Cross-linking for Corneal Ulcers Treatment Trial (CLAIR)
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Cross-Linking Assisted Infection Reduction

CLAIR

Manual of Operations and Procedures (MOP)

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1. INTRODUCTION AND BACKGROUND

Cross-linking (CXL), a process by which collagen fibrils form covalent bonds with adjacent molecules, was first discovered in the corneas of diabetic patients who cross-link through glycosylation and have a decreased incidence of keratoconus.[1] It is currently used as a treatment for corneal ectatic disorders such as keratoconus and post-LASIK ectasia. CXL involves applying riboflavin to the cornea and photo-chemically activating it with UV light. This has been shown to strengthen the cornea and allow it to retain its normal shape.[1-4] There has been recent interest in corneal CXL as an adjuvant therapy for infectious keratitis.[5] *There are two mechanisms by which CXL may benefit patients with infectious corneal ulcers; antimicrobial effects, and increased resistance of corneal tissue to enzymatic degradation.*

***In vitro* efficacy of UV-A + riboflavin against the most common bacterial ocular pathogens, such as *Pseudomonas aeruginosa* and *Streptococcus pneumonia* has been demonstrated.**[6] In addition to these *in vitro* data, multiple case reports have suggested potential benefits of collagen CXL for treatment of bacterial and fungal keratitis, such as symptomatic improvement, resolution of resistant infection, and halting of progressive melting.[7-10] One small study treated a series of patients with bacterial keratitis exclusively with photo-chemically activated riboflavin, which resolved infection in all patients.[11] Interestingly, only 2 of the 16 study participants required topical antibiotic treatment. *Thus, riboflavin + UV-A could potentially decrease antibiotic resistance and ocular surface toxicity that can complicate the current management of bacterial keratitis.*

Filamentous fungal corneal ulcers continue to have a poor prognosis and further investigation into their treatment is warranted. Unfortunately, *in vitro* collagen CXL alone has failed to demonstrate fungal pathogen inactivation.[6,12] However, one study showed improved inhibition of fungus *in vitro* with amphotericin plus riboflavin+UV-A compared with amphotericin alone.[6] The authors suggest that CXL may improve diffusion of antifungal medications or inhibit fungal proliferation through more complex mechanisms. Although the evidence for cross-linking as a treatment for fungal keratitis is less robust, clinicians have already begun using UV-A + riboflavin in conjunction with antifungals in hopes that there will be some benefit in this challenging disease. Thus, scientific investigation into this treatment approach is necessary.

Research Question: Is collagen cross-linking a useful adjuvant in the treatment of infectious corneal ulcers?

1.1 Specific Aims:

1. To determine if patients with culture positive bacterial and fungal keratitis clear their repeat cultures more quickly with the addition of collagen cross-linking.

- We hypothesize that there will be an increased rate of negative cultures immediately post procedure in the CXL group compared with sham CXL.

2. To determine if CXL can decrease enzymatic degradation and prevent corneal thinning and perforation.

- Hypothesis 2A) There will be a decreased rate of perforation among study participants treated with CXL.
- Hypothesis 2b) There will be less corneal thinning as measured by optical coherence tomography in the CXL group at 3 months.

3. To determine what effect CXL has on visual acuity, corneal astigmatism, and 3-month infiltrate/scar size.

- Hypothesis 3A) There will be improved visual acuity with reduced 3-month infiltrate/scar size among study participants treated with CXL.
- Hypothesis 3B) There will be no difference in irregular astigmatism (as measured by topography) between participants in the CXL group versus antibiotics alone.

1.2 Study Outcomes:

Primary Outcome:

- Microbiological cure on repeat culture
    - *CLAIR I.* Bacterial ulcers – Culture at 4 hours
    - *CLAIR II* Fungal ulcers – Culture at 24 hours

Secondary Outcomes:

- BSCVA at 3 months
- Scar size/depth, as measured by clinical exam, slit lamp photographs and OCT
- Adverse events including rate of perforation/need for TPK
- Thinning as measured by pachymetry and OCT
- Topography and corneal higher-order aberrations
- Change in IND-VFQ from *baseline* to *3 months*.

