Publications using C-Eye device and Ribo-Ker

(since 2017)

September 7, 2025

Peer-reviewed publications

1. **2025.** Lu, N.J.*, et al.* Transmission rates of UV-A and green light in an ex vivo corneal crosslinking model for infectious keratitis. J Cataract Refract Surg 51, 249-256

PURPOSE: To investigate the light transmission (LT) of UV-A and green light through infected corneas saturated with riboflavin or rose bengal in an ex vivo porcine model for infectious keratitis. SETTING: University of Zurich, Zurich, Switzerland, and Empa, St. Gallen, Switzerland. DESIGN: Laboratory study. METHODS: Ex vivo porcine eyes (n = 162) were divided into 3 groups: control eyes, eyes infected with Staphylococcus aureus , and eyes infected with Pseudomonas aeruginosa . Corneas remained either uninfected or were infected with S. aureus and P. aeruginosa and were either left untreated or were instilled with 0.1% riboflavin or 0.1% rose bengal. Corneal buttons were prepared, and corneal LT was measured at 365 nm and 522 nm using a spectrophotometer. LT values were calculated and compared. Transmission electron microscopy (TEM) was used to visualize structural damage and bacteria within infected corneas. RESULTS: Riboflavin-saturated corneas infected by S. aureus or P. aeruginosa (LT = 0.77% [0.41-1.87] and 0.81% [0.23, 1.46]) exhibited 3.18-fold and 3.02-fold lower LT values, respectively, than uninfected corneas (LT = 2.45% [2.15, 5.89]) (both P -values < 0.001). No LT difference was found between rose bengal-saturated corneas infected by S. aureus or P. aeruginosa and uninfected corneas (all LT values = 0.01% [0.01-0.01]; both P -values = 0.08). TEM showed bacteria on corneal stroma borders and occasionally inside the stroma. CONCLUSIONS: The results indicate that the amount of light arriving at the corneal endothelium is substantially reduced in infected corneas. The total fluence of clinical photoactivated chromophore for keratitis corneal crosslinking protocols can be safely increased substantially while maintaining a low risk of corneal endothelial damage.

2. **2025.** Hafezi, F.*, et al.* Same-session dual chromophore riboflavin/UV-A and rose bengal/green light PACK-CXL in Acanthamoeba keratitis: a case report. Eye Vis (Lond) 12, 2

BACKGROUND: Acanthamoeba keratitis (AK) is the most challenging corneal infection to treat, with conventional therapies often proving ineffective. While photoactivated chromophore for keratitis-corneal cross-linking (PACK-CXL) with riboflavin/UV-A has shown success in treating bacterial and fungal keratitis, and PACK-CXL with rose bengal/green light has demonstrated promise in fungal keratitis, neither approach has been shown to effectively eradicate AK. This case study explores a novel combined same-session treatment approach using both riboflavin/UV-A and rose bengal/green light in a single procedure. CASE PRESENTATION: A 44-year-old patient with active AK in the left cornea, unresponsive to 10 months of conventional treatment according to American Academy of Ophthalmology (AAO) guidelines, was treated using same-session sequential PACK-CXL with riboflavin/UV-A (365 nm) irradiation (10 J/cm(2)) and rose bengal/green light (522 nm) irradiation (5.4 J/cm(2)) in a single setting. The procedure was repeated twice due to persistent signs of inflammation and infection. After three combined same-session PACK-CXL treatments, the patient's cornea converted to a quiescent scar, and symptoms of ocular pain, photophobia, epiphora, and blepharospasm resolved. Confocal microscopy revealed no detectable Acanthamoeba cysts. The patient currently awaits penetrating keratoplasty. CONCLUSIONS: The same-session combination of riboflavin/UV-A and rose bengal/green light PACK-CXL effectively treated a patient with confirmed AK that was resistant to conventional medical therapy, suggesting that using two chromophores in a single procedure may represent a future treatment alternative for AK.

3. **2025.** Aydemir, M.E.*, et al.* Corneal Resistance to Enzymatic Digestion After Rose Bengal and Combined Rose Bengal/Riboflavin Cross-Linking Is Oxygen Independent. Transl Vis Sci Technol 14, 1

PURPOSE: To assess corneal resistance to enzymatic digestion after rose bengal (RB)/green light and RB/green light followed by riboflavin (RF)/ultraviolet A (UV-A) cross-linking (CXL), with or without oxygen. METHODS: Ex vivo porcine corneal buttons (n = 144) underwent CXL with RB/green or RB/green-RF/UV-A under atmospheric

21% oxygen conditions or in a nitrogen chamber with 0.1% oxygen (hypoxic conditions) to test 10- and 15-J/cm2 fluences. After CXL, corneas were digested with 0.3% collagenase A, and mean digestion times (MDTs) were recorded. RESULTS: For the non-irradiated control group, the MDT was 19.75 ± 1.34 hours. Under atmospheric oxygen conditions, RB/green CXL yielded MDTs of 33.69 ± 1.4 and 34.38 ± 1.31 hours with fluences of 10 and 15 J/cm2, respectively. RB/green + RF/UV-A showed MDTs of 39.56 ± 1.93 and 51.94 ± 4.2 hours for combined fluences of 10 + 10 J/cm2 and 15 + 15 J/cm2, respectively. Hypoxic RB/green MDTs were 33.88 ± 1.02 and 34.06 ± 1.57 hours, and RB/green + RF/UV-A MDTs were 39.62 ± 2.5 and 50.35 ± 1.59 hours for the same respective fluences. No significant differences were observed between the control groups and corresponding intervention groups (all P > 0.05). CONCLUSIONS: CXL via RB/green and RB/green-RF/UV-A significantly enhanced corneal collagenase digestion resistance, irrespective of oxygen presence. These findings could help optimize infectious keratitis therapy CXL protocols. TRANSLATIONAL RELEVANCE: Our findings aid the understanding of the molecular mechanisms underlying the therapeutic effect of CXL and may contribute to refining accelerated PACK-CXL protocols and other CXL treatment strategies.

