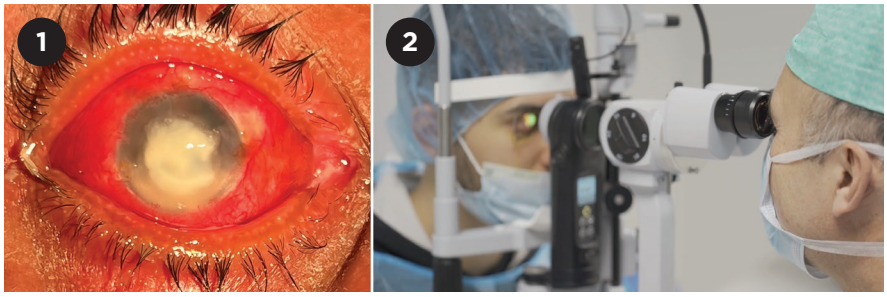
# MD Roundtable: The Role of Cross-Linking in Infectious Keratitis

Although corneal cross-linking (CXL) is well known as a treatment to strengthen the cornea and halt the progression of keratoconus and other ectasias, it has also been used in the management of infectious keratitis. However, reports about the efficacy of CXL in this latter application have been inconsistent.

For this roundtable, Bennie H. Jeng, MD, at the Scheie Eye Institute, hosts two of the world's experts in CXL to discuss recent studies and the current status of this therapy for infectious keratitis: Farhad Hafezi, MD, PhD, at the ELZA Institute in Dietikon, Switzerland, and Jennifer Rose-Nussbaumer, MD, at Stanford University in California. *(This discussion has been edited for length and clarity.)*




***CXL.*** *(1) An eye with bacterial infectious keratitis. (2) PACK-CXL at the slit lamp.*

## Multiple Effects of CXL

***Dr. Jeng:*** *Infectious keratitis is an important global cause of blindness. In the U.S., it is mainly caused by contact lens wear, but worldwide, trauma is the leading culprit. Despite good medications, these infections sometimes get out of control, and we need other options. What are the proposed mechanisms that could make CXL useful for infectious keratitis?*

**Dr. Hafezi:** The best-known effect of CXL with riboflavin and UV-A is inducing biomechanical stiffening, and that is why it is used for corneal ectasia. But, in fact, CXL has several other effects.

The second effect is increased resistance to enzymatic digestion of collagen, which occurs through the mechanism of steric hindrance (this can reduce corneal melting). The third is oxidative stress damage; CXL creates many reactive oxygen species. And last, photoactivated riboflavin binds to the nucleic acids in the DNA and RNA of anything that lives within the cornea, including microorganisms, and disrupts them.

When I perform keratoconus treatment, the most important effect is stiffening and, to lesser extent, increased resistance to digestion. But when using the same procedure for infectious keratitis, I'm more interested in resistance to digestion and the antimicrobial effect in the cornea.

**Dr. Rose-Nussbaumer:** In addition to these direct antimicrobial effects from the photochemically activated photosensitizer, which can be riboflavin or rose bengal, I'm interested in two other aspects of this process in infectious keratitis. As Dr. Hafezi mentioned, CXL damages keratocytes, and I think it also affects inflammatory cells that have infiltrated the cornea; we can use that to our benefit to reduce inflammation, which also causes damage in these patients.

I'm also actively investigating the effect of CXL on the nerve plexus. The anterior nerves are damaged by CXL, which may relieve some of the pain that patients experience with acute infectious keratitis.

## Contradictory Trial Results

***Dr. Jeng:*** *Ever since the first reports of CXL for infectious keratitis came out, this has been a hot and somewhat controversial topic. Can you explain why there are such contradictory results in the literature?*

**Dr. Rose-Nussbaumer:** One problem is that the trials are studying all kinds of different organisms and using different protocols, and many of them are retrospective studies. Although some randomized clinical trials have been done, their design is problematic.

**Dr. Hafezi:** I was on the team of Theo Seiler, the inventor of cross-linking, and

ROUNDTABLE HOSTED BY **BENNIE H. JENG, MD,** WITH **FARHAD HAFEZI, MD, PHD,** AND **JENNIFER ROSE-NUSSBAUMER, MD.**

Dr. Boris Knyazer and Prof. Farhad Hafezi

we were the first to use CXL to halt corneal melt in infectious keratitis in humans back in 2008.[1] We used the Dresden protocol at that time, which was 3 milliwatts (mW)/cm$^2$ for 30 minutes. If you look at the total irradiance, the fluence that is produced by the factors of intensity and time is the famous 5.4 joules (J)/cm$^2$. Most of the studies performed from 2008 to the present day have simplified the basic science by repeating the same low fluence.

