

# Corneal Collagen Cross-Linking for Keratoconus and Ectasia

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Welcome to the final installment of *Cataract & Refractive Surgery Today's* three-part series highlighting corneal ectasia. This article discusses corneal collagen cross-linking with riboflavin, the latest addition to our armamentarium for the treatment of keratoconus and for stabilizing corneal ectasia after keratorefractive surgery.

Internationally, cross-linking is widely becoming an accepted treatment for these corneal pathologies. Clinical trials in the United States are coming to an end and, when completed, I hope will increase the availability of cross-linking in the near future. Because cross-linking can be performed in the clinical setting, it offers physicians and patients the benefits of speed and comfort. As we gain more experience with cross-linking, I believe we will significantly reduce the need for penetrating keratoplasty among most patients with progressive corneal thinning.

Cross-linking strengthens the cornea by increasing the number of covalent bonds between collagen fibers. When riboflavin is activated by ultraviolet-A light (UVA; 3 mW/cm<sup>2</sup>), it promotes a free radical pathway that cross-links collagen and increases the cornea's strength by more than 300%. Riboflavin cross-linking is currently recommended only for corneas that are at least 400 μm thick, but a modified method that uses dextran-free riboflavin allows physicians to cross-link thinner corneas safely.<sup>1</sup>

A debate about the most efficient method for infusing riboflavin into the corneal stroma continues. The original clinical trials called for epithelial debridement of the central 7 to 9 mm of the cornea. Recently reported studies, however, suggest that applying tetracaine to the intact corneal surface preoperatively sufficiently disrupts the tight junctions between epithelial cells to promote the induction of riboflavin into the stroma. With either approach, the cornea should appear completely yellow under the slit lamp, and the aqueous should also demonstrate a yellow tint. The instillation of riboflavin generally takes about 30 minutes, after which the solution is activated by UVA (Peschke UV-X; Peschke Meditrade GmbH, Nuremberg, Germany). In many patients, this procedure not only stabilizes the process of ectasia, but also improves BCVA by one or two lines.

I hope you enjoy this installment of "Peer Review," and I encourage you to seek out and review the articles in their entirety at your convenience.

—Mitchell C. Shultz, MD, Section Editor

In 2003, Wollensak et al introduced corneal collagen cross-linking as an alternative to penetrating keratoplasty for treating progressive keratoconus.

Instead of removing the weakened stroma, cross-linking uses photosensitive riboflavin and UVA light to create new connections between existing collagen fibers. This process increases the cornea's biomechanical

strength by approximately 300% and halts the progressive thinning that occurs with keratoconus.<sup>2</sup>

The cross-linking technique developed by Wollensak et al comprises two distinct stages. First, surgeons remove an area of epithelium measuring 7 to 9 mm in diameter from the central cornea and apply riboflavin to the exposed stroma every 3 to 5 minutes for 30 min-

utes. Next, they irradiate the debrided area with UVA (wavelength = 370 nm) for 30 minutes while continuing to instill riboflavin into the eye every 3 to 5 minutes.<sup>2</sup>

Cross-linking was approved for clinical use in Europe in January 2007, but the procedure is still undergoing FDA trials in the United States.

### EFFECTS ON OCULAR ANATOMY AND PHYSIOLOGY

An experimental model developed by Ahearne et al demonstrated the effect of cross-linking on corneal biomechanics. The investigators found that treating manufactured collagen hydrogels with riboflavin cross-linking and UVA (n = 4) significantly increased the material's Young modulus (a measure of corneal stiffness). They also found that the quantity of improvement in biomechanical strength depended on how long the riboflavin was exposed to UVA. The investigators did not observe a similar change in hydrogels exposed to UVA alone (n = 4). In fact, in the absence of riboflavin, UVA degraded collagen and reduced its integrity. Because the modulus of the hydrogels in the riboflavin group did not increase significantly after 45 minutes of exposure to UVA, the investigators suggested that "the majority of cross-linking occurred in the first 30 to 45 minutes and a longer exposure time might be unnecessary" to achieve an optimal outcome.<sup>3</sup> Exposure to UVA also reduced the number of viable human corneal fibroblast cells that had been seeded into the artificial hydrogel during the manufacturing process. Confocal microscopy showed that the number of live cells decreased from 83% at baseline to 16% and 37% after 15 and 30 minutes of UVA exposure, respectively.<sup>3</sup>

