Collagen Cross-Linking: The Promise Keeps Growing

Studies continue to show good results while researchers develop new ways to expedite the procedure and utilize its effects.

Christopher Kent, Senior Editor

Despite not yet having U.S. Food and Drug Administration approval, interest in corneal collagen cross-linking is widespread. The procedure, which uses riboflavin and UV light to increase the links between collagen fibrils and strengthen the cornea, offers real hope for people suffering from keratoconus or ectasia, and may be useful for other conditions as well.

Seven Year Follow-up

A. John Kanellopoulos, MD, clinical associate professor of ophthalmology at NYU Medical School and director of the Laservision.gr Institute in Athens, Greece, has used the cross-linking procedure, with epithelium removed, on more than 1,000 eyes. His group is currently investigating more than 20 cross-linking protocols.

"The good news with the standard cross-linking procedure is that we now have cases with a follow-up of seven years," he says. "It appears that the effect holds. In fact, we've noticed a progressive effect, even at five years out. After studying pachymetries and Pentacam maps and OCTs, we attribute that to the cornea tissue re-expanding for the first five years after the initial shrinkage is caused by the cross-linking procedure. The periphery expands more, creating the topographic effect of the peak of the cornea becoming flatter and flatter. This can add another half-diopter—maybe even 1 D—to the initial 1.5- to 2-D-correction that we and other investigators are seeing in ectatic corneas."

Several clinical trials are ongoing in the United States as well, including both physician-sponsored trials and company-sponsored multicenter trials. "Based on what I know, and what's been presented and published, the results of these trials have pretty much mirrored the findings from the international trials that have been published," says R. Doyle Stulting, MD, PhD, professor of ophthalmology at Emory University in Atlanta, who has participated in several trials. "For example, the results of the physician-sponsored trial that I conducted confirmed the results reported by Christine Wittig-Silva and her group in Australia." (Preliminary results from Dr. Wittig-Silva's randomized, controlled study of 66 eyes with documented progression of keratoconus found that K-max in the treated eyes flattened by an average of 0.74 D at three months, 0.92 D at six months and 1.28 D at 12 months—all significantly better than the untreated control group. The treated group also displayed a trend toward improved best-corrected visual acuity; in contrast, the control eyes trended toward decreased BCVA during the same period.1)

One reason corneal cross-linking continues to generate interest is that the effect doesn't appear to regress in most patients. "Out of about 1,000 cases we've treated, only three required retreatment," says Dr. Kanellopoulos. He acknowledges, however, that not every center is achieving equally good numbers. "We're aware of work done in Zurich, where they found a higher percentage of regression and retreatments," he notes. "The explanation could lie in our protocols. Our riboflavin solution has the same concentration—0.1-percent riboflavin—but ours is prepared as a sodium phosphate dilution, and it's a slightly hypotonic, 340-millimolar solution. In our experience, this formulation penetrates the stroma more readily, which probably results in a more uniform procedure.

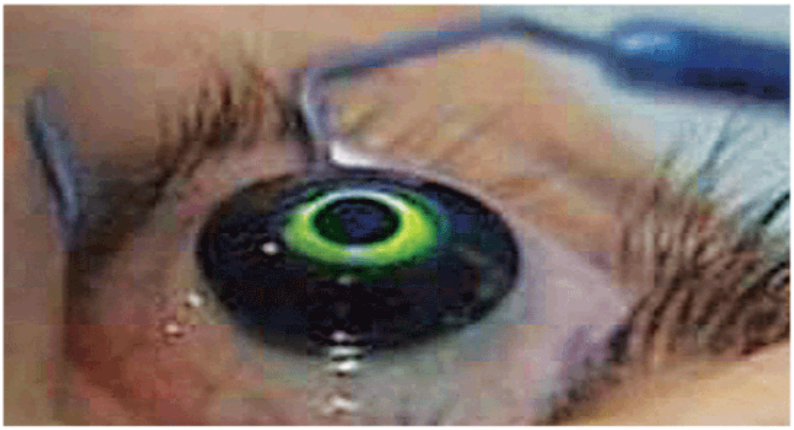
"The other difference is that the Zurich and Dresden protocols use manual removal of the epithelium; we remove the epithelium using a Wave- Light laser," he continues. "We perform a

photodynamic keratotomy at 6.5-mm diameter to a depth of 50 µm. In most keratoconic eyes, this probably goes deeper than the epithelium and affects Bowman's membrane as well. Riboflavin is a very large molecule which has difficulty passing through Bowman's membrane, so we theorize that this facilitates its entry into the stroma."