4. **2024.** Aydemir, M.E.*, et al.* Combining Riboflavin/UV-A Light and Rose Bengal/Green Light Corneal Cross-Linking Increases the Resistance of Corneal Enzymatic Digestion. Transl Vis Sci Technol 13, 30

PURPOSE: The purpose of this study was to determine if concurrent riboflavin/UV-A light (RF/UV-A) and rose Bengal/green light (RB/green) epi-off PACK-CXL enhances corneal resistance to enzymatic digestion compared to separate chromophore/light treatments. METHODS: Ex vivo porcine corneas were allocated as follows. Group A corneas were soaked with riboflavin (RF) and were either not irradiated (A1, controls) or were irradiated with 10 (A2) or 15 J/cm(2) (A3) UV-A light at 365 nm, respectively. Group B corneas were soaked with RB and either not irradiated (B1, controls) or were illuminated with 10 (B2) or 15 J/cm(2) (B3) green light at 525 nm, respectively. Corneas in group C were soaked with both RF and RB and were either not irradiated (C1, controls) or were subjected to the same session consecutive 10 J/cm2 (C2) or 15 J/cm2 (C3) UV-A and green light exposure. Following treatment, all corneas were exposed to 0.3% collagenase A to assess digestion time until corneal button dissolution. RESULTS: A1 to A3 digestion times were 21.38, 30.5, and 32.25 hours, respectively, with A2 and A3 showing increased resistance to A1. B1-3 had digestion times of 31.2, 33.81, and 34.38 hours, with B3 resisting more than B1. C1 to C3 times were 33.47, 39.81, and 51.94 hours; C3 exhibited superior resistance to C1 and C2 (both P < 0.05). CONCLUSIONS: Same-session combined RF/UV-A and RB/green PACK-cross-linking significantly increases corneal enzymatic digestion resistance over standalone treatments. TRANSLATIONAL RELEVANCE: Combining RF-based and RB-based PACK-CXL considerably increases corneal collagenase digestion resistance, potentially minimizing ulcer size in clinical contexts.

5. **2023.** Torres-Netto, E.A.*, et al.* Corneal crosslinking with riboflavin using sunlight. J Cataract Refract Surg 49, 1049-1055

PURPOSE: To assess whether sunlight might be used to induce a biomechanical stiffening effect in riboflavin-soaked corneas similar to the effect observed in corneal crosslinking (CXL) using riboflavin and UV-A light. SETTING: Center for Applied Biotechnology and Molecular Medicine, University of Zurich, Zurich, Switzerland. DESIGN: Experimental study. METHODS: 52 porcine eyes were assayed. The concentration of riboflavin in the corneal stroma was estimated using UV-A transmission in a preliminary experiment. Then, the duration of sunlight exposure to achieve a fluence of 7.2/cm 2 was calculated. Finally, de-epithelialized corneas were divided equally into 3 groups and soaked with riboflavin 0.1% (control group and Group 1) or 0.5% (Group 2). Eyes from Groups 1 and 2 were then exposed to sunlight. The elastic modulus was calculated as an indicator of stiffness. RESULTS: Riboflavin concentration in Group B was higher by a factor of 2.8 than Group A. According to live illuminance measurements and stromal riboflavin concentration, the sunlight exposure duration varied between 16 minutes and 45 minutes. Groups 1 and 2 had higher elastic modulus than controls (P < .0001) but did not differ between them (P = .194). The stiffening effect was 84% and 55%, respectively. CONCLUSIONS: Sunlight exposure of ex vivo corneas soaked in both riboflavin 0.1% and 0.5% resulted in increased corneal stiffness. Specifically, riboflavin 0.1% with longer UV-A exposure showed a trend for a greater stiffening effect, which might open new alleys for the use of oral riboflavin and fractioned sunlight exposure as less invasive CXL techniques.

6. **2023.** Olshaker, H.*, et al.* Accelerated high fluence photoactivated chromophore for infectious keratitis-corneal cross-linking (PACK-CXL) at the slit lamp: a pilot study. Front Pharmacol 14, 1229095

Introduction: Photoactivated Chromophore for Infectious Keratitis-Corneal Cross-Linking (PACK-CXL) has garnered substantial interest among researchers and ophthalmologists due to its high promise as a potential treatment for infectious keratitis. The aim of this study is to evaluate the efficacy and safety of high fluence PACK-CXL, using 10.0 J/cm(2) (30 mW/cm(2), 5 min, and 33 s) at the slit lamp. Methods: This prospective interventional, nonrandomized cohort study included 20 eyes of 20 patients with bacterial, fungal, or mixed origin keratitis who underwent high fluence PACK-CXL treatment as an adjunct therapy to conventional antimicrobial therapy per American Academy of Ophthalmology treatment guidelines. The re-epithelization time was recorded, and corneal endothelial cell density was counted before and after treatment. Results: The average re-epithelization time was 8.2 +/- 2.8 days (range 3-14 days). After PACK-CXL treatment, eight patients (40%) were directly discharged, while the remained patients stayed in the hospital for an average of 5.6 +/- 3.5 days. No eyes required keratoplasty. Endothelial cell density counts before and after the PACK-CXL procedure were 2,562.1 +/- 397.3, and 2,564.8 +/- 404.5 cells/mm(2), respectively (p = 0.96). Conclusion: although it was not a randomized control trial, we conclude that high fluence PACK-CXL as an adjuvant therapy is safe with no complications observed, and efficient as time to re-epithelization was less than 14 days for all patients and no patients underwent tectonic keratoplasties. Further research is needed to compare it to the current standard of care.