1.3 Study Design:

Cross-Linking Assisted Infection Reduction (CLAIR) I for bacterial ulcers and CLAIR II for fungal ulcers is a series of randomized, masked, clinical trials. The purpose of these studies is to determine differences in microbiological cure for 1-day repeat cultures between different medical antimicrobial treatments alone versus antimicrobial treatment plus collagen cross-linking. There will be 1:1 randomization to each of these treatment groups:

CLAIR I. Bacterial Ulcers:

1) Topical 0.5% Moxifloxacin alone
2) Topical 0.5% Moxifloxacin plus cross-linking

CLAIR II. Fungal Ulcers:

1) Topical 5% natamycin alone
2) Topical 5% natamycin plus cross-linking
3) Topical 0.15% amphotericin alone
4) Topical 0.15% amphotericin plus cross-linking

2. ORGANIZATION

Aravind Eye Hospital, Madurai along with the University of California San Francisco (UCSF) will jointly execute these clinical trials. Aravind Eye Hospital will mainly be responsible for recruitment and enrollment, intervention implementation, and follow-up visits. UCSF will take the lead on all data analysis, writing of study-related materials, and writing journal publications.

2.1 Collaborating Institutions:

Aravind Eye Hospital, India

Dr. N. Venkatesh Prajna will be the lead investigator at Aravind Eye Hospital. All recruitment, treatment/intervention, and follow-up visits will be done at the Aravind Eye Hospitals. All study personnel assisting with the research will be adequately informed about the protocol, the research procedures, and their duties and functions through ongoing communication between the 2 sites.

Aurolab at Aravind Eye Hospital in Madurai will be responsible for preparing and distributing study medications. The microbiology lab in Madurai will perform the cultures and serve as a bank for all specimens collected. Madurai microbiology lab will serve as a reference lab that will assist with any difficult microbiological identification.

Francis I. Proctor Foundation, University of California, San Francisco, USA

The Proctor Foundation is an organized research unit at the University of California, San Francisco. The Foundation has a 56-year history of research in ocular infectious and inflammatory disease and runs one of the leading corneal fellowship training programs in the United States. Proctor Foundation faculty have been involved in prevention of blindness research in developing countries since the Foundation's inception. The impetus for establishing the Foundation in 1947 was to eradicate trachoma in the American Southwest and other parts of the world.

UCSF will perform the data analysis. UCSF will not consent any participants, perform surgeries, or collect study data. UCSF will have the most current protocol, consent documents and HIPAA authorization for reference. UCSF has received IRB approval for this study (IRB number: 14-14918). All modifications to either IRB of record will be communicated between sites. UCSF will ensure protection of all study-related data by using de-identified information over a secure server. All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy. There will be ongoing communication of problems, interim results, and study closure between both sites.

Jennifer Rose-Nussbaumer, MD is the co-PI at UCSF for this study. Dr. Rose-Nussbaumer is a Cornea fellowship trained Ophthalmologist at F. I. Proctor Foundation. In addition to her clinical work in cataract and corneal transplant surgery, she is an NIH funded clinical researcher. She is studying corneal ulcer treatment in India and Nepal and corneal transplant outcomes in Ethiopia. She is the principle investigator on the Corneal Preservation Time Study (CPTS) and Descemets Endothelial Thickness Comparison Trial (DETECT) at UCSF. Her previous vision research in Ophthalmology includes work with the World Health Organization on Trachoma, as well as investigating the ocular manifestations of HIV disease. Dr. Rose-Nussbaumer's role in this study will be to perform data analysis, to collaborate with surgery site and eye bank regarding study design and protocols.