Epithelium On or Off?

As clinicians and researchers work to find a maximally effective protocol for corneal cross-linking, a significant question is whether the epithelium really needs to come off in order to get optimum results. "The epithelium blocks absorption of riboflavin," Dr. Stulting points out. "Studies have shown that it doesn't get through an intact epithelium during an exposure time of 30 minutes—but unless it gets into the corneal stroma, cross-linking can't take place. So, the epithelium either has to be removed or treated in such a way that it no longer blocks absorption of the riboflavin."

"We've seen a lot of work from the United States and Italy using techniques that leave the epithelium on," notes Dr. Kanellopoulos. "There's definite value to this approach. It's obviously more comfortable and has a shorter healing time. The question is whether you're getting enough cross-linking. Unfortunately, we don't have an in vivo tool to titrate how much cross-linking we're achieving with any of these techniques. I've personally seen several breakthrough ectasias in eyes that have been cross-linked with the epithelium on; in fact, we're preparing a series reporting these cases."



Peter S. Hersh, MD, FACS

A limbal protecting ring is applied to the cornea as part of Avedro's KXL fast cross-linking treatment (done with epithelium removed). Treatment time may be as short as five minutes, including presoak.

Despite these concerns, the advantages of leaving the epithelium intact appeal to many clinicians. "When you remove the epithelium you increase the risk of corneal infections," notes Brian S. Boxer Wachler, MD, director of the Boxer Wachler Vision Institute in Beverly Hills, Calif. Dr. Boxer Wachler was the first surgeon to perform transepithelial cross-linking, which he refers to as Holcomb C3-R. He's been using the procedure for the past six and a half years. "You certainly can have pain, because you're inducing a corneal abrasion. And I recall a study presented at the Academy of Ophthalmology meeting that found corneal nerve desensitization following epithelium removal that took six months to recover. Furthermore, there can be cases of delayed epithelialization and haze. Leaving the epithelium intact, the only side effect we've seen is a little scratchiness for a day or two. When you leave the epithelium intact, you make this a pretty benign procedure."

Dr. Kanellopoulos admits that epithelial removal has some downsides. "These patients take a long time to heal," he says. "The best-case scenario is about a week; the worst-case scenario is two months. These patients also require special care compared to our routine LASIK and PRK cases, to ensure that some of the delayed healers don't develop more serious complications such as infection or permanent scarring of the cornea. I have these patients use a lot of lubrication; in some cases I

prescribe daily bandage contact lens use. In a few cases we've even employed some extreme measures, such as autologous serum. Fortunately, these are the exceptions."

"In addition, the epithelium-off procedure is painful, similar to what a patient experiences with PRK," he adds. "However, we've had very bad experiences with using topical anesthetics, so I would strongly advise against using them in this situation, even though they may offer comfort. The risk of creating a severe epitheliopathy is significant enough for me to avoid them.

"In general, I don't think pain is a major issue with the procedure, as long as patients are well-informed and prepared," he adds. "I think the patient's fear is more of an issue than the pain." (Dr. Kanellopoulos notes that one alternative would be the use of an intramuscular painkiller.)

