation: logMAR, logarithm of the minimum angle of resolution.

Table 4. Corneal thickness (μm) in eyes cured after corneal collagen cross-linking treatment.

	Baseline	1 week	2 week	1 month	3 month	6 month
n = 61	841.68 $\pm$ 173.88	725.44 $\pm$ 93.87	650.68 $\pm$ 90.52	612.77 $\pm$ 86.60	572.65 $\pm$ 55.50	568.58 $\pm$ 40.01
Z		$-$5.67	$-$4.20	$-$4.03	$-$3.82	$-$0.49
P	–	$<$0.001***	$<$0.001***	$<$0.001***	$<$0.001***	0.626

Data are shown as mean $\pm$ standard deviation. Values were compared by Wilcoxon rank sum test; P values indicate comparison with previous time point.

$P < 0.001$ for all). Notably, mean corneal thickness values were markedly reduced at each follow-up time point, compared with baseline ($P < 0.001$). Furthermore, corneal thickness was significantly reduced at the 1-week, 2-week, 1-month, and 3-month follow-up time points, compared with each previous time point (Table 4, $P < 0.001$ for all).

Changes in hyphae in eyes with fungal keratitis

In vivo confocal microscopy was used to observe the growth of hyphae in eyes with fungal keratitis. For 48 eyes with fungal keratitis cured by CXL, hyphae showed a significant reduction at 1 week postoperatively, compared with baseline ($P < 0.01$; Figure 1). Furthermore, 42 eyes exhibited

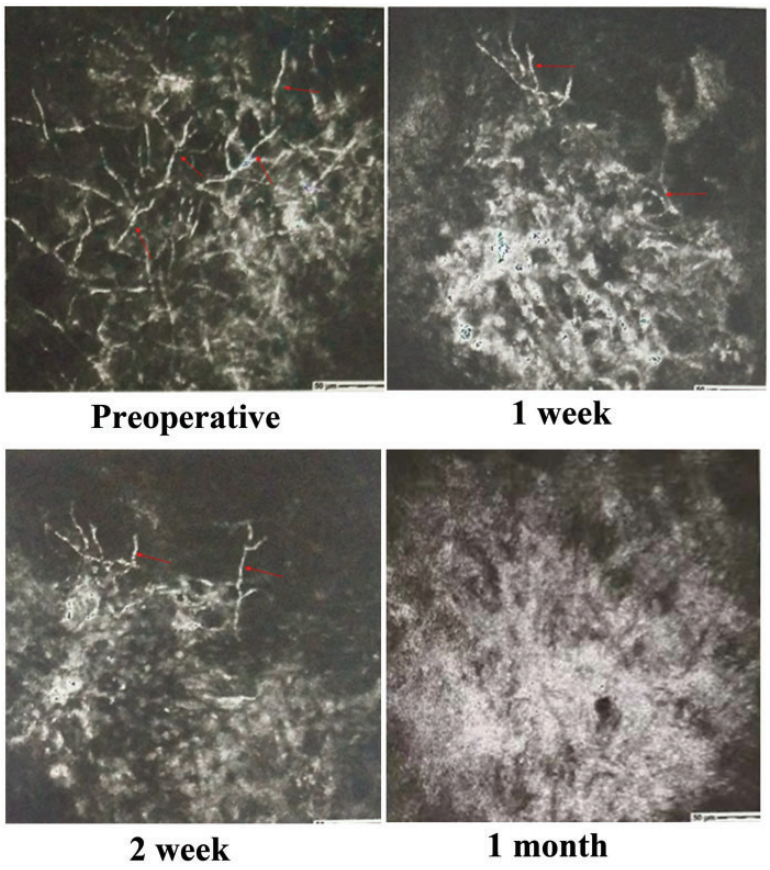

Figure 1. Confocal microscopy images of eyes with fungal keratitis at baseline, 1 week, 2 weeks, and 1 month postoperatively, showing that hyphae disappeared completely and formed corneal scars at 1 month postoperatively (red arrows indicate hyphae).

no hyphae growth at 2 weeks postoperatively; all 48 eyes exhibited no hyphae growth at 1 month postoperatively.

Changes in corneal biomechanical parameters

Corneal tissue exhibits biological viscoelasticity, and the study of its biological characteristics is of considerable importance for the diagnosis and treatment of corneal diseases.[20] Thus, corneal biomechanical parameters were compared at baseline and after surgery. Table 5 shows that postoperative CCT was significantly thinner than baseline CCT ($P < 0.001$), while postoperative CCT did not significantly differ relative to the control group. Additionally, postoperative DA was significantly smaller than baseline DA (Table 5, $P < 0.05$).