7. **2023.** Lu, N.J., Koliwer-Brandl, H., Hillen, M., Egli, A. & Hafezi, F. High-Fluence Accelerated PACK-CXL for Bacterial Keratitis Using Riboflavin/UV-A or Rose Bengal/Green in the Ex Vivo Porcine Cornea. Transl Vis Sci Technol 12, 14

PURPOSE: To investigate and compare the efficacy of high-fluence accelerated photoactivated chromophore for keratitis-corneal cross-linking (PACK-CXL) using either riboflavin/ultraviolet (UV)-A light or rose bengal/green light to treat Staphylococcus aureus or Pseudomonas aeruginosa infections in an ex vivo porcine cornea model. METHODS: One hundred and seventeen ex vivo porcine corneas were injected with clinical isolates of S. aureus or P. aeruginosa, divided into eight groups, and cultured for 24 hours. Then, either riboflavin with UV-A light irradiation (30 mW/cm2; 8 minutes, 20 seconds; 15 J/cm2) or rose bengal with green light irradiation (15 mW/cm2, 16 minutes, 40 seconds; 15 J/cm2) was applied; unirradiated infected groups served as controls. All corneas were incubated for another 24 hours. Next, corneal buttons were obtained and vortexed to release the bacterial cells. The irradiated and unirradiated solutions were then plated and incubated on agar plates. The amount of colony-forming units was quantified and the bacterial killing ratios (BKRs) resulting from different PACK-CXL protocols relative to non-treated controls were calculated. RESULTS: Riboflavin/UV-A light PACK-CXL resulted in median BKRs of 52.8% and 45.8% in S. aureus and P. aeruginosa, respectively, whereas rose bengal/green light PACK-CXL resulted in significantly greater BKRs of 76.7% and 81.0%, respectively (both $P < 0.01$). CONCLUSIONS: Both accelerated PACK-CXL protocols significantly decreased S. aureus and P. aeruginosa bacterial loads. Comparing the riboflavin/UV-A light and rose bengal/green light PACK-CXL approaches in the same experimental setup may help develop strain-specific and depth-dependent PACK-CXL approaches that could be used alongside the current standard of care. TRANSLATIONAL RELEVANCE: Our study used an animal model to gain insight into the efficacy of high-fluence accelerated PACK-CXL using either riboflavin/UV-A light or rose bengal/green light to treat Staphylococcus aureus or Pseudomonas aeruginosa infections.

8. **2023.** Lu, N.J.*, et al.* The Antibacterial Efficacy of High-Fluence PACK Cross-Linking Can Be Accelerated. Transl Vis Sci Technol 12, 12

PURPOSE: To determine whether high-fluence photoactivated chromophore for keratitis cross-linking (PACK-CXL) can be accelerated. METHODS: Solutions of Staphylococcus aureus and Pseudomonas aeruginosa with 0.1% riboflavin were prepared and exposed to 365 nm ultraviolet (UV)-A irradiation of intensities and fluences from 9 to 30 mW/cm2 and from 5.4 to 15.0 J/cm2, respectively, representing nine different accelerated PACK-CXL protocols. Irradiated solutions and unirradiated controls were diluted, plated, and inoculated on agar plates so that the

bacterial killing ratios (BKR) could be calculated. Additionally, strains of Achromobacter xylosoxidans, Staphylococcus epidermidis, and Stenotrophomonas maltophilia were exposed to a single accelerated PACK-CXL protocol (intensity: 30 mW/cm2, total fluence: 15.0 J/cm2). RESULTS: With total fluences of 5.4, 10.0, and 15.0 J/cm2, the range of mean BKR for S. aureus was 45.78% to 50.91%, 84.13% to 88.16%, and 97.50% to 99.90%, respectively; the mean BKR for P. aeruginosa was 69.09% to 70.86%, 75.37% to 77.93%, and 82.27% to 91.44%, respectively. The mean BKR was 41.97% for A. xylosoxidans, 65.38% for S. epidermidis, and 78.04% for S. maltophilia for the accelerated PACK-CXL protocol (30 mW/cm2, 15 J/cm2). CONCLUSIONS: The BKR of high-fluence PACK-CXL protocols can be accelerated while maintaining a high, but species-dependent, BKR. The Bunsen to Roscoe law is respected in fluences up to 10 J/cm2 in S. aureus and P. aeruginosa, whereas fluences above 10 J/cm2 show strain dependence. TRANSLATIONAL RELEVANCE: The high-fluence PACK-CXL protocols can be accelerated in clinical practice while maintaining high levels of BKR.