Getting Through the Barrier

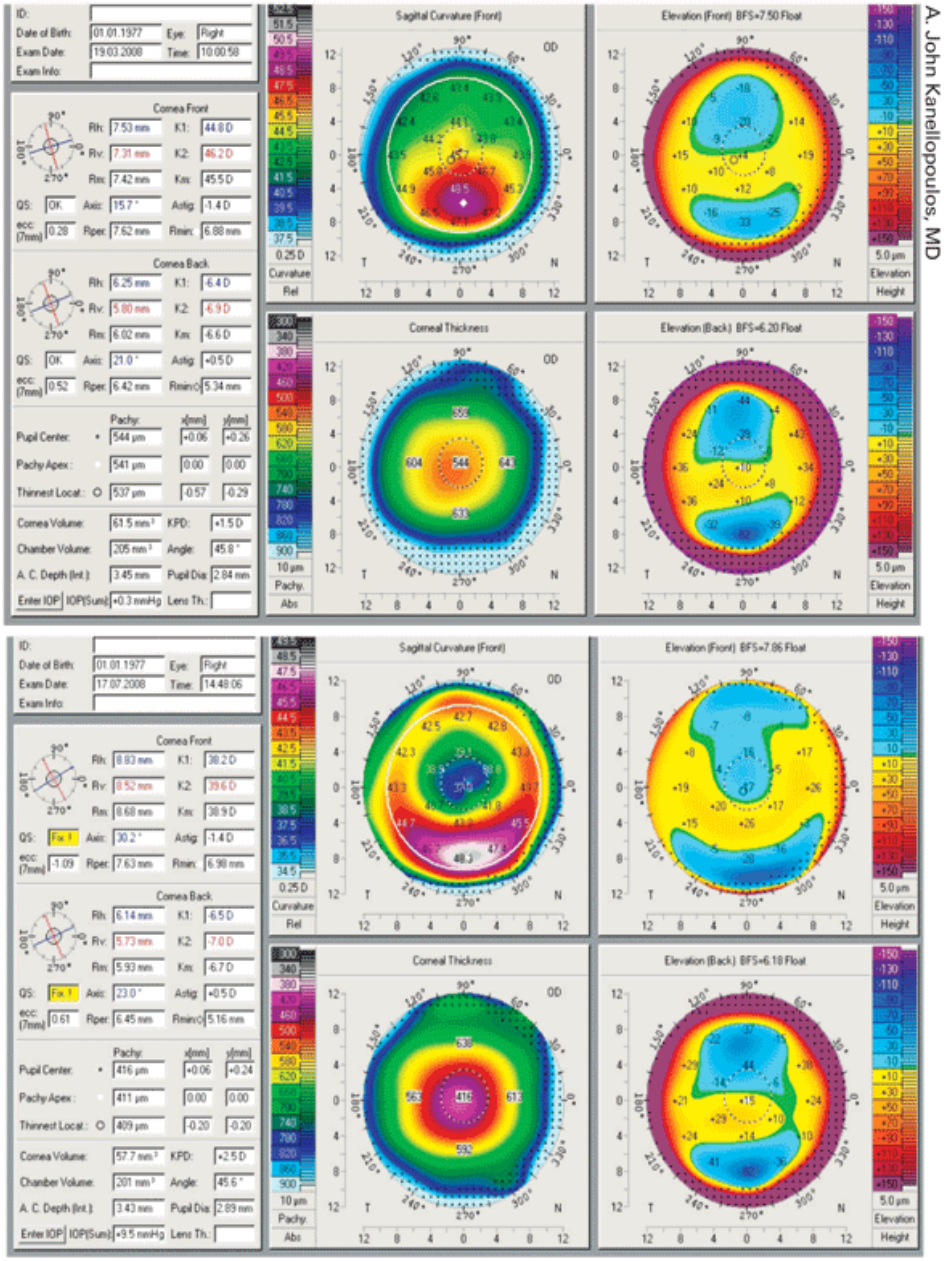
Dr. Stulting observes that many practitioners around the world are using or developing cross-linking protocols that leave the epithelium intact. "To the best of my knowledge, all of them incorporate some sort of technique that makes the epithelium more permeable," he says. "This could be anything from limited mechanical insult, such as scratching the epithelium, to chemical manipulation of the epithelium using topical anesthetics or other compounds. In fact, there's a riboflavin formula commercially available outside the United States that's labeled and tested for use with an intact epithelium."