3. PATIENT FLOW

3.1 Study Timeline

When the patient presents to clinic with infectious keratitis and undergoes corneal scraping by the treating ophthalmologist, a specimen will be submitted for microbiological testing (KOH and Gram stain bacterial or fungal). This is a routine practice already in place at the hospital. If the patient has a positive KOH or Gram stain, the patient will be approached by study personnel to be enrolled in study.

If enrolled, study subjects will follow the procedures below - if bacteria positive on Gram stain, the subject will undergo procedures outlined for CLAIR I. Bacterial ulcers; if positive for fungus on KOH, subject will undergo procedures outlined for CLAIR II. Fungal ulcers.

Study participants will be required to have 4 follow-up visits, one for the cross-linking procedure and re-scraping, one at 3 days for re-scraping, one at 3 weeks (2.5 – 5 weeks) and one at 3 months (visit window: 2.5 -3.5 months). Additional visits may be needed and will be determined by the physician/investigator.

3.2 Eligibility Requirements

Only those who meet all of the inclusion criteria and none of the exclusion criteria will be enrolled in this study.

Inclusion criteria:

- Corneal ulcer that is smear positive for either bacteria, filamentous fungus
- Pinhole visual acuity worse than 6/21 (20/70) in the affected eye
- Age over 18 years
- Basic understanding of the study as determined by the physician
- Commitment to be hospitalized for 3 days
- Commitment to return for follow up visits

Exclusion criteria:

- No evidence of concomitant infection on exam or gram stain (i.e. herpes, both bacteria and acanthameoba on gram stain)
- Impending or frank perforation at recruitment
- Involvement of sclera at presentation
- Non-infectious or autoimmune keratitis
- History of corneal transplantation or recent intraocular surgery
- No light perception in the affected eye
- Pinhole visual acuity worse than 20/200 in the unaffected eye
- Participants who are decisionally and/or cognitively impaired
- Involvement of posterior 1/3 of stroma at presentation for fungal patients

3.3 Randomization

Each study eye will be randomly assigned to the treatment group. Block randomization will be performed using a computer program (Statistical package R; Version 2.12; R Foundation for Statistical Computing, Vienna, Austria) by the coordinating site.

Once an eye is enrolled in the study, the study coordinator will assign the study participant's eye an ID (alpha-numeric code) and organize the procedure in the operating room within 3 hours for bacterial ulcers, and within 24 hours for fungal ulcers. Once the study participant has been assigned a study participant ID and randomized to treatment group, they will be included in the intention to treat analysis.

3.4 Study visits

3.3.1 Baseline Visit. During this visit, eligible patients will be enrolled in the study and will give consent. The baseline patient form will be completed and baseline topography will be obtained.

CLAIR I. Bacterial Ulcers:

Study participants will be randomized to one of two possible treatment arms: 1) topical 0.5% moxifloxacin alone 2) topical 0.5% moxifloxacin plus CXL. Those randomized to CXL will undergo the procedure in the operating theatre within 4 hours. Both arms will undergo repeat scraping at 4 hours post enrollment. Thereafter, all study participants will start 0.5% moxifloxacin every hour. All patients will be admitted to the inpatient setting until the 3rd day.

CLAIR II. Fungal Ulcers:

They will be randomized to one of four possible treatment arms 1) topical 5% natamycin alone, 2) topical 5% natamycin plus CXL, 3) topical 0.15% amphotericin alone or 4) topical 0.15% amphotericin plus CXL. All study participants will begin medical therapy immediately (either natamycin or amphotericin) every hour and those randomized to CXL will undergo the procedure in the operating theatre within 24 hours. All study participants will undergo repeat scrapping at 24 hours from enrollment. All patients will be admitted to the inpatient setting until the 3rd day.

3.3.2 Procedure visit. All study participants randomized to CXL will have the procedure in the operating theater. This will occur within 4 hours for bacterial ulcers and within 24 hours of the baseline visit for fungal ulcers. Once a study participant has been randomized to treatment, he will have the treatment even if there is a delay in the operating theatre that results in delayed treatment, however re-scraping will occur at 4 hours for bacterial ulcers and 24 hours for fungal ulcers even if they have not yet had cross-linking. If the cross-linking occurs after repeat scrapping, a third smear and culture should be performed.