9. **2023.** Lu, N.J.*, et al.* Effect of fluence levels on prophylactic corneal cross-linking for laser in situ keratomileusis and transepithelial photorefractive keratectomy. Acta Ophthalmol 101, e185-e196

PURPOSE: The purpose of this study is to assess the effect of various fluence levels on prophylactic corneal cross-linking (CXL) combined with femtosecond laser in situ keratomileusis (FS-LASIK-Xtra) or transepithelial photorefractive keratectomy (TransPRK-Xtra) on biomechanics, demarcation line (DL), and stromal haze. METHODS: Prospective analysis where two prophylactic CXL protocols (lower/higher fluence [LF/HF]: 30 mw/cm(2) , 60/80 s, 1.8/2.4 J/cm(2)) were performed as part of either an FS-LASIK-Xtra or TransPRK-Xtra procedure. Data were collected preoperatively and at 1 week and 1, 3, and 6 months postoperatively. Main outcome measures were (1) dynamic corneal response parameters and the stress-strain index (SSI) from Corvis, (2) actual DL depth (ADL), and (3) stromal haze on OCT images analysed by a machine learning algorithm. RESULTS: Eighty-six eyes from 86 patients underwent FS-LASIK-Xtra-HF (21 eyes), FS-LASIK-Xtra-LF (21 eyes), TransPRK-Xtra-HF (23 eyes), and TransPRK-Xtra-LF (21 eyes). SSI increased similarly by around 15% in all groups 6 months postoperatively (p = 0.155). All other corneal biomechanical parameters were statistically significant worsening postoperatively, but the change was similar in all groups. At 1 month postoperatively, there was no statistical difference in mean ADL among four groups (p = 0.613), mean stromal haze was similar between the two FS-LASIK-Xtra groups, but higher in the TransPRK-Xtra-HF group compared with the TransPRK-Xtra-LF group. CONCLUSIONS: FS-LASIK-Xtra and TransPRK-Xtra lead to a similar ADL and improve SSI equally. Lower fluence prophylactic CXL might be recommended as it achieves similar mean ADL with potentially less induced stromal haze, especially in TransPRK. The clinical relevance and applicability of such protocols remains to be assessed.

10. **2023.** Hafezi, F.*, et al.* Rates of infectious keratitis and other ocular surface adverse events in corneal cross-linking for keratoconus and corneal ectasias performed in an office-based setting: a retrospective cohort study. Eye Vis (Lond) 10, 36

BACKGROUND: This study aimed to compare the complication rates of epithelium-off corneal cross-linking (epi-off CXL) performed in an office-based setting with those of epi-off CXL performed in an operating room. METHODS: A retrospective cohort study, comprising 501 consecutive epi-off CXL procedures, performed in a non-sterile procedure room without laminar flow ventilation at the ELZA Institute in Zurich, Switzerland, between November 2015 and October 2021, was conducted. RESULTS: No cases of postoperative infectious keratitis were observed, while sterile infiltrates occurred in 10 out of 501 (2.00%) patients, all of whom responded well to topical steroid therapy. Delayed epithelialization (> 7 days) occurred in 14 out of 501 (2.79%) patients. No other adverse events were noted. CONCLUSIONS: Office-based epi-off CXL does not appear to be associated with an increased risk of complications when compared to operating room settings.

11. 2022. Hafezi, F., Munzinger, A., Goldblum, D., Hillen, M. & Tandogan, T. Repeated High-Fluence Accelerated Slitlamp-Based Photoactivated Chromophore for Keratitis Corneal Cross-Linking for Treatment-Resistant Fungal Keratitis. Cornea 41, 1058-1061

PURPOSE: The purpose of this study was to report a case of fungal keratitis resistant to standard-of-care antimicrobial treatment and successful resolution, thanks to the repeated high-fluence accelerated photoactivated chromophore for keratitis-corneal cross-linking (PACK-CXL). METHODS: This was a case report. RESULTS: A 79-year-old male patient with previous Descemet membrane endothelial keratoplasty presented with a corneal ulcer that was resistant to topical antimicrobial therapy and amniotic membrane placement. Fungal keratitis was diagnosed, and the cornea was on the verge of perforation. After over a month of topical and systemic therapy without marked improvement, the patient underwent 2 repeated high-fluence accelerated CXL procedures (7.2 J/cm2 using a UV irradiation of 30 mW/cm2 for 4 minutes) over an interval of 8 days (accumulated fluence of 14.4 J/cm2), which resulted in significant clinical improvement, with consolidation into a quiescent scar. CONCLUSIONS: PACK-CXL protocols delivering a total UV fluence of 5.4 J/cm2 (as per the original Dresden protocol for corneal ectasia cross-linking) can be an effective primary therapy for initial or superficial corneal infections because approximately half of the energy is absorbed in the first 100 mum of a riboflavin-soaked cornea. However, fungal keratitis may require higher fluences than 5.4 J/cm2 because, unlike ectatic corneas, corneal ulcers are not transparent, and the infection may involve deep stroma. This case illustrates how repeated high-fluence accelerated PACK-CXL can be used to successfully treat fungal keratitis resistant to conventional topical and systemic medications.

12. 2022. Hafezi, F*., et al.* Demarcation Line Depth in Epithelium-Off Corneal Cross-Linking Performed at the Slit Lamp. J Clin Med 11

We aimed to evaluate the depth of the demarcation line following accelerated epithelium-off corneal cross-linking (A-CXL) performed at the slit lamp with the patient sitting in an upright position. Twenty-three eyes from twenty patients, undergoing epi-off A-CXL (9 mW/cm(2) for 10 min) using a CXL device at the slit lamp in the upright position. Demarcation line depth was assessed at 1 month after the procedure using anterior segment optical coherence tomography (AS-OCT) and specialized software. Surgery was uneventful in all cases. The average postoperative demarcation line depth achieved was 189.4 microm (standard deviation: 58.67 microm). The demarcation line depth achieved with patients sitting upright, receiving CXL at the slit lamp, is similar to published data on CXL performed in the supine position, suggesting that demarcation line depth is not dependent on patient orientation during CXL.