Linear regression analysis revealed that non-contact tonometry IOP and CCT were significantly positively correlated at baseline (Figure 2, $P < 0.05$). However, because of the small sample size, the scatter plot was atypical. Moreover, postoperative pachymetry IOP was markedly higher than baseline pachymetry IOP (Table 6, $P < 0.05$), whereas the difference between postoperative non-contact tonometry IOP

Table 5. Baseline, postoperative, and control corneal biomechanical parameters.

	Baseline	Postoperative	Control
CCT (μm)	639.95 ± 104.96	535.29 ± 49.91	540.76 ± 24.97
t	4.13		0.05
P	<0.001*** (baseline vs postoperative)	–	0.655 (control vs postoperative)
DA (mm)	1.24 ± 0.18	1.06 ± 0.05	1.13 ± 0.10
t	−4.89		3.09
P	<0.001*** (baseline vs postoperative)	–	<0.05* (control vs postoperative)

Data are shown as mean ± standard deviation. Values were compared by paired t-test.
Abbreviations: CCT, central corneal thickness; DA, deformation amplitude.

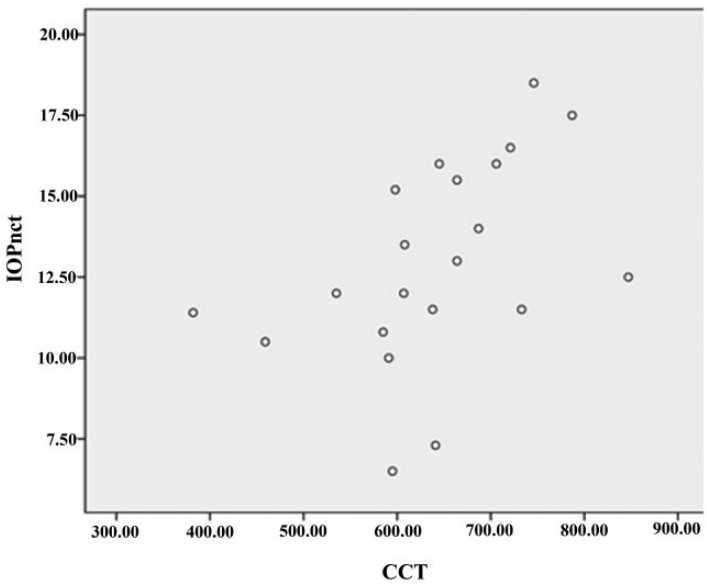

Figure 2. Positive correlation between non-contact tonometry intraocular pressure and central corneal thickness before corneal collagen cross-linking.
Abbreviations: CCT, central corneal thickness; CXL, corneal collagen cross-linking; IOPnct, non-contact tonometry intraocular pressure.

Table 6. Baseline and postoperative intraocular pressure measurements.

	Baseline	Postoperative	t	P
Non-contact tonometry IOP (mmHg)	12.94 ± 3.14	11.35 ± 2.35	1.754	0.095
Pachymetry IOP (mmHg)	9.20 ± 3.14	11.25 ± 2.38	−2.179	0.04

Data are shown as mean ± standard deviation. Values were compared by paired t-test.
Abbreviations: IOP, intraocular pressure.

and baseline non-contact tonometry IOP was not statistically significant.

Discussion

Infectious keratitis is a vision-threatening disease with an increasing incidence, as well as a high recurrence rate after current treatment;[21] thus, new treatment methods are needed. In the present study, accelerated CXL was performed in 65 eyes of 65 patients who had been diagnosed with infectious keratitis; the overall cure rate was 93.85%. After 6 months of follow-up, the VA of cured eyes was significantly improved, while corneal thickness was significantly reduced. The hyphae growth in eyes with fungal keratitis was completely inhibited at 1 month postoperatively. Corneal biomechanical parameters (CCT, DA, and pachymetry IOP) were also significantly improved, compared with baseline.

As a new technology for treatment of corneal diseases, CXL has become a research hotspot in the past 20 years. This surgery uses riboflavin and ultraviolet-A to increase the biomechanical strength of the cornea through photochemical cross-linking of individual collagen fibers in the anterior stroma.[9] Initially, CXL was proposed for treatment of keratoectasia (e.g., progressive keratoconus), in which it demonstrated good results.[22,23] Riboflavin/ultraviolet-A combination therapy has better germicidal efficacy than ultraviolet-A treatment alone.[24] With the continuous development of this technology, CXL has been identified as a safe and effective treatment for infectious keratitis. Iseli et al.[25] performed CXL treatment on patients with fungal keratitis in whom antibiotic treatment was ineffective; they reported that five patients were cured and exhibited no primary recurrence during 9 months of follow-up. Al-Sabai et al.[26] performed CXL treatment in one patient who exhibited *Pseudomonas aeruginosa* keratitis, which had been resistant to drug treatment. After healing, a corneal scar was formed and corneal perforation was avoided. Additionally, CXL has achieved good results in the treatment of corneal ulcers with unclear infection type, for which antibiotic treatment was ineffective.[27] These findings indicated that CXL could be used to treat infectious keratitis; however, these publications all constituted case reports. Various literature reviews have also been performed. Papaioannou et al.[18] published a systematic review and meta-analysis of CXL in the treatment of infectious corneal ulcers; notably, the effective rate of CXL treatment reached 85.7%. In a review of 12 studies, Alio et al.[28] found that CXL was able to control corneal ulcer development in 85% of eyes. To the best of our knowledge, there have been few reports regarding large samples. The present study used a large sample size to evaluate the clinical efficacy of CXL in the treatment of various aspects of infectious keratitis. This analysis revealed that CXL was equally effective in the treatment of fungal keratitis and bacterial keratitis.