3.3.3 Day 3 visit. During this visit, study participants will have BSCVA recorded, IND-VFQ, slit lamp examination and undergo repeat scraping and culture of the corneal ulcer. Thereafter, all additional treatments including but not limited to topical antimicrobials, or oral antimicrobials, will be at the clinician's discretion. At this point patients may be discharged from the inpatient setting.

3.3.4 *3 week visit.* During this visit study participants will have BSCVA recorded, slit lamp examination, and they will have digital photography.

3.3.5 Final Visit. At the 3- Month visit study participants will have their BSCVA and slit lamp examination recorded, in addition to repeat topography and be administered the IND-VFQ.

3.5 Study Schedule

	Visit 1 Baseline Exam	Visit 2 Procedure & Repeat Culture	Visit 3 3-Day Repeat Culture	Visit 4 3 week Follow-up	Visit 5 Month 3 Final
Forms					
Consent and Authorization form	X				
Baseline form	X				
NEI-VFQ			X		X
Follow-up form			X	X	X
Final form					X
Procedures					
Cross-linking		X			
Tests					
IOP	X		X	X	X
Pachymetry U/S	X		X	X	X
Pentacam Topography	X				X
Slit Lamp Photography*	X			X	X
BSCVA/ETDRS/MRx		X		X	X
Total visit time	2 hours	2 hours	2 hours	1 hour	1 hour
					* Slit lamp photography also taken upon an adverse event

3.6 Adverse Events (AEs)

During each study visit, the subject will be questioned about AEs in a non-leading manner. All AEs, whether observed by the Investigator, elicited by the Investigator, or spontaneously reported by the subject, will be documented in the subject's chart and the adverse event form. Slit lamp photography will also be completed after any adverse event takes place.

All AEs should be followed until they are resolved or until a stable clinical endpoint is reached. Each AE is to be classified by the Investigator as SERIOUS (SAE) or NONSERIOUS (NSAE).

If a SAE occurs, the investigator at Aravind Eye Hospital, Madurai must fill out the serious adverse event form and email it to Stephen Mcleod at UCSF within 24 hours of the occurrence of the SAE. The investigator must provide written follow-up reports until the SAE or clinically significant AE has resolved or until a stable clinical endpoint is reached. Notification of an SAE or clinically significant AE must also be submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) in accordance with its requirements. All AEs must be reported from the time that the subject provides informed consent through the last study visit.

4. PROCEDURES

4.1 Masking

Due to the nature of the intervention, the study participant, surgeon and technician performing cross-linking will not be masked, however, the physician performing repeat scraping as well as the microbiologist will be masked to treatment arm. The refractionist performing the BSCVA will also be masked. UCSF study personnel will not be given any identifying information.

4.2 Procedures

4.2.1 Corneal Gram Stain and Culture

After patient history and slit lamp examination at the enrollment visit (Day 0), corneal scraping is performed to determine the eligibility of the patient (positive fungal stain and negative bacterial stain). Corneal scraping is recommended as standard of care for all corneal ulcer patients at the Aravind Eye Hospitals. There is a small risk to patients of inducing a perforation, worsening an epithelial defect, or contaminating an ulcer, and patients are duly informed of these risks prior to the taking of the corneal scrape.

Corneal scraping will be obtained as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye to be examined.
- Aseptic technique is used to obtain each corneal scrape. A flame-sterilized Kimura spatula is used, with the aid of slit lamp magnification, to obtain a scrape from the leading edge and base of the corneal ulcer. The Kimura spatula is again flame sterilized between the takings of each sample.
- Two scrapings are smeared directly on to two separate glass microbiology slides for Gram stain and for KOH wet mount (if necessary, Giemsa or Gram Stain can be used to identify fungal elements as well).
- Three further scrapings are taken and directly inoculated on to sheep's blood agar, chocolate agar, potato dextrose agar or Sabouraud's agar for bacterial and fungal culture.