13. 2022. Hafezi, F., Hillen, M., Kollros, L., Hafezi, N. & Torres Netto, E.A. Corneal Cross-linking in Thin Corneas: From Origins to State of the Art. Reviews in Ophthalmology, 1-4

14. 2022. Hafezi, F. Corneal Cross-Linking: Epi-On. Cornea 41

15. 2022. Abdshahzadeh, H*., et al.* Repeated application of riboflavin during corneal cross-linking does not improve the biomechanical stiffening effect ex vivo. Exp Eye Res 224, 109267

PURPOSE: To evaluate whether repeated application of riboflavin during corneal cross-linking (CXL) has an impact on the corneal biomechanical strength in ex-vivo porcine corneas. DESIGN: Laboratory investigation. METHODS: Sixty-six porcine corneas with intact epithelium were divided into three groups and analyzed. All corneas were pre-soaked with an iso-osmolar solution of 0.1% riboflavin in a phosphate-buffered saline (PBS) solution ("riboflavin solution"). Then, the corneas in Groups 1 and 2 were irradiated with a standard epi-off CXL (S-CXL) UV-A irradiation protocol (3 mW/cm(2) for 30 min); while the corneas in Group 3 were not irradiated and served as control.

During irradiation, Group 1 (CXL-PBS-Ribo) received repeated riboflavin solution application while corneas in Group 2 (CXL-PBS) received only repeated iso-osmolar PBS solution. Immediately after the procedure, 5-mm wide corneal strips were prepared, and elastic modulus was calculated to characterize biomechanical properties. RESULTS: Significant differences in stress-strain extensiometry were found between two cross-linked groups with control group (P = 0.005 and 0.002, respectively). No significant difference was observed in the normalized stiffening effect between Groups 1 and 2 (P = 0.715). CONCLUSIONS: The repeated application of riboflavin solution during UV-A irradiation does not affect the corneal biomechanical properties achieved with standard epi-off CXL. Riboflavin application during CXL may be omitted without altering the biomechanical stiffening induced by the procedure.

16. **2021.** Wu, D.*, et al.* Corneal Cross-Linking: The Evolution of Treatment for Corneal Diseases. Front Pharmacol 12, 686630

Corneal cross-linking (CXL) using riboflavin and ultraviolet A (UVA) light has become a useful treatment option for not only corneal ectasias, such as keratoconus, but also a number of other corneal diseases. Riboflavin is a photoactivated chromophore that plays an integral role in facilitating collagen crosslinking. Modifications to its formulation and administration have been proposed to overcome shortcomings of the original epithelium-off Dresden CXL protocol and increase its applicability across various clinical scenarios. Hypoosmolar riboflavin formulations have been used to artificially thicken thin corneas prior to cross-linking to mitigate safety concerns regarding the corneal endothelium, whereas hyperosmolar formulations have been used to reduce corneal oedema when treating bullous keratopathy. Transepithelial protocols incorporate supplementary topical medications such as tetracaine, benzalkonium chloride, ethylenediaminetetraacetic acid and trometamol to disrupt the corneal epithelium and improve corneal penetration of riboflavin. Further assistive techniques include use of iontophoresis and other wearable adjuncts to facilitate epithelium-on riboflavin administration. Recent advances include, Photoactivated Chromophore for Keratitis-Corneal Cross-linking (PACK-CXL) for treatment of infectious keratitis, customised protocols (CurV) utilising riboflavin coupled with customised UVA shapes to induce targeted stiffening have further induced interest in the field. This review aims to examine the latest advances in riboflavin and UVA administration, and their efficacy and safety in treating a range of corneal diseases. With such diverse riboflavin delivery options, CXL is well primed to complement the armamentarium of therapeutic options available for the treatment of a variety of corneal diseases.

17. **2021.** Peyman, A. & Pourazizi, M. Corneal Cross-linking at the Slit Lamp: Concern About Risk of Corneal Ulcer. J Refract Surg 37, 572

18. **2021.** Knyazer, B., Hillen, M. & Hafezi, F. Corneal Cross-linking for Infectious Keratitis at the Slit Lamp in Wheelchair Users. J Refr Surg Case Rep 1, e36-e39

19. **2021.** Hafezi, F.*, et al.* Corneal Cross-linking for Keratoglobus Using Individualized Fluence. J Refr Surg Case Rep 1, e10-e14

20. **2021.** Hafezi, F., Richoz, O., Torres-Netto, E.A., Hillen, M. & Hafezi, N.L. Corneal Cross-linking at the Slit Lamp. J Refract Surg 37, 78-82

PURPOSE: To describe a new surgical technique where corneal cross-linking (CXL) (to treat corneal ectasias) and photo-activated chromophore for keratitis-CXL (PACK-CXL) are performed while the patient is seated in an upright position at the slit lamp. METHODS: Topical anesthesia is applied in the waiting room, 10 minutes before the procedure. Once in the office or procedure room, eyelids and periorbital areas are disinfected with chloramphenicol and the patient is seated at the slit lamp. Epithelial debridement is performed with a cotton swab soaked in freshly prepared 40% ethanol, using 70 seconds of tapping, followed by gentle pressure to remove the epithelium. The patient is placed in the supine position for riboflavin application for 10 minutes. Stromal thickness is assessed using ultrasound pachymetry after 5 and 10 minutes. Finally, the patient is returned to the slit lamp to

receive ultraviolet irradiation. RESULTS: CXL at the slit lamp is an easy-to-perform technique that substantially reduces the infrastructure needed to perform CXL and PACK-CXL procedures. CONCLUSIONS: A significant advantage of allowing CXL treatment at the slit lamp is that CXL technology can now be used in clinics that do not have easy access to an operating room infrastructure. Slit-lamp CXL can also reduce procedure costs by eliminating the technical fees related to the use of an operating room, making this treatment not only more accessible for patients, but also affordable. [J Refract Surg. 2021;37(2):78-82.].

