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Collagen Cross-linking

What you should know about this potential new treatment for keratoconus and ectasia.

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Despite intensive biochemical and genetic research, we do not know what causes keratoconus, although research in these disciplines will ultimately provide answers which could lead to a medical therapy.^{1,2} We do know that keratoconus typically commences at puberty, progressing until the patient's mid thirties, when it typically arrests. During this period, progression may stop and start at any time. It is not possible to predict when keratoconus will progress or stop without intervention.

A variant of this disorder, pellucid marginal degeneration, typically starts in the mid thirties and can progress or arrest any time thereafter. Post-LASIK ectasia results in corneal thinning. It is most common in individuals who had forme fruste keratoconus or were predisposed to developing keratoconus prior to their LASIK procedures. For all these ectatic disorders, the treatment of choice is rigid contact lenses. Intacs implants (Addition Technology) and corneal transplants are surgical alternatives for individuals who are contact-lens intolerant.³⁻⁶

We also know that in keratoconus the cornea is biomechanically unstable. Some literature suggests this may be the result of a decrease in cross-links between and within collagen fibers in the anterior corneal stroma. If there were a way to increase these cross-links, it could provide a means to stiffen the cornea and confer biomechanical stability with the potential to slow the progression of the disorder.⁶ In this article, I will detail a procedure that shows promise in achieving these goals.

Cross-linking 101

Collagen cross-linking provides a potential means for stiffening the cornea — but what is it? Well known in material sciences, it is an enzymatic process whereby there is an increase of molecular bonds to increase the mechanical strength of tissue. Cross-links can be induced enzymatically by means of aldehydes, chemical fixatives and by means of photosensitizing radiation. *In vivo* experiments have revealed that a combination of UV radiation and riboflavin is the most effective and least harmful procedure for inducing collagen cross-linking in the human cornea (**Figure 1**). In 2003 Wollensak, Spoerl and Seiler

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reported on 22 patients with progressive keratoconus upon whom they performed collagen cross-linking and followed for 2 to 4 years. They demonstrated no progression of keratoconus in all eyes, improved visual acuity in 15 of 22 eyes and flattening of K-max by 2 diopters in 16 of 22 eyes.⁷

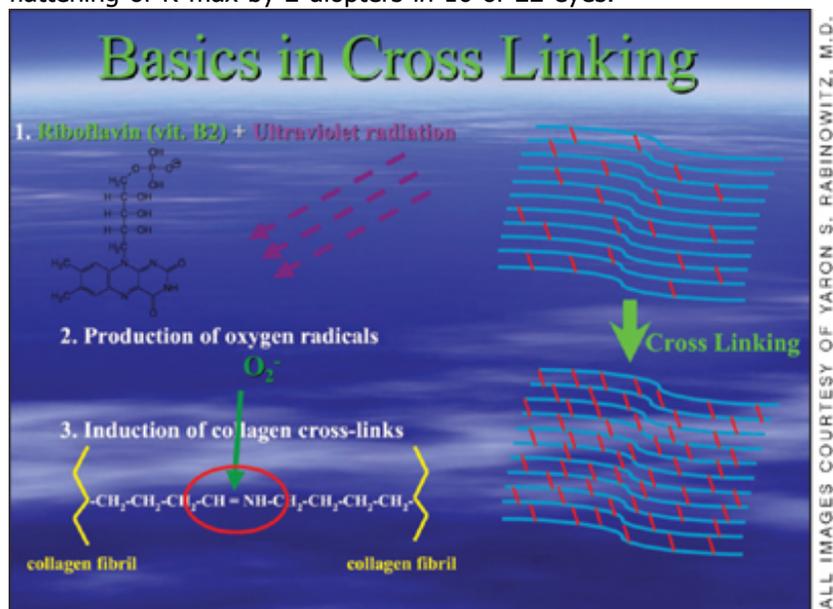


Figure 1. Combining UV radiation and riboflavin is the most effective method to induce collagen cross linking.

The procedure as initially described by this group is relatively simple. Using topical anaesthesia, a corneal abrasion is created to facilitate riboflavin diffusion into the cornea. One drop of riboflavin 0.1% ophthalmic solution is instilled topically in the eye every 2 minutes for 30 minutes. At the end of the 30-minute pretreatment period, the eye is examined with blue light for the presence of a yellow flare in the anterior chamber, indicating adequate riboflavin saturation of the corneal tissue.