Dr. Kanellopoulos reports that several investigators, including his group, have tried using a riboflavin solution spiked with higher concentration of the preservative BAK.
"The thought process behind that is that the preservative tends to break down the junctions between epithelial cells," he explains. "So it may be creating passages for the large riboflavin molecule to go through the epithelium more readily. Our preliminary data are confirming this theory. In terms of the patient's experience, these eyes are less comfortable than when we use a preservative-free solution—but they are definitely more comfortable than if we take the epithelium off. Of course, the ultimate problem is that we still don't have an in vivo device to measure the cross-linking results in the three categories: epithelium on; chemically treated epithelium on; and epithelium off."

Dr. Boxer Wachler says that for the past six and a half years his group has used anesthetic drops to enhance the passage of the riboflavin through the cornea. "The preservative in the drops loosens up the epithelial tight junctions," he says, noting that preservatives serve the same function in many other ocular medications as well. He also points out that other factors can affect the ability of the riboflavin to be absorbed. "What carrier solution is the riboflavin in?" he asks. "If it's in Dextran, which is very thick—the consistency of honey—the solution almost binds the riboflavin, making it more difficult to achieve penetration. If the riboflavin is in a more watery solution, it's more likely to penetrate the cornea."

Comparing Epi On and Off

A research study coordinated by the CXL USA Study Group, headed by Roy S. Rubinfeld, MD, MS, is now actively recruiting under Institutional Review Board protocol. This physician-sponsored study is comparing a proprietary cross-linking procedure done with the epithelium on to a similar protocol done with the epithelium removed.
William B. Trattler, MD, who practices at the Center for Excellence in Eye Care in Miami and is a volunteer assistant professor of ophthalmology at the University of Miami's Bascom Palmer Eye Institute, has participated in the FDA multicenter study on corneal collagen cross-linking and is currently taking part in the CXL USA trial. "In this protocol, the surgeon can decide to treat patients either by removing the epithelium or by keeping the epithelium intact during the treatment," he says. "The study is examining the outcomes of cross-linking for patients with keratoconus, forme fruste keratoconus, pellucid marginal degeneration and post-LASIK ectasia, and in radial keratotomy patients with diurnal vision fluctuations."



Prophylactic cross-linking can be done at the end of LASIK in "high-risk" cases. In this 31-year-old patient, BCVA preop (top) was 20/30; at five months (bottom), UCVA was 20/20. Out of 25 cases studied, no ectasia or refractive surprises were seen (mean follow-up: 18 months).

Dr. Rubinfeld, who is in private practice with Wash-ington Eye Physicians & Surgeons in Chevy Chase, Md., clinical associate professor at Georgetown University Medical Center and on staff at The Washington Hospital Center in Washington, D.C., notes that the comparison of epi-on and off makes this study unique. "However, we're not including an untreated control group," he says. "I think the risks of not treating patients are far greater than the risks related to performing cross-linking, and I'd feel very uncomfortable watching individuals in the control group progressively worsen—as many keratoconus patients do."

Dr. Trattler admits that leaving the epithelium on lengthens the procedure time. "It may take an hour or longer to get enough riboflavin into the corneal stroma," he says. "In my experience, the longest it has taken was about an hour and a half of placing riboflavin drops." He notes that because riboflavin fluoresces with a cobalt blue filter, the surgeon can determine the amount of riboflavin absorption by checking periodically at the slit lamp.

Despite the time concern, Dr. Trattler sees the benefits of leaving the epithelium intact as a worthy trade-off. "Theoretically, without a large corneal abrasion there should be a reduced risk of developing a corneal infection, and we expect there will be a reduced risk of cornea haze," he says.

