REVIEW of Ophthalmology

Cross-linking: Finding The Right Parameters

Strengthening the cornea has a host of potential uses—but the ideal parameters are still being worked out.

Christopher Kent, Senior Editor
9/8/2015

The process of cross-linking the corneal stroma to strengthen it, using riboflavin and UVA light, continues to be the focus of researchers' attention in laboratories around the world. It's clear that the process is beneficial in numerous situations, most notably in preventing or minimizing the progression of keratoconus. But because the procedure has only been around for a few years and is not approved for clinical use by the U.S. Food and Drug Administration, some basic issues about the most effective ways to do the procedure remain unanswered.

Here, several surgeons with experience using the procedure discuss what they've learned about cross-linking with the epithelium on (rather than off); which parameters of light, riboflavin and timing work the best; and how these answers are modified when cross-linking is used in different ways.

Epi-on vs. Epi-off

In order for stromal cross-linking to take place, riboflavin must make it past the epithelium and permeate the corneal stroma. Because the corneal epithelium is an effective barrier to most formulations of riboflavin, the original protocol for cross-linking, now known as the Dresden protocol, required removing the epithelium before applying the riboflavin solution to the cornea. While this protocol is undeniably effective, from the outset researchers hoped to find a way to make the procedure work without having to remove the epithelium.

Independent Research

The problems with epi-on cross-linking center around the difficulty of getting riboflavin through the intact corneal epithelium. However, different formulations have different levels of success because of their chemical structure, and it appears that a consortium of American doctors interested in studying cross-linking, known as CXL-USA, may have discovered a formulation that penetrates the epithelium quite rapidly.

“CXL-USA is an IRB-approved group of physicians conducting multiple studies, working on innovating and optimizing the science and practice of corneal strengthening,” explains Roy S. Rubinfeld, MD, MS, in private practice in Rockville, Md., and Fairfax, Va., a clinical associate professor at Georgetown University Medical Center in Washington, D.C., and a member of the consortium. “CXL-USA has been up and running and treating patients actively since 2009. We’ve done our best to make cross-linking available in our clinical trials, and we continue to innovate and publish, spreading information and sharing our experiences. We’re focused on the science part of this, not the commercial side.”

Dr. Rubinfeld says it’s been very helpful to do this research without commercial involvement. “In a commercial trial, whatever protocol you go in with is what you have to continue to do for the next few years,” he says. “Once you set up an FDA trial, for example, you have to stay with the chosen technique, metrics and timing. In general, no matter what you learn during the years of conducting the trial, you can’t use any of that knowledge to innovate and improve. You have to follow the pre-established protocol.

“In our world, because we write our protocols with some flexibility in them and the ability to incorporate innovation, we haven’t had that limitation,” he says. “When we’ve figured out that something works better, we’ve switched to it. For example, we tried many riboflavin formulations that took one to three hours to load into the stroma through intact epithelium. We just kept modifying them and the delivery techniques until we found ways to consistently, reliably and homogenously load through intact corneal epithelium in 20 minutes or less, and then we switched to that protocol.

We've been able to be much more innovative and agile than commercial trials can be."

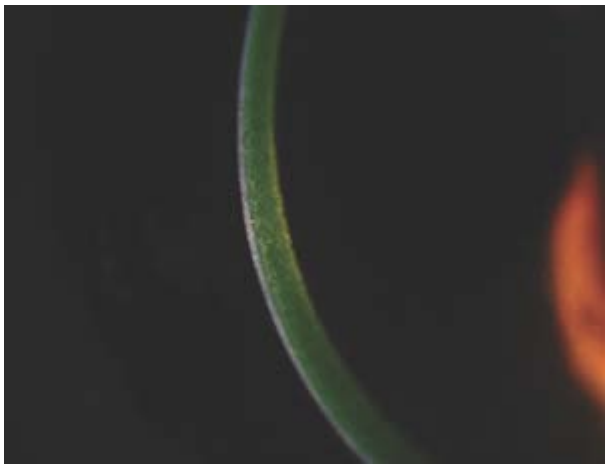
Perfecting Epi-on

Dr. Rubinfeld notes that finding a formulation of riboflavin that could readily penetrate intact epithelium has been a central focus of the CXL-USA group. "If you can do cross-linking without scraping off the epithelium, anybody would prefer that," notes Dr. Rubinfeld. "Even people who have strongly defended the epi-off approach are now saying, 'If you can get the riboflavin in, and the UVA light gets in and the oxygen is present in the corneal stroma, you're going to get good cross-linking.' And everyone agrees that it would be great if patients didn't have to undergo four to seven days of discomfort and the risk of infection, haze, scarring and perforation—all of which have been reported with epi-off. Epi-on is an inherently noninvasive treatment, and everybody wants epi-on to work.