21. **2021.** Hafezi, F., Richoz, O., Torres-Netto, E.A., Hillen, M. & Hafezi, N. Reply: Corneal Cross-linking at the Slit Lamp: Concern About Risk of Corneal Ulcer. J Refract Surg 37, 572-573

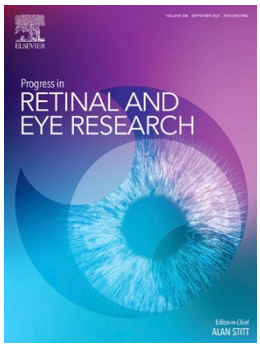
22. **2021.** Abrishamchi, R.*, et al.* High-Fluence Accelerated Epithelium-Off Corneal Cross-Linking Protocol Provides Dresden Protocol-Like Corneal Strengthening. Transl Vis Sci Technol 10, 10

Purpose: To assess whether optimized technical settings for accelerated epithelium-off corneal cross-linking may lead to increases in biomechanical stiffness similar to the benchmark 30-minute epithelium-off Dresden protocol. Methods: Three-hundred porcine eyes were divided equally into six groups for analysis. All samples underwent epithelial debridement and soaking with 0.1% iso-osmolar riboflavin solution for 20 minutes. Corneal cross-linking (CXL) was performed using epithelium-off protocols varying in acceleration and total fluence (intensity in mW/cm(2) * time in minutes, total fluence in J/cm(2)): standard (S)-CXL (3*30, 5.4), accelerated (A)-CXL (9*10, 5.4), A-CXL (9*13'20", 7.2), A-CXL (18*6'40", 7.2), and A-CXL (18*9'15", 10). Control corneas were not irradiated. The elastic modulus of 5-mm wide corneal strips was measured as an indicator of corneal stiffness. Results: All irradiated groups had significantly higher elastic modulus than controls ($P < 0.05$), with a stiffening effect of 133% S-CXL (3*30, 5.4), 122% A-CXL (9*10, 5.4), 120% A-CXL (9*13'20", 7.2), 114% A-CXL (18*6'40", 7.2) and 149% A-CXL (18*9'15", 10). The high-fluence accelerated epithelium-off protocol (18*9'15", 10) showed the highest stiffening effect. Elastic modulus at 5% strain (1%-5% strain) showed significant differences between A-CXL (18*9'15", 7.2) and three other accelerated protocols: A-CXL (9*10, 5.4; $P = 0.01$), A-CXL (9*13'20", 7.2; $P = 0.003$), and A-CXL (18*6'40", 10; $P = 0.0001$). Conclusions: An accelerated high-fluence epithelium-off CXL protocol (18 mW/cm(2) for 9'15") was identified to provide a significantly greater stiffening effect than any other accelerated protocols and is indistinguishable from the Dresden protocol, with accelerating irradiation times ranging from 30 to 9 minutes; by combining gentle acceleration with higher fluence, such a protocol does not require supplemental oxygen. Translational Relevance: This A-CXL (18*9'15", 10) protocol has the potential to become a new standard in epithelium-off CXL, delivering Dresden protocol-like strengthening over a shorter period.

23. **2017.** Salmon, B.*, et al.* CXL at the Slit Lamp: No Clinically Relevant Changes in Corneal Riboflavin Distribution During Upright UV Irradiation. J Refract Surg 33, 281

Book chapters and Reviews

24. **2025.** Hafezi, F.*, et al.* Corneal cross-linking. Prog Retin Eye Res 104, 101322



First introduced over 20 years ago as a treatment for progressive keratoconus, the original "Dresden" corneal cross-linking (CXL) protocol involved riboflavin saturation of the stroma, followed by 30 min of 3 mW/cm(2)-intensity ultraviolet-A (UV-A) irradiation. This procedure generates reactive oxygen species (ROS) that cross-link stromal molecules, thereby stiffening the cornea and counteracting the ectasia-induced weakening. Due to their large size, riboflavin molecules cannot readily pass through the corneal epithelial cell tight junctions; thus, epithelial debridement was performed. Moreover, the Dresden protocol necessitates a minimal corneal thickness of 400 mum to protect the endothelium from UV-induced damage. While the Dresden protocol is highly effective at enhancing corneal biomechanical strength, there was a strong desire for CXL procedures that would deliver Dresden-like strengthening in a shorter time, in corneas thinner than 400 mum, and without requiring epithelial debridement. This review explores the advancements and scientific discoveries that have enabled such improvements. Accelerated CXL protocols, utilizing our increased knowledge about the role of oxygen and photochemical reactions in the cornea have shortened and simplified the procedure duration while maintaining efficacy and safety, improving clinical workflow and patient compliance. CXL is not confined to improving biomechanics in corneal ectasia, but rather represents a technique that modulates corneal physiology and biochemistry on multiple levels. Accordingly, CXL indications have expanded to include treating other corneal ectasias, corneal neovascularization, corneal sterile melting, inflammatory dry eye and importantly, infectious keratitis in a procedure termed photoactivated chromophore for keratitis-CXL (PACK-CXL). In PACK-CXL, ROS have a direct pathogen-killing effect, and cross-linking enhances the cornea's resistance to pathogen-produced protease digestion through steric hindrance. The distinct requirements of PACK-CXL compared to ectasia treatment have led to the development of different CXL protocols, including higher UV fluences and other chromophore/light combinations, specifically rose bengal and green light. Additionally, combining CXL with vision-enhancing procedures like individualized wavefront- or topographically-guided excimer ablation can regularize a biomechanically stable cornea, improve visual acuity, and potentially eliminate the need for corneal transplantation, leading to long-term improvements in quality of life.