Since then, we've done a series of lab experiments and published a paper in 2020 showing that when you increase the fluence from 5.4 J to 10 to 12 or 15 J, you exponentially kill more microorganisms.[2] So, we go from killing 50% of an antibiotic-resistant *Staphylococcus aureus* strain to killing 99%. We've also treated fungal ulcers of 6 mm with triple fluence of 15 J, and they fade away.

I think it's a great pity that studies currently listed on clinicaltrials.gov are still using low-fluence protocols. So in the next two or three years, we could see more papers reporting little effect of CXL on infectious keratitis, but this might be because the fluence is too low.

## Results Vary by Organism and Chromophore

***Dr. Jeng:*** *Do the results of CXL differ according to the type of organism?*

**Dr. Rose-Nussbaumer:** It is important to note that in vitro data may not translate to in vivo results. However, most in vitro evidence suggests that CXL with riboflavin is most effective for bacterial keratitis and that rose bengal photodynamic therapy (RB-PDT), which is essentially CXL using rose bengal as the photosensitizer, is much more effective for organisms such as fungus and *Acanthamoeba*. Thus, using different photosensitizers and light to activate them is probably where our research should be heading for these challenging organisms. I'd also like to see a head-to-head comparison of high-fluence CXL with riboflavin versus other photosensitizers.

**Dr. Hafezi:** I completely agree. When we coined the term PACK-CXL (photoactivated chromophore for keratitis CXL), we chose not to call it photoactivated riboflavin for a reason. Recently, we have been working intensely—we should have at least half a dozen papers coming out in the next six months—on the relationship between high-fluence CXL using riboflavin, high-fluence CXL using rose bengal, and most important, the combination of both. But if you look at the chromophores separately, rose bengal seems to be more effective in fungal than bacterial keratitis when performed at the same fluence as riboflavin, but their penetration is very different.

Thus, in clinical use you might have a superior effect at the same fluence from rose bengal but it stops at about 150 µm, whereas the riboflavin-mediated damage goes much deeper. So the question is, What is the best fluence for each chromophore and how do we combine them?

***Dr. Jeng:*** *The bane of our existence is parasitic disease such as* Acanthamoeba*. Do you have any thoughts about the utility of CXL for this entity?*

**Dr. Rose-Nussbaumer:** It's extremely challenging to study this important question in a thoughtful, prospective fashion because the number of cases at any institution is quite low. We've included *Acanthamoeba* patients in our RB-PDT study and are hoping to have a large enough subgroup to really look at this question.

All of these technologies are somewhat dependent on how deep the light can penetrate, so with *Acanthamoeba* the earlier you can identify patients and get them treatment, the better. If you have a very scarred, very opacified end-stage cornea, it's really tough. Thus far, I don't think there's a role for riboflavin CXL in the treatment of *Acanthamoeba*.

## Case Study of High-Fluence Combined Treatment

**Dr. Hafezi:** In the case of *Acanthamoeba*, I agree that CXL with riboflavin alone would require fluences greater than the cornea could withstand. The absorption spectra of riboflavin and rose bengal almost do not overlap. So we looked at performing procedures using both photosensitizers at the same time and at the same setting. What I can already disclose is that resistance to enzymatic digestion goes through the roof—it's roughly four times higher than with riboflavin at 5.4 J. It's also much higher than rose bengal alone, but we used fluences as high as 15 J.

We very recently had a patient referred to us with *Acanthamoeba* keratitis, confirmed by PCR and confocal microscopy, who had been unsuccessfully treated with appropriate medical therapy for almost a year. We received approval from our local ethics committee to do compassionate use therapy combining riboflavin UV-A treatment and rose bengal green-light treatment in a prototype with fluences up to 15 J.

The first time we saw the patient, he had suffered extreme pain for 10 months, had photophobia and blepharospasm, and barely opened his eyes. The first combined treatment calmed down the eye a bit, but *Acanthamoeba* cysts were still present. We did a second treatment after two months and a third treatment after five months. We saw him a month ago, and the eye is completely calm, with a quiescent central scar. There are no detectable cysts, and he's on a waiting list for a penetrating keratoplasty. This is absolutely mind-blowing and made us very excited about what happens when you combine the two chromophores and repeat treatment.

Now, back at the lab, we're trying to determine the optimal sequence of events, knowing that rose bengal is more superficial and riboflavin goes deeper. Probably it makes more sense to start with the deeper treatment and then continue with the more superficial one. We also need to learn what fluences at different wavelengths mean in terms of endothelial damage because 5.4 J at 522-nm green light is nothing like 5.4 J at 365-nm UV-A.

I think that combined treatment could really be the answer because rose bengal works super well. The downside is its depth of penetration. We need something to go deeper; otherwise, these cysts just retreat to greater depth.

**Dr. Rose-Nussbaumer:** The photochemical reaction goes deeper than the 100 µm or so that the rose bengal penetrates, but apart from that, I agree with you.