"At CXL-USA we have developed a proprietary, patent-pending riboflavin formula that's specifically formulated for rapid, consistent, homogeneous riboflavin stromal loading with the epithelium intact," he explains. "We've tried and observed many other formulations in our many studies, including two commercial formulations available in Europe that are specifically designed for transepithelial cross-linking. However, we were disappointed with the results they produced. Using our new formulation, our investigators across the United States have been able to load both eyes in 20 minutes or less. We also have a proprietary, patent-pending loading delivery system that does not involve iontophoresis. Based on our formulation and protocol, we're consistently able to load the stroma quickly and easily and get very effective cross-linking. (See example, above.) Of course I'm biased, but if I were having cross-linking, this would be my preferred approach. In fact, William Trattler, MD, one of our key investigators, did treat his 12-year-old daughter with our epi-on technology. It not only stopped her disease progression but improved her acuity as well."

Dr. Rubinfeld says the surgeons in CXL-USA started with epithelium-off treatments at the outset. "Like everyone else, we simply didn't believe that epi-on could work," he notes. "With those early formulations, it took two to three hours or more to get adequate stromal loading through the epithelium in the first set of corneas we treated. It was not a pleasure for the patients or

the staff. Over time we've adjusted key characteristics of the formulation until we developed the current version. It makes the loading process work consistently, smoothly and easily. Patients really appreciate a short procedure—lying there for 20 minutes listening to music, as opposed to an hour or more. The next day, they're back to their normal activities because we haven't removed their epithelium. They're seeing well. Usually they drive to the office for their one-day-postoperative visit."



A new proprietary riboflavin formula created by CXL-USA successfully penetrates the epithelium quickly without soaking it. Above: The new formulation used on the eye of a 20-year-old patient with keratoconus. After 15 minutes of epithelium-on soaking, this slit lamp photo shows a well-loaded stroma (green) and a clear epithelium (white). (Photo used with permission of EyeWorld; image by Roy S. Rubinfeld, MD, MS.)

Dr. Rubinfeld notes that all of the cross-linking approaches are possible in part because of specific characteristics associated with riboflavin. "One of the good clinical aspects of riboflavin is that it's easy to see," he says. "It's a bright yellow-green color that's readily visible. So, after loading, anyone can take a slit lamp and see where the riboflavin is, how much is there and whether or not it's evenly and adequately distributed. Then when you apply UV light, it fluoresces. It's essentially its own marker. If it didn't have that vivid color, we'd have to have some sort of advanced technology to measure where it is.

"The other nice thing is that once you get the riboflavin through the epithelium, it diffuses nicely through the stroma," he adds. "The epithelium has a lot of lipids and other barriers to penetration of water-soluble solutions on the surface, whereas the stroma is mostly water and is completely water-soluble. Riboflavin is also incredibly water-soluble, so it diffuses easily throughout the stroma. That's one reason other innovations like femto pockets or channels or partial-scraping procedures work. They just need to

get the riboflavin through the intact epithelium; then it spreads throughout the stroma. Of course, with our riboflavin formulation, laser pockets and channels are not necessary. Those approaches were developed because other riboflavin formulations are unable to penetrate the epithelium well."

Dr. Rubinfeld agrees that riboflavin in the epithelium can theoretically block some of the UV light from reaching the stroma. "Not only will that block some UVA light, it will also consume some of the oxygen needed in the stroma for cross-linking to occur," he says. "We don't want that. But in our proprietary technique the epithelium is crystal clear after loading, indicating that the riboflavin isn't getting trapped there. At the same time, the stroma is well-loaded with green in a homogeneous concentration. So our protocol avoids this problem."

Combining Cross-linking and Conductive Keratoplasty

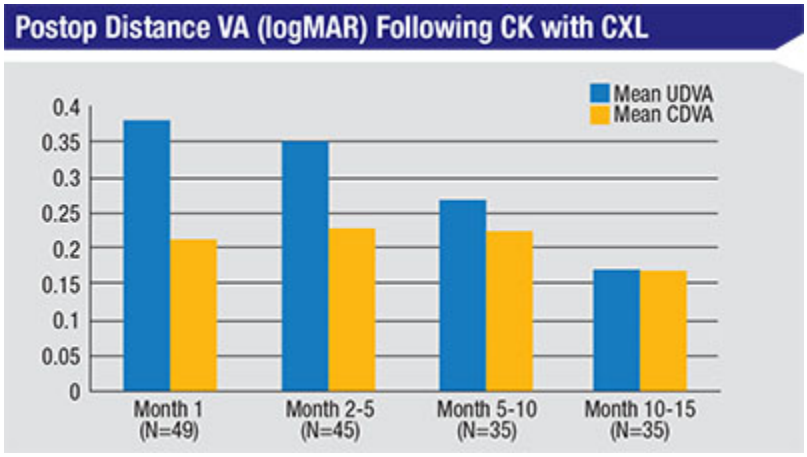
One of the applications for cross-linking currently being investigated is using it to stabilize the refractive changes produced by conductive keratoplasty. In CK, the surgeon uses a probe inserted into the cornea to a depth of about 500 µm to increase the temperature in a circumferential series of eight or more spots placed 6, 7 or 8 mm from the corneal center. The heat causes controlled shrinkage of the tissue, resulting in a tightening effect on the mid-peripheral cornea, increasing refractive power. CK has been used to treat astigmatism, decentered ablations, keratoconus and trauma, as well as to produce a moderate refractive correction. In general, the procedure has not been widely adopted due to the tendency for the changes to regress over time. Researchers realized, however, that cross-linking might minimize or eliminate that drawback.