25. **2024.** Singh, R.B.*, et al.* Keratoconus. Nat Rev Dis Primers 10, 81

nature REVIEWS DISEASE PRIMERS

Keratoconus is a progressive eye disorder primarily affecting individuals in adolescence and early adulthood. The ectatic changes in the cornea cause thinning and cone-like steepening leading to irregular astigmatism and reduced vision. Keratoconus is a complex disorder with a multifaceted aetiology and pathogenesis, including genetic, environmental, biomechanical and cellular factors. Environmental factors, such as eye rubbing, UV light exposure and contact lens wearing, are associated with disease progression. On the cellular level, a complex interplay of hormonal changes, alterations in enzymatic activity that modify extracellular membrane stiffness, and changes in biochemical and biomechanical signalling pathways disrupt collagen cross-linking within the stroma, contributing to structural integrity loss and distortion of normal corneal anatomy. Clinically, keratoconus is diagnosed through clinical examination and corneal imaging. Advanced imaging platforms have improved the detection of keratoconus, facilitating early diagnosis and monitoring of disease progression. Treatment strategies for keratoconus are tailored to disease severity and progression. In early stages, vision correction with glasses or soft contact lenses may suffice. As the condition advances, rigid gas-permeable contact lenses or scleral lenses are prescribed. Corneal cross-linking has emerged as a pivotal treatment aimed at halting the progression of corneal ectasia. In patients with keratoconus with scarring or contact lens intolerance, surgical interventions are performed.

26. **2024.** Lu, N.J., Hafezi, F., Koppen, C. & Aslanides, I.M. Editorial: Research and advances on medication for corneal diseases and surgery. Front Pharmacol 15, 1434180

27. **2023.** Hafezi, F., Torres-Netto, E.A. & Hillen, M. Expanding indications for corneal cross-linking. Curr Opin Ophthalmol 34, 339-347

PURPOSE OF REVIEW: The aim of this study was to summarize the recent developments in corneal cross-linking (CXL) and its indications, including corneal ectasias, refractive surgery and infectious keratitis. RECENT FINDINGS: Advances in CXL technology, such as the use of higher-intensity LED ultraviolet (UV) light sources and a better understanding of the UV-riboflavin photochemical reaction, have enabled safer and more effective methods of cross-linking thin and ultra-thin corneas, and more effective accelerated transepithelial/'epi-on' CXL procedures that are beginning to supplant the Dresden protocol as the 'gold standard' CXL method. CXL is also being used in combination with laser surgery, not only to expand the patient base who can receive refractive surgery, but also to help rehabilitate vision in patients with ectasia. CXL, and CXL combined with photorefractive keratectomy (PRK), can result in corneal flattening of 1-2D, and corneal regularization of 4-5D, respectively. Finally, photoactivated chromophore for keratitis-corneal cross-linking (PACK-CXL) has been shown to be an effective therapy for infectious keratitis, both alone, and in combination with antimicrobial drugs. SUMMARY: CXL has evolved from a single technique to treat a single corneal ectasia, keratoconus, to several techniques with several indications, spanning a spectrum of corneal ectasias, as well as visual rehabilitation, refractive procedures and infectious keratitis treatment.

28. Hafezi, F. & Hillen, M. in *Keratoconus* (ed. Das, S.) 183-193 (Springer Nature, Cham, Switzerland, 2022).

29. Torres Netto, E.A., Hosny, M. & Hafezi, F. in *Keratoconus* (ed. Armia, A., Mazzotta, C.) 149-59 (Springer Nature, Cham, Switzerland, 2021).

30. Hafezi, F. & Torres Netto, E.A. in *Keratoconus* (ed. Armia, A., Mazzotta, C.) 159-67 (Springer Nature, Cham, Switzerland, 2021).