In the past 10 years, research has continued regarding CXL. PACK-CXL (a version of CXL that involves linking with photoactivated riboflavin) was proposed at the 9th CXL conference in Dublin (Ireland); this method is mainly used to treat infectious keratitis.[17] Said et al.[29] found that the use of PACK-CXL in the treatment of concomitant corneal dissolution and infectious corneal disease could not shorten the course of disease and improve vision correction; however, it could significantly reduce the incidence of complications. Nevertheless, the efficacy and safety of PACK-CXL in the treatment of fungal keratitis remain uncertain; it should be used with caution in eyes with severe deep fungal infection, and its usefulness in eyes

with *Acanthamoeba* keratitis is unclear.[30] Our analysis of accelerated CXL demonstrated significant improvement of VA, as well as safety and efficacy in treatment of fungal keratitis. Additionally, one eye diagnosed with *Acanthamoeba* keratitis achieved good outcomes in the present study, which suggests that accelerated CXL is also an effective treatment for *Acanthamoeba* keratitis. However, the effectiveness of accelerated CXL in the treatment of *Acanthamoeba* keratitis is not fully established and should be confirmed in a large study.

The crosslinker used in this study was produced by Avedro, Inc., in the United States. The crosslinker has a rapid crosslinking mode, which is significantly enhanced compared with that of the traditional crosslinker. Specifically, the traditional crosslinking instrument requires 30 minutes of irradiation,[31,32] whereas the accelerated crosslinking instrument only requires 160 s to reach the required energy level of 7.2 J/cm^2; this greatly reduces the duration of surgery and associated discomfort in the patient. When determining the irradiation distance, the accelerated crosslinker focuses on the double "ten" words, which comprises adjustment of the crosslinking instrument irradiation head, alignment of the two "ten" characters to the corneal lesion area, and subsequent adjustment of irradiation distance to establish the lesion area. This approach provides greater accuracy than the traditional crosslinker when determining irradiation distance, thereby achieving a better crosslinking effect. In the present study, the VA was significantly improved in eyes that underwent CXL, compared with baseline; no corneal endothelium decompensation occurred.

In this study, although most eyes with infectious keratitis were cured after CXL treatment, four eyes did not achieve effective control, including one eye with bacterial keratitis and three eyes with fungal keratitis. During follow-up treatment, one eye with bacterial keratitis and one eye with fungal keratitis underwent keratoplasty; the remaining two eyes with fungal keratitis were subjected to silver nitrate cauterization and achieved cure. Thus, for patients with CXL treatment failure, drug control should be considered initially. If the condition does not improve, bacterial keratitis should be treated with keratoplasty, while fungal keratitis can be treated with silver nitrate cauterization or keratoplasty. The lesion will increase in size after cauterization; however, the scope of cauterization will gradually diminish, resulting in improved vision. Importantly, silver nitrate exhibits considerable corrosiveness.[33] After cauterization, patients experience strong irritation symptoms within 1 to 2 days, and the corneal ulcer forms a severe corneal scar after healing. If the lesion is located in the central area of the cornea, the patient's vision will be substantially impacted by cauterization. Although the corneal scar will gradually fade, this process is slow, and the patient will experience reduced quality of life during recovery. Hence, silver nitrate treatment is not the first choice for eyes with fungal keratitis.[34]

In conclusion, accelerated CXL is a safe and simple surgical method for eyes with infectious keratitis. It can shorten the course of disease and improve vision for cured patients. Our findings illustrate that accelerated CXL can be used as an effective supplementary treatment for eyes with infectious keratitis, thus reducing the incidence of keratitis complications, improving the cure rate of keratitis, and reducing the need for therapeutic keratoplasty. However, this study mainly focused on the use of CXL in patients with infectious keratitis that was resistant to drug treatment. Additional research is needed to determine whether CXL should be used in eyes with early-stage infectious keratitis.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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