"Patients are more comfortable, and have faster visual recovery. Pain isn't necessarily eliminated altogether—the ocular surface does get very irritated, and patients can occasionally develop some epithelial sloughing. And although an 8-mm circular abrasion is avoided, the patient can still end up with a small 1-mm epithelial defect. Nevertheless, patients have in general reported less discomfort when the epithelium is not removed. Furthermore, some patients have experienced improvement in uncorrected visual acuity as early as the first postoperative day." (Dr. Trattler notes that these are early results, and the long-term data is still under analysis.)

Dr. Rubinfeld agrees that so far they've seen some great results. "At this point in our study we have clinical impressions, and we need the complete data to make sure we've got it right," he says. "I can only tell you that so far our group, like Drs. Pinelli and Boxer Wachler, has had excellent results in terms of getting the riboflavin to penetrate into the cornea."

The Athens Protocol

One variation on the cross-linking procedure pioneered by Dr. Kanellopoulos in Greece combines cross-linking with a small amount of refractive laser ablation. "In our experience, cross-linking works best when combined with laser," says Dr. Kanellopoulos.
"I realize that it sounds like a radical measure to employ laser with a thin cornea, but even if we're only talking about removing 8 or 10 µm, we're removing Bowman's and getting better riboflavin penetration.

"We've found that topography-guided intervention in the direction of normalizing the cone is effective," he continues. "We've also compared three variations on this protocol: cross-linking alone; cross-linking with possible laser procedure at a second time; or combining the two together. Overwhelmingly, a partial topography-guided PRK and cross-linking done in a sequential, combined manner, is best; it appears to have a synergistic effect in the visual rehabilitation of these patients. As a result, this is our standard procedure now, even in corneas that are very thin. Our standard treatment is up to 50 µm in thickness, which would be considered negligible for a normal myopic eye. In our experience, the positive results definitely overcome the potential disadvantages."

Dr. Kanellopoulos's group refers to this as the Athens protocol, noting that the intent is not just to remove the epithelium, but to normalize the very irregular cornea and at the same time have some refractive effect, even though it may be slight. "We use the PTK procedure first; then we go directly into a partial topography-guided PRK using the WaveLight platform," he explains.
"We've set an arbitrary maximum ablation limit of 50 µm—minimum, 10 µm. We correct as much cylinder and sphere within that range as we can. In most cases, this represents 30 to 40 percent of their refraction. So this is really a therapeutic, not refractive PRK, because the purpose is just to normalize the big troughs and valleys in the cornea.

"After this we only need to use riboflavin drops for 10 minutes because the riboflavin gets into the cornea right away," he continues. "Then we do collagen cross-linking using 7 mW/cm2 for 15 minutes, rather than the standard 3 mW for a half hour. We've made this change during the past two years, and we believe we're getting a better effect. The corneal keratocytes, in theory, survive better under higher UV exposure over a shorter time."

Dr. Kanellopoulos notes that, at first, his group was heavily criticized for using this protocol. "The Athens protocol, essentially, thins an already thin cornea," he says. "But the combination of topography-guided partial ablation and cross-linking has been extremely fruitful. We're very pleased and proud that it's now been adopted by several international centers."

Does the Athens protocol work well for everyone? "In very thin and severely ectatic corneas, we don't have as good a response to the treatment," says Dr. Kanellopoulos.
"We've em-ployed this protocol even in very thin corneas as a compassionate use measure before a patient undergoes PK—even in some corneas that were 360 µm before treatment. Some of these patients still had breakthrough ectasias. But if you can avoid grafting in more than 50 percent of these patients, it's a tremendous help in terms of their long-term prognosis."

Dr. Kanellopoulos also notes that vision isn't always better post-treatment. "If you cross-link the cornea in some younger patients with keratoconus, you definitely get a stabilization effect," he says. "However, some of these young people have worse uncorrected vision afterwards. We were puzzled by this finding early on. Our theory is that they may be losing some of the multifocality that comes with a keratoconic cornea.
Or, the more flexible cornea in younger patients may allow them to alter their operative visual acuity favorably—and of course, we're trying to make these corneas stiffer. So, if you treat a patient like this, don't promise that his vision will be better after the treatment. It may not be—at

least uncorrected."