4.2.2 Collagen Cross-Linking

All study participants randomized to collagen cross-linking will have cross-linking within 4 hours for bacterial ulcers and within 24 hours for fungal ulcers. They will be taken to the operating theatre and the procedure will be performed under sterile conditions.

Cross-linking will be performed as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye.
- A 30-minute loading dose of 0.1% topical riboflavin and 20% dextran T500 drops will be administered every 2 minutes.
- The cornea will be exposed to UV-A light at a wavelength of 365nm with irradiance of 3mW/cm^2 for a total of 30 minutes.
- Throughout the UV-A treatment the study participant will continue to receive topical riboflavin Q5 min intervals during treatment.
- The study participant will be transferred back to the microbiology laboratory for repeat scraping and culture (see section 4.4.1).

4.3 Pentacam Protocol

Cornea densitometry and topography will be documented using the Pentacam HR (high resolution) Scheimpflug camera at baseline and 3 months. The images will be captured using standardized protocols by study certified ophthalmic technicians. The data will then be shared with UCSF via Dropbox.

In order to obtain the highest quality Pentacam HR Scheimpflug camera examination, the technician will scan the appropriate eye according to the Pentacam HR instruction manual.

4.3.1 Imaging Procedures

Corneal densitometry and topography measurement

- The examination will be performed in a designated diagnostic room with a uniform ambient light level.
- To begin the corneal densitometry measurement, the technician will select the examination scan for cornea densitometry measurement, per the Pentacam HR manual.
- The cornea densitometry measurement will consist of a measurement protocol of 25 images under the examination mode of 3D scan.

4.3.2 Check Scan Quality

- If Quality Specifications ("QS") field reads "OK," then the measurement is correct and reproducible.
- If the "QS" field is highlighted YELLOW, the examination will be repeated until an "OK," reading is obtained.
- If the "QS" field is RED, then the examination must be repeated.
- If an "OK" reading is not obtainable, then the best of 3 examinations will be saved.

4.3.3 Saving Image

- Download data to excel or save to USB drive
- De-identify data
    - Patient names or any other identifying information such as birthday should not appear within any of the image files.
- Rename data
    - The image files will be designated with: 1) the eye ID number, 2) the timing of visit '3m', '6m', or '12m' respectively for the 3, 6, and 12-month follow-up visits, and 3) image number of the series. An image renamed to C101_3m_1.jpg will be the first image for eye number C101's 3 month follow-up visit. An image renamed to C222_12m_3.jpg will be the third image for eye 222's 12 month follow-up visit.
- Batch upload images to Dropbox. Procedures for uploading images to Dropbox:
    - Nagivate to Dropbox → eye's folder → relevant study visit
    - Upload images to the eye's relevant study visit folder

5. STUDY MEDICATIONS

5.1 Medical management protocol & Treatment Schedule

All study participants will receive the same medical management in the initial 3 day study period. **Table 2** outlines the dosing schedule for medications for CLAIR I and CLAIR II. Study participants will be randomized to medical management plus CXL versus medical management alone. This study requires 5 visits over 3 months including baseline visit, procedure visit, 3-day follow-up, 1 and 3-month follow-up. After the 3-day repeat culture visit additional treatments may be added per clinician preference including but not limited to topical and oral antimicrobials.

Table 2: Dosing schedule for Medications in the first 3 days.

	CLAIR I	CLAIR II
Moxifloxacin 0.5%	Q 1 hours x 3 days	
Natamycin 5%		Q 1 hour x 3 days*
Amphotericin 0.15%		Q 1 hour x 3 days*
Homatropine	TID x 3 days	TID x 3 days

*50% randomized to Natamycin 5% and 50% randomized to Amphotericin 0.15%

5.2 Possible Side Effects of Study Medications

Amphotericin: eye irritation, swelling, pain and/or redness, discharge

Homatropine: eye irritation, swelling, pain, or redness; Eye dilation may result in temporary blurred vision, light sensitivity, and increased heart rate.