A thermographic analysis of the cornea's response to UVA demonstrated the procedure's intraoperative safety. The investigators found that the mean corneal temperature in six eyes increased by a maximum of 2.6°C during irradiation with UVA. Because the ocular surface measured approximately 30°C preoperatively, the observed rise in temperature with UVA was well below the 50°C threshold at which thermal damage disorganizes collagen, induces stromal edema, and damages keratocytes.<sup>4</sup>

To determine if artificially increasing corneal rigidity interfered with the accuracy of IOP measurements, Romppainen et al evaluated 10 cadaveric corneas with Goldmann applanation tonometry (GAT), the Pascal Dynamic Contour tonometer (DCT; Ziemer Ophthalmics, Port, Switzerland), and the Tono-Pen XL (Medtronic ENT, Jacksonville, FL) before and after cross-linking. The donated corneas were mounted on artificial chambers and perfused to simulate IOPs of

10, 15, 20, 25, 30, 35, and 40 mm Hg. Before cross-linking, the measurements obtained with the three tonometers were "almost identical with the reference pressure in the perfused anterior chamber." After the corneas were debrided and treated with cross-linking, the investigators noted that all of the tonometers overestimated IOP relative to the known perfusion pressure. Of all the tonometers, the Tono-Pen obtained the least accurate results after cross-linking (+3.1 ± 8.3 mm Hg vs +2.9 ± 6.1 mm Hg with GAT and +1.8 ± 3.5 mm Hg with DCT). Compared with DCT and the Tono-Pen, however, the IOPs obtained with GAT had a greater range of variation relative to the reference measurements. Based on this finding, the investigators suggested that GAT may underestimate IOP after cross-linking and thus may not be ideal for measuring the IOP of artificially stiffened corneas.<sup>5</sup>

"Several studies show the efficacy and long-term stability of cross-linking and support its use for treating keratoconus and ectasia."

The literature reports very few adverse effects from cross-linking. Angunawela et al described a rare exception in which a 40-year-old patient developed peripheral sterile infiltrates 5 days postoperatively. The infiltrates eventually resolved on a regimen of preservative-free levofloxacin and dexamethasone, but the patient had residual thinning of the stroma (approximately 30% of the total corneal thickness at maximum) 2 months after the cross-linking procedure. Hypothesizing that the infiltrates were caused by the deposition of staphylococcal antigen "in areas of static tear pooling beneath the bandage contact lens," the investigators now carefully debride areas of heaped up epithelium from the eye before applying a protective lens at the end of the procedure.<sup>6</sup>

### OUTCOMES OF CROSS-LINKING

Several studies show the efficacy and long-term stability of cross-linking and support its use for halting or reversing the progression of keratoconus and post-LASIK ectasia.

#### Keratoconus

By 1 year postoperatively, 53% (127 of 241) of eyes treated with cross-linking gained one line of BSCVA from baseline. During the same period, the treatment

also significantly reduced apical corneal curvature in 62% (149 of 241) and decreased astigmatism by a mean of 0.93 D in 50% (120 of 241) of eyes. Keratometry and astigmatism remained unchanged after cross-linking in 17% (41 of 241) and 36% (86 of 241) of eyes, respectively. Based on these data, the investigators concluded, "The improvement in vision after cross-linking is caused by a decrease in astigmatism and corneal curvature, as well as by topographical homogenization" secondary to increased corneal rigidity.<sup>7</sup>

An analysis of 102 patients by Grewal et al also supported the utility of cross-linking for stabilizing keratoconus. By 1 year postoperatively, the investigators did not observe any statistically significant changes from baseline in the patients' mean BCVA ( $P=.89$ ), spherical equivalent ( $P=.22$ ), central corneal thickness ( $P=.647$ ), IOP ( $P=.85$ ), or anterior corneal curvature ( $P=.893$ ).<sup>8</sup>

#### Ectasia

Hafezi et al used cross-linking to stabilize progressive corneal thinning in 10 patients who developed ectasia after LASIK. At 25 months' follow-up, four patients had gained more than two lines of BSCVA, and all of them had significantly reduced measurements of maximum keratometry. The investigators initially observed the latter change approximately 12 months after cross-linking.<sup>9</sup>