## Considerations and Cautions

***Dr. Jeng:*** *Would you consider using CXL alone to manage a patient, or as an adjunct to antimicrobials?*

**Dr. Rose-Nussbaumer:** I think at this point it's adjunctive. Some of the work being done is really promising, but we first have to establish that the procedure alone is truly beneficial, and for which organisms, before we can start to treat patients earlier with CXL as primary therapy.

**Dr. Hafezi:** I agree. For the sake of better understanding the effects, we published a prospective randomized controlled trial a year ago, looking at the two treatments independently.[3] I would use CXL only as an adjunct treatment.

On the other hand, I would always use it in appropriate cases because we should remember that CXL does something that no antimicrobial medication can: it increases the tissue's resistance to digestion, and that's a huge advantage beyond the direct killing effect. Theoretically, we should have a smaller corneal scar because the access of the collagenases into the cleavage site is impaired.

I think it's also shown in a meta-analysis that the healing time is faster, and the earlier the treatment the better.[4] I would appeal to everybody's sound judgment that if you use CXL in a very advanced ulcer, you cannot expect a miracle; but if you do it very early in the game, you might see a faster time to healing and a smaller scar.

**Dr. Rose-Nussbaumer:** Just as a counterpoint to that: in a randomized clinical trial in fungal keratitis, we actually saw larger scars in those who had riboflavin CXL, though this was with the lower-fluence protocol. We looked at it in various ways. We did contact lens overrefraction because we thought that maybe it affected the astigmatism or was just a difference in melts, but even with overrefraction, the patients had worse vision with CXL.[5]

We also measured it objectively with Pentacam, and densitometry showed that the cornea was much more opacified in the patients who had CXL with riboflavin for fungal keratitis. Also, we didn't see a benefit in terms of perforation or need for therapeutic penetrating keratoplasty. In our hands, there was no benefit with the lower-fluence riboflavin protocol for fungal ulcers.

**Dr. Hafezi:** With all due respect, I disagree, and this is a nice point-counterpoint because I think when you use the methods at a low fluence in huge ulcers, you are not really comparing apples with apples. If there is a large ulcer, I first want to appease the cornea. I want to see a quiescent scar, and then I can do the visual rehabilitation later. So I would be much more interested in the outcomes and scar size in smaller ulcers using high fluence. I think then it's a good comparison because it's relatively hard to take visual acuity as an indicator in a cornea that can have spots of infection all over the visual axis.

I also wanted to mention something to you, Dr. Rose-Nussbaumer, because you focus on fungal keratitis. We have seen that a fractioned approach—doing a CXL and repeating it 48 hours later and even 72 hours later a third time—has a benefit in fungal keratitis. It might have to do with immunomodulation of something we destroy in the cornea that then has time to react, and this fractioned approach was clearly beneficial in our hands for fungal, but not bacterial, keratitis.

**Dr. Rose-Nussbaumer:** In response to your first point, we did primary treatment of smaller ulcers in the trial I mentioned—we didn't do very severe ulcers. We were capturing many different outcomes, and visual acuity was one of them. Our primary outcome was actually microbiological, and we did not see improvement in microbiological cure among the CXL group, which we found surprising because we had anticipated that it would at least sterilize the surface.

In terms of your comment on repeated treatments—it's really, really interesting, and I would love to study it, but I've found that developing a protocol around that has been difficult because there's no consensus. It sounds like you're working on what a protocol would be, and I'm interested in that research going forward.

***Dr. Jeng:*** *What are the risks of doing CXL for infectious keratitis? Any special considerations?*

**Dr. Hafezi:** I would avoid anything that looks suspicious for a viral infection; and given the current state of knowledge, I would keep my hopes down for *Acanthamoeba*. As far as bacteria and fungus (or mixed organisms), I think that the earlier the better, using at least 10 or even 15 J/cm$^2$.

I wouldn't use it for very deep fungal ulcers because there we had some perforations. I wouldn't go into an 8-mm fungal ulcer for now; we first have to learn with normal-size ulcers.

**Dr. Rose-Nussbaumer:** For me, for now, riboflavin CXL has no role in fungus and *Acanthamoeba*. The organism is a key indicator, and I agree that early and superficial ulcers are more likely to respond to riboflavin CXL. Otherwise, I don't use it. I'm really looking forward to hearing more about the fluence over time because I think higher-fluence protocols have the potential, and I love the idea of studying the two photosensitizers together.


1 Iseli HP et al. *Cornea*. 2008;27(5):590-594.
2 Kling S et al. *Cornea*. 2020;39(8):1020-1026.
3 Hafezi F et al. *Eye Vis* (Lond). 2022;9(1):2.
4 Ting DSJ et al. *Ocul Surf*. 2019;17(4):624-634.
5 Prajna NV. *Ophthalmology*. 2021;128(6):950-952.



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