Moxifloxacin: eye irritation, swelling, pain, or redness

Natamycin: eye irritation, swelling, pain and/or redness, discharge

6. PROTECTION OF HUMAN SUBJECTS

6.1 Institutional Review Board Approval

6.1.1 Aravind Eye Hospital, Ethics Review Committee The Ethics Committee with review the study protocol annually for ethical approval.

6.1.2 University of California, San Francisco Committee on Human Research (CHR) The UCSF CHR will review the study protocol annually for ethical approval.

6.2 Informed Consent

Aravind personnel will obtain full written consent from each patient. The primary surgeon will screen participants and determine their eligibility. He/she will clearly explain the process and risks involved and will also ask the patient to sign any necessary consent documents. The study participant will have 4 visits total per eye included in the study over 3 months; visits include hospitalization for the first 3 days and a procedure visit. The patient has the

ability to withdraw at any time and will not be forced into anything with which he/she is not comfortable. Consent documents have been uploaded to the IRB application.

6.3 Risks to Study Participants

Blindness from infectious corneal ulcers is a worldwide public health problem. If our hypothesis is correct, and corneal crosslinking provides an additive benefit to topical antimicrobials in the treatment of corneal infections, there could potentially be a profound societal benefit. The process of corneal cross-linking is painless. Adverse events associated with corneal cross-linking appear to be exceedingly rare. A recent publication of 3-year follow-up of 100 keratoconus eyes, found two adverse events: one instance of corneal infection and one case of corneal edema, which resolved within one week.[13] In a series of 16 patients with bacterial ulcers and presenting average visual acuity of approximately 20/100 (including one patient with 20/20 vision) no complications or side effects of treatment were observed.[11] Additionally, a meta-analysis of the literature concerning cross-linking for infectious keratitis concluded that the available evidence supports the use of cross-linking for the treatment of infectious keratitis.[5] All procedures will be performed by ophthalmologists board certified in the study country who are well versed in all planned treatment procedures.

We anticipate minimal risk for study patients. This fact, coupled with the potential benefit the data provided by the study could provide in terms of guiding future therapies for corneal infections, makes the minimal risks posed to the patient entirely reasonable.

There may be some discomfort during follow-up testing (BSCVA, IOP, slit lamp, Pachymeter, Topography, and photo imaging of the eye), but this will be kept to a minimum. The participant will be asked to tell the doctor if any of this testing feels painful. There may be a medication reaction such as eye irritation, swelling, pain, redness, or discharge. If this occurs the risks of stopping the medication must be weighed against the severity of infection and other treatment options.

6.4 Privacy, Confidentiality and Data Security

All data will be stored and transferred using a secure server with de-identified information.

We will take steps to keep the participant's personal information confidential. Health information will be kept secure and separate from information which identifies participants. Upon enrollment, participants will be assigned a code that will be used instead of their name, medical record number or other personally identifying information. Electronic files for data analysis will contain only the participant code. The code will be stored at Aravind on a secure server and will only be accessed by study personnel – the PI, study coordinator, or other member of the study team). Access to study-related patient information will be limited only to members of the study team.

Only a small number of researchers related to this study will have direct access to the participant's medical information. Paper files will be stored in locked filing cabinets in restricted access offices at Aravind. Access to data/specimens is restricted to study personnel.

Information may be released to others outside of Aravind who are involved in coordinating or overseeing research, but information which identifies the participant will be kept secure. To ensure the success of this study, Aravind has partnered with University of California, San Francisco (UCSF)'s F.I. Proctor Foundation, which is a center that specializes in ophthalmology research. The coordinating center at UCSF will have access to de-identified information using a code number. UCSF's role is to support Aravind on the data analysis portion of this study and assisting with the development of consent and study-related materials. UCSF has obtained IRB approval to take part in this study (IRB number: #14-14918).