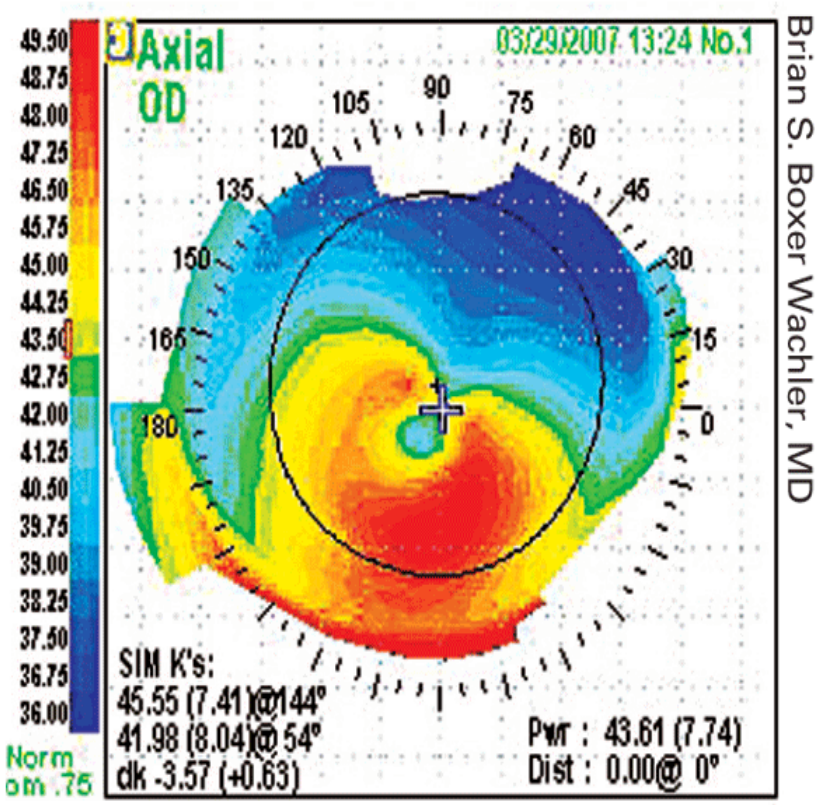
Nevertheless, Dr. Kanellopoulos points out that some outcomes are spectacular. "Our star Athens-protocol patient is a 25-year-old helicopter pilot from New York who had post-LASIK ectasia," he says. "After being decommissioned because of his visual condition, he was recommended for a corneal transplant. Instead, he came to Athens to have cross-linking done. Two years out, he's not only able to fly again—he's joined the Air Force and become a fighter pilot. Obviously, the majority of patients don't experience results this spectacular, but this demonstrates the incredible potential this procedure has. As a clinician, I see no bigger reward than experiencing this."