If the yellow flare is not detected, riboflavin is continued at one drop every 2 minutes for an additional two to three drops, then the anterior chamber is rechecked for yellow flare. This process is repeated as necessary. After the presence of the yellow flare is confirmed on slit lamp examination, ultrasonic pachymetry is performed. If the corneal thickness is less than 400 microns, two drops of hypotonic riboflavin 0.1% are instilled every 10 to 15 seconds until the corneal thickness increases to at least 400 microns. During irradiation, instillation of riboflavin is continued every 2 minutes.

When the yellow flare in the anterior chamber is confirmed, the eye is aligned under the UV-X light (**Figure 2**) with the treatment plane at 50 mm from the UV-X beam aperture. The correct aperture setting is selected for the size of the eye; the eye is irradiated for 30 minutes, during which time instillation of riboflavin is continued (one drop every 2 minutes).



Figure 2. The eye is irradiated for 30 minutes as riboflavin is instilled.

After completion of the procedure, an antibiotic drop is given and a bandage contact lens is placed on the eye. The contact lens is removed once the abrasion has healed. Postoperative medications include an antibiotic and a steroid for 2 weeks postoperatively.

Safety and Efficacy

There are reports of the procedure being performed without removing the epithelium. This is attractive to patients since they would forgo the pain caused by the abrasion, as well as decrease their risk for infection due to an open wound. However, recent immunofluorescent confocal microscopy studies by Bottos et al. demonstrate that the epithelium is a barrier to cross-linking and very little cross-linking occurs in the presence of epithelium (**Figure 3**).⁸ These findings suggest that for the treatment to be effective, the epithelium should always be removed as initially described by Seiler's group.

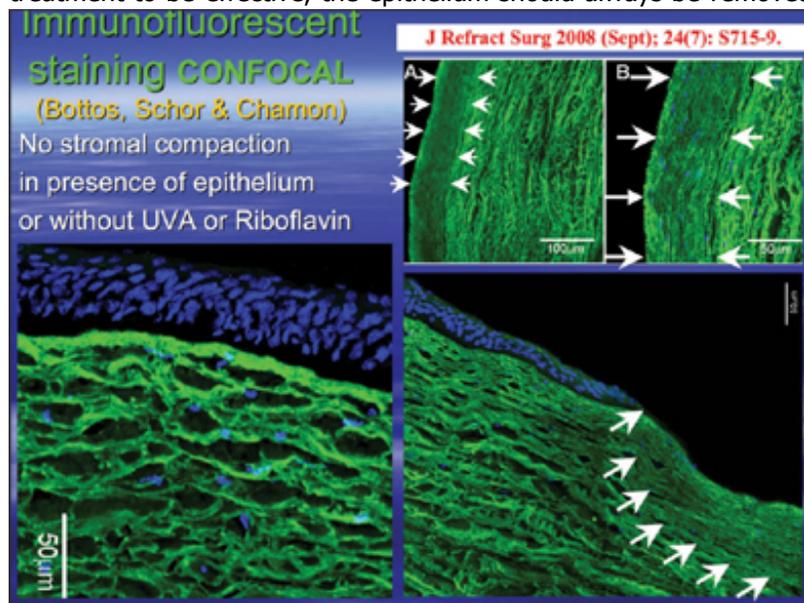


Figure 3. The epithelium has been found to act as a barrier to cross linking.

The procedure appears to be relatively safe. The only adverse event reported to date after cross-linking has been corneal edema in an eye with a pretreatment corneal thickness of less than 400 microns, presumably caused by UV damage to the corneal endothelium.⁵ Subsequent experiments led to the conservative recommendation that corneas not be treated with UVA/riboflavin unless they are thicker than 400 microns after epithelial debridement. Other complications reported in the literature are a case of HSV keratitis and DLK in a case of post-LASIK ectasia. Both resolved without any long term-effects on the patients.

Prior to clinical application of this procedure, there was significant laboratory investigation to demonstrate the safety of the procedure and determine the optimal photosensitizing agent/dosage and irradiance/exposure time. Laboratory studies also focused on characterizing the biomechanical and biochemical effects of cross linking on corneal collagen tissue using rabbit, porcine and human corneal tissue models. Other studies demonstrated toxicity to keratocytes and endothelial cells as well as thermal effects and effects on collagenase resistance.

Wollensak's group, using stress-strain measurements on porcine and human corneas, demonstrated a 328% increase in rigidity of the human cornea after being cross-linked with UVA and riboflavin (**Figure 4**).⁹ Subsequent experiments on rabbit corneas demonstrated that the cross-linking effect can last up to 8 months. Confirmation of cross-linking was demonstrated with gel electrophoresis, which demonstrated a significant increase in the diameter of collagen fibres in rabbit corneas. The presence of the cross-linking effect has