Trade Journal articles

31. Torres Netto E. A., Hillen M., Hafezi F. Advancing Keratoconus Management: ECO-CAIRS, Biomechanics, and the Evolution of Corneal Allogenic Intrastromal Ring Segments. **Touch Ophthalmology**. 2025. 3:

32. Jacobs S., Hafezi F. Experts explore CAIRS through landmarks that shaped its evolution. **Ocular Surgery News European Edition**. 2025. 2: 1-3

33. Hafezi F., Hillen M., Torres Netto E. A. ECO-CAIRS. Optimizing Allogenic Ring Segment Rigidity for Safer, More Predictable Keratoconus Surgery. **Ocular Surgery News**. 2025. 4: 1-6

34. Hafezi F. ELZA-PACE: Verbesserung des Sehvermögens bei Keratokonus ohne Gewebeentfernung. **Zeitschrift Praktische Augenheilkunde**. 2025. 46: 1-5

35. Hafezi F. ELZA epi-on cross-linking protocol shows comparable outcome to accelerated epi-off CXL. **Ocular Surgery News US Edition**. 2025. online: 1-4

36. Hafezi F. CXL an der Spaltlampe. **Ophthalmologische Nachrichten**. 2025. 3: 15-19

37. Hafezi-Kristoffersen NL. Scanning the Eye Care Horizon - Sitting down with Nikki Hafezi. **The Ophthalmologist**. 2024. 4: 1-3

38. Hafezi F., Torres Netto E. A. The ELZA approach: Rehabilitating Vision after Keratoconus using transPRK, PACE and All Femto-CAIRS. **The Ophthalmologist**. 2024. 4:

39. Hafezi F., Hillen M., Torres Netto E. A. Corneal Cross-Linking (CXL): From Origins to Modern Innovations. **ESCRS Daily**. 2024: Supplement 7

40. Hafezi F., Hillen M., Torres Netto E. A. Corneal Cross-Linking (CXL): Leaving the Epithelium On. **EuroTimes**. 2024. 2: 33-39

41. Hafezi F. Transforming the eye with a new standard for corneal cross-linking. **Ophthalmology Times Europe**. 2024. 9: 26-28

42. Hafezi F. Korneales Crosslinking: Neueste Fortschritte. **Zeitschrift Praktische Augenheilkunde**. 2024. 45(9): 405-08

43. Hafezi F. Slit Lamp CXL for Infectious Keratitis. **Corneal Physician**. 2024. 1: 7-10

44. Hafezi F. ELZA-PACE: Improving vision in keratoconus without tissue removal. **Ophthalmology Times Europe**. 2024. online:

45. Hafezi F., Hillen M., Kollros L., Torres Netto E. A., Hafezi N. Was tun bei dünner Hornhaut und Progression? **Ophthalmologische Nachrichten**. 2023. 9: 1-3

46. Hafezi F. Oogwrijven alléén verergert keratoconus niet. **De Oogarts**. 2023. 1: 1-3

47. Hafezi F. Global Update on CXL. **Cataract and Refractive Surgery Today**. 2023. 1: 25-29

48. Hafezi F. Therapeutic Refractive Laser Surgery as a Means to Reconstruct Corneal Optical Properties. **Cataract and Refractive Surgery Today**. 2023. 4: 1-3

49. Hafezi F. MD Roundtable: The Role of Cross-Linking in Infectious Keratitis. **EyeNet Magazine**. 2023. 7: 1-4

50. Hafezi F. Korneales Crosslinking: Neueste Fortschritte. **Ophthalmo-Chirurgie**. 2023. 35: 273-76

51. Hafezi F. Corneal Cross-Linking: what's new in 2023? **The Ophthalmologist**. 2023. 11: 1-6

52. Hafezi F. Fortschritte in der Cross-Linking-Therapie der Hornhautektasien. **Ophthalmologische Nachrichten**. 2022. 4: 1-2

53. Hafezi F. Improving the gold standard for CXL. **Review of Oprometry**. 2022. 4: 50-52

54. Hafezi F. Cross-linking advancements continue in US, Europe. **Ocular Surgery News European Edition**. 2022. Nov/Dec:

55. Hafezi F. Review on CXL Technology. **Ophta**. 2022. 4: 1-6

56. Hafezi F. Advancing CXL technology. **Ocular Surgery News**. 2022. 12: 1-2

57. Przewlocka K., Kanklerz P. Aktualne wskazania oraz nowe możliwości zastosowania cross-inkingu w leczeniu chorób rogówki (Current and new indications for corneal cross-linking). **Ophthatherapy**. 2021. 8(3(31)): 153-60

58. Ostrovsky A. US corneal cross-linking specialists aim to bridge the innovation gap with Europe. **Ocular Surgery News US Edition**. 2021. 6: 1-7

59. Norris T. C-Eye, un salto evolutivo. **EyeSee**. 2021: 1-6

60. Leonard C. The epic future of epi-on. **Review of Ophthalmology**. 2021. 28(10): 28-34

61. Hafezi N.L. The Eclectic Approach to Becoming a CEO. **CAKE Magazine**. 2021(3): 24-25

62. Hafezi N.L. When the Rubber Hits the Road. **The Ophthalmologist**. 2021(3):

63. Hafezi F. Cross-linking at the Slit Lamp—Why Moving Corneal Cross-linking from the Operating Room to an Office-based Procedure Makes a Difference. **US Ophthalmic Review**. 2021(1): 55-57

64. Gerste R. Korneales Crosslinking: Vom Operationsraum an die Spaltlampe. **Zeitschrift für praktische Augenheilkunde**. 2021. 42: 267-69

65. Agarwal A. Cross-linking can be performed at the slit lamp. **Ocular Surgery News US Edition**. 2021. 6: 8-12

66. Hafezi F., Torres Netto E.A. Democratização do crosslinking corneano. **Oftalmologia em foco**. 2020. 183(1): 29-31

67. Hafezi F. CXL beyond the OR. **Cataract & Refractive Surgery Today Europe**. 2020(3): 27-29

68. Hillen M. CXL out of the OR. **CAKE Magazine**. 2019(5): 8-10

69. Hafezi N.L. CXL at the slit lamp: the way of the future? **Cataract & Refractive Surgery Today Europe**. 2017(3): 56-58