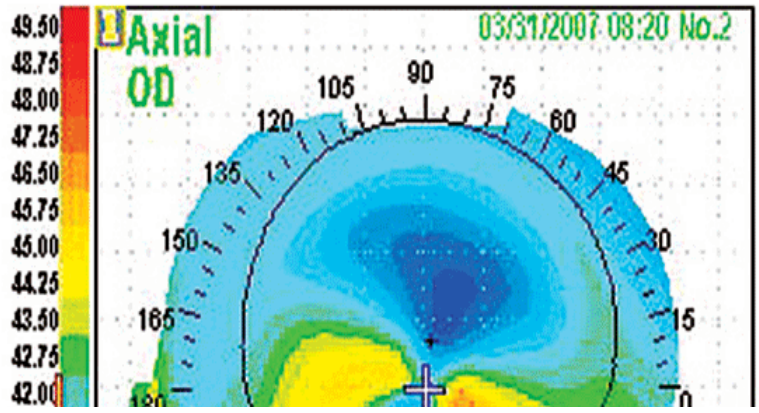
Alternative Protocols

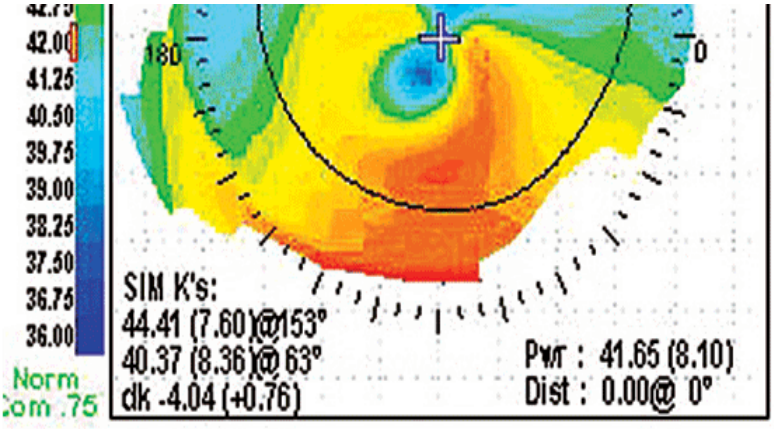
In addition to epi-on vs. epi-off, numerous other variations on the cross-linking procedure have emerged in recent years. For example, a few years ago Dr. Kanellopoulos's group pioneered the concept of using a femtosecond laser to create a pocket inside the cornea into which riboflavin could be placed. However, the positive results from the Athens protocol have put this idea on the back burner. "With the Athens protocol we not only get cross-linking of larger portions of the cornea, the patients end up with better visual acuity," he says.

Other current variations include:

• **Cross-linking combined with Intacs corneal implants.** Studies suggest that performing cross-linking after using Intacs enhances the desired flattening effect, leading to more regular topography and improved vision.







In this eye a single Intacs segment was placed inferiorly, followed by cross-linking. (Preop, top; postop, bottom.)

Dr. Boxer Wachler, one of the pioneers of this approach, reports that he's recently adopted a new twist on this combination procedure, pioneered by Aylin Ertan, MD, at the Kudret Eye Hospital in Ankara, Turkey. "Dr. Ertan came up with the idea of putting riboflavin in the Intacs channel before putting the Intac segment in," he explains. "We've found that this augments the results of combining Intacs and cross-linking. Dr. Ertan has really made a significant contribution with this technique."

• **Pulsing the UV light.** Dr. Kanellopoulos's group is experimenting with this option. "There's a self-limiting part of the photochemical cross-linking reaction which is determined by the availability of oxygen," he explains. "Higher fluence of UV light for a shorter period of time helps by requiring less available oxygen, and pulsing the UV light may do the same thing, providing time between pulses during which the tissue can recover some of its oxygen reserves."

• **Combining cross-linking with other cornea-altering treatments.** The intention here is to generate desired changes in corneal structure using a different protocol and then "lock in" the changes using cross-linking. At least two variations on this idea are under investigation.

Peter S. Hersh, MD, FACS, clinical professor and director of cornea and refractive surgery at the Institute of Ophthalmology and Visual Science, New Jersey Medical School, and director of the Cornea and Laser Eye Institute in Teaneck, N.J., is participating in the clinical trial of a new Keraflex cross-linking procedure developed by Avedro Inc. (Waltham, Mass.). "The procedure requires the use of a Keraflex microwave device [the Vedera KXS] and a special applicator for the riboflavin solution," he explains.

According to the company, the KXS system delivers a low-energy microwave pulse into the corneal stroma via a sterile, disposable emitter that's placed in contact with the corneal surface. The brief pulse—shorter than one second—raises the temperature of that area of stroma to about 65 C [149 F], shrinking the collagen and forming a toroidal lesion in the upper 150 µm of the stroma. (Bowman's membrane is not affected, according to the company.) The shrinkage flattens the central cornea; adjusting the amount of energy applied alters the resulting effect. The cross-linking appears to "lock in" the changes.