#### ALTERNATIVE APPROACHES TO CROSS-LINKING

As described earlier, the traditional cross-linking technique requires the repeated instillation of riboflavin and 30 minutes of UVA irradiation. An experimental technique developed by Rocha et al, however, appears to stiffen corneal tissue as effectively as riboflavin/UVA cross-linking with a single drop of a fast-curing hydrogel substrate and only 30 seconds of irradiation with UVA. In an analysis of enucleated porcine eyes, mean corneal stiffness (as measured with surface wave velocity) increased from  $90.87 \pm 15.26$  to  $109.2 \pm 21.76$  m/sec in the riboflavin group ( $n = 10$ ) and from  $83.66 \pm 12.30$  to  $109.2 \pm 18.42$  m/sec in the flash-linking group ( $n = 10$ ).<sup>10</sup>

Hafezi et al successfully used a modified technique to stabilize advanced keratoconus in 20 patients whose corneas were too thin to undergo traditional cross-linking. In this study, the investigators induced stromal swelling by repeatedly instilling hypo-osmolar riboflavin into the debrided eyes at predetermined intervals. When the corneas achieved a minimum thickness of 400  $\mu\text{m}$ , they were treated with isomolar riboflavin and UVA in the customary manner. Because the experimental technique halted the progression of keratoconus without adverse effects,

the investigators concluded that "preoperative swelling of the cornea safely broadens the spectrum of [cross-linking] indications to corneas that would otherwise not be eligible for treatment due to low minimum stromal thickness."<sup>11</sup>

#### THE ROLE OF THE EPITHELIUM IN CROSS-LINKING

Controversy has erupted over the importance of epithelial debridement in cross-linking. Although several investigators have reported achieving satisfactory cross-linking through intact corneas pretreated with tetracaine, the only peer-reviewed data available for this technique were collected from keratoconic eyes that were also implanted with Intacs corneal segments (Addition Technology, Inc., Des Plaines, IL).<sup>11</sup>

Spectrophotometry of enucleated porcine eyes treated with traditional and modified cross-linking techniques showed that only debrided eyes saturated with riboflavin ( $n = 12$ ) impeded the passage of light waves ranging between 400 and 510 nm. None of the eyes in the other groups, including those treated with transepithelial cross-linking ( $n = 6$ ), showed a similar dip in transmission spectra. These results suggest that the corneal epithelium impedes the penetration of riboflavin into the stroma and may "impair the efficacy of the cross-linking process."<sup>12</sup>

In a letter to the *Journal of Cataract & Refractive Surgery*, Yuen et al disputed the assertion by Hayes et al that the "epithelium must be completely removed to allow adequate penetration of riboflavin into the stroma" prior to corneal cross-linking.<sup>12</sup> Yuen et al cited differences in the thickness of porcine and human corneal epithelium, the investigators' use of light transmission versus direct clinical observation to assess the penetration of riboflavin into the stroma, and an inherent observer bias. Yuen et al then suggested that, by "extrapolating porcine results to human corneas," the study conducted by Hayes et al "is methodically flawed."<sup>13</sup>

A study by Bottos et al appears to support the results published by Hayes et al.<sup>12</sup> Immunofluorescence confocal microscopy showed that corneal debridement prior to riboflavin/UVA cross-linking created a  $182.5 \pm 22.5$   $\mu\text{m}$  wide zone of fluorescence in the anterior stroma of enucleated porcine eyes. This zone was characterized by the presence of highly organized collagen fibers. Because the investigators did not observe similar changes in the porcine eyes treated with transepithelial cross-linking, they concluded, "Corneas treated with riboflavin/UVA without previous deepithelialization had a diminished cross-linking effect compared with those that had the epithelium removed."<sup>14</sup>

A recent study by Wollensak and Iodina suggested that traditional riboflavin/UVA cross-linking increases the rigidity of corneal tissue more effectively than the transepithelial technique. The investigators found that the Young modulus of debrided rabbit eyes ( $n = 5$ ) increased by 102.45% relative to untreated controls ( $n = 4$ ) after cross-linking ( $19.86 \pm 1.04$  vs  $9.81 \pm 1.36$  MPa). In contrast, the same parameter in intact eyes ( $n = 5$ ) increased by only 21.3% relative to untreated controls ( $11.9 \pm 1.22$  vs  $9.81 \pm 1.36$  MPa). Wollensak and Iodina therefore wrote, "We do not recommend [transepithelial cross-linking] for the routine treatment of keratoconus, but rather for cases with a corneal thickness less than  $400 \mu\text{m}$ ."<sup>15</sup> ■

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