6.5 Exclusion of Vulnerable Populations

The following populations will not be enrolled in this study: children (18 years and under), pregnant women, decisionally impaired adults, and prisoners.

6.6 Compensation to Participants

There is no additional cost or compensation for the study participant.

7. DATA COLLECTION AND MANAGEMENT

7.1 Data Collection and Entry

All data will be collected using hardcopy patient forms at Aravind during baseline and follow-up visits. De-identified data will be uploaded to a Box account in order to share the data with the UCSF study coordinator. De-identified data will be input into a database using double data entry at UCSF.

7.2 Data Consistency, Validity, and Monitoring

Data monitoring reports will be prepared using STATA and Excel. If the forms are not filled out completely, the UCSF study coordinator will contact the person responsible for completing the form to provide the missing data, or clarify any inconsistent data. The Aravind study coordinator is the only person who is authorized to add missing data or make any changes to the study forms. All changes should be made with a red ink pen, and then signed and dated.

7.3 Data Storage and Security

De-identified data will then be input into a database using double data entry at UCSF. Data will be stored at UCSF for about 10 years.

8. STATISTICAL ANALYSIS PLAN

Please see the **Statistical Analysis Plan (SAP)** for details on the statistical analysis.

9. REFERENCES

1. Keating A, Pineda R, 2nd, Colby K. Corneal cross linking for keratoconus. *Seminars in ophthalmology.* Sep-Nov 2010;25(5-6):249-255.
2. Lamy R, Netto CF, Reis RG, et al. Effects of corneal cross-linking on contrast sensitivity, visual acuity, and corneal topography in patients with keratoconus. *Cornea.* May 2013;32(5):591-596.
3. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *Journal of cataract and refractive surgery.* May 2008;34(5):796-801.
4. Vinciguerra P, Albe E, Trazza S, Seiler T, Epstein D. Intraoperative and postoperative effects of corneal collagen cross-linking on progressive keratoconus. *Archives of ophthalmology.* Oct 2009;127(10):1258-1265.
5. Alio JL, Abbouda A, Valle DD, Del Castillo JM, Fernandez JA. Corneal cross linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. *Journal of ophthalmic inflammation and infection.* 2013;3(1):47.
6. Martins SA, Combs JC, Noguera G, et al. Antimicrobial efficacy of riboflavin/UVA combination (365 nm) in vitro for bacterial and fungal isolates: a potential new treatment for infectious keratitis. *Investigative ophthalmology & visual science.* Aug 2008;49(8):3402-3408.
7. Panda A, Krishna SN, Kumar S. Photo-activated riboflavin therapy of refractory corneal ulcers. *Cornea.* Oct 2012;31(10):1210-1213.
8. Iseli HP, Thiel MA, Hafezi F, Kampmeier J, Seiler T. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. *Cornea.* Jun 2008;27(5):590-594.
9. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. *Cornea.* Dec 2010;29(12):1353-1358.
10. Shetty R, Nagaraja H, Jayadev C, Shivanna Y, Kugar T. Collagen crosslinking in the management of advanced non-resolving microbial keratitis. *The British journal of ophthalmology.* Aug 2014;98(8):1033-1035.
11. Makdoumi K, Mortensen J, Sorkhabi O, Malmvall BE, Crafoord S. UVA-riboflavin photochemical therapy of bacterial keratitis: a pilot study. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie.* Jan 2012;250(1):95-102.
12. Sauer A, Letscher-Bru V, Speeg-Schatz C, et al. In vitro efficacy of antifungal treatment using riboflavin/UV-A (365 nm) combination and amphotericin B. *Investigative ophthalmology & visual science.* Aug 2010;51(8):3950-3953.
13. Wittig-Silva C, Chan E, Islam FM, Wu T, Whiting M, Snibson GR. A randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus: three-year results. *Ophthalmology.* Apr 2014;121(4):812-821.