"Early trials of the procedure with both keratoconus patients and ectasia patients have been carried out in Istanbul, Turkey," says Dr. Hersh. "We only have data for a few months of follow-up, but the early results are encouraging. We've seen good flattening of the cone in keratoconus patients—up to 6 D—along with regularization of topography and a decrease in myopia. Trials are now beginning in Europe, where the procedure recently received the CE Mark, and we're anticipating a multicenter clinical trial in the United States." The company says it plans to file for an FDA Investigational Device Exemption this year.

Dr. Kanellopoulos's group is investigating a similar procedure, called TRXL, proprietary to California-based Seros Medical. "In this procedure we combine cross-linking with the use of a continuous-wave infrared laser that shrinks the anterior stroma of the cornea without affecting the

epithelium," he explains. "We then perform epithelium-on cross-linking using high-BAK riboflavin. We've done some initial clinical cases with eight-month follow-up, and this appears to have great promise.

"One reason this has so much potential is that you can design the laser treatment, and thus the shrinkage of the cornea, on the computer before you apply it," he continues.
"That means that in theory you may not only be able to treat ectatic and irregular corneas, but myopic, hyperopic and astigmatic eyes. The problem with treatments like this in the past, such as conductive keratoplasty, was that they tended to regress. It seems that combining a procedure of this type with cross-linking results in a more permanent effect. The results so far are remarkable."

• **Using different equipment and parameters to speed the process.** Dr. Hersh notes that Avedro is also working on a cross-linking technique that should dramatically reduce the time required to perform the basic procedure. "In KXL a limbal protecting ring is applied to the cornea," he explains. "The KXL protocol has successfully reduced procedure time from about one hour to several minutes. Clinical trials of KXL are now starting in Europe,
and we hope to perform clinical trials in the United States."

Cross-linking and LASIK

Because ectasia following LASIK is a major concern for refractive surgeons, the potential for cross-linking to aid in prevention has attracted considerable attention. (See images, p. 39.) Furthermore, in addition to managing ectasia, cross-linking might actually enhance the LASIK procedure.
"Placing a few drops of riboflavin on the stromal bed and then putting the flap back on as if we were finishing a normal LASIK procedure disperses riboflavin readily within the underlying and overlying stroma," says Dr. Kanellopoulos.
"Then we expose the eye to 7 mw of UV light for just 10 minutes. Corneal CT scans have shown that this causes increased interweaving of the flap to the anterior stroma, which we're hoping will be a potential prophylactic measure for future ectasia.

"Furthermore, there is laboratory evidence from cadaver LASIK eyes that the interface between the flap and stroma never actually touches," he says. "So there may be some amorphous material collecting there over time, which could change the refractive effect of the procedure and become a permanent cistern for interstitial keratitis in the future. Cross-linking the flap to the underlying stroma might result in a more physiological cornea after LASIK."

Champing at the Bit

Given the positive outcomes associated with cross-linking so far, surgeons in the United States are anxious to see the procedure approved. "By Sep-tember 2006, all 25 European Union countries had approved the procedure, and I believe there are now at least 130 peer-reviewed articles describing its benefits," notes Dr. Rubinfeld. "So this has been frustrating for those of us in the United States. In my medical opinion, there's no question that cross-linking is an effective and low-risk procedure, especially compared to a corneal transplant. In fact, I don't know a single ophthalmologist who'd rather perform a transplant than use cross-linking. I think cross-linking is one of the most important corneal treatments to come along in a long time."

Drs. Kanellopoulos and Boxer Wachler have no financial ties to any companies related to cross-linking or Intacs. Dr. Trattler is a consultant for CXL USA, and Dr. Rubinfeld has a financial interest in CXL USA. Dr. Hersh is a paid medical monitor for Avedro. Dr. Stulting was consultant and medical monitor for Peschke Meditrade and is currently consultant and medical monitor for Topcon.

1. Wittig-Silva C, Whiting M, et al. A Randomized Controlled Trial of Corneal Collagen Cross-linking in Progressive Keratoconus: Preliminary Results. J Refractive Surg 2008:24;7; S720-S725.

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