"Over time, we found it frustrating for patients (and surgeons) to do a cross-linking procedure which stopped vision loss but did not do much to improve the patient's poor vision," says Roy S. Rubinfeld, MD, MS, a clinical associate professor at Georgetown University Medical Center in Washington, D.C., and a member of CXL-USA. "Over the past several years, to improve vision in these patients we've been doing a lot of conductive keratoplasty to regularize the corneal shape, followed by cross-linking, both to lock in the beneficial effects of the CK and to stabilize the cornea. We call this 'CK-plus' or refractive CXL. CK is a noninvasive, very well-tested and safe procedure that's been around for a long time, but one of its limitations has always been the tendency for the improved visual results to regress. When we combine it with cross-linking, the corneal changes seem to stick. We have one- and two-year data now that demonstrates substantial, statistically significant clinical improvement in both uncorrected and best-corrected vision. (See chart, p. 32.) It's been really fulfilling for both patients and surgeons."

Dr. Rubinfeld says it took investigators some time to figure out the sequence in which the procedures should be done. "The literature shows that when you do CK at the same time as cross-linking, the results are probably going to regress," he notes. "But our colleague Arthur Cummings, MD, in Dublin found that leaving an interval between the CK and cross-linking helped to prevent the effect from regressing. So, we wait a day after the CK procedure before doing the cross-linking, and that seems to make all the difference. We don't have final data yet, but the people who had the procedures a day apart have had notably better long-term data that those who were done on the same day."

Dr. Rubinfeld adds that they perform the CK with real-time monitoring. "I'm able to watch the intraoperative keratometry when I do the CK," he explains. "If I find I'm ending up with an oval-shaped or pear-shaped ring on the cornea because of astigmatism, I can make it round with an extra spot or two. I sometimes take a Pentacam before the procedure and again after a few spots and then decide if it's enough. It's a great technique, because we can see what we're doing while we're doing it."
—CK

Dr. Rubinfeld says he is not an advocate of high fluence. “More is not always better,” he notes. “The rate-limiting factor in the cross-linking reaction is oxygen, and the more UV you use, the more you deplete that rate-limiting agent. I think of it as being like a recipe. If the recipe says to bake a pan of brownies for 30 minutes at 300 degrees, trying to bake it for three minutes at 3,000 degrees probably won’t produce the result you’re hoping for. You might be able to improve upon the original formula, but there are bound to be limits. We [at CXL-USA] have aimed to create a highly effective corneal strengthening procedure that’s the least inflammatory and most respectful of the health of the corneal cells and the patient’s comfort.”



Results of combining cross-linking with conductive keratoplasty in eyes with keratoconus or corneal ectasia, with preop corrected distance visual acuity of 20/40 or worse. More recent follow-up data suggests that the benefits seen at months 10 to 15 have been maintained. Similar tests conducted without cross-linking have regressed more quickly and produced significantly worse long-term results. (Image courtesy Roy S. Rubinfeld, MD, MS.)

What’s Next?

And what about the ongoing debate regarding epi-on vs. epi-off treatment? Asked whether he thinks epi-on will eventually replace epi-off as the cross-linking procedure of choice, Dr. Rubinfeld gives a qualified yes. “If the patient just needs cross-linking to stabilize the cornea, then I think epi-on will become the standard over time,” he says. “Cross-linking is great at stopping the progression of vision loss. So, if someone comes into the office and has early keratoconus and hasn’t yet lost vision, cross-linking is the procedure for that person. In that situation, if epi-on works fine, why wouldn’t you use it? But if a patient is only correctable to 20/80 because of advanced keratoconus and

irregular astigmatism, then you'll want to try and find a way to not just stop the progression but also make the patient see better. Cross-linking alone is not very good at that, for most patients. That's where you want to combine cross-linking with something else, such as Intacs, topography-guided ablation or standard PRK. If you're going to do PRK, you're going to have to remove the epithelium anyway, so in some situations, epi-off will continue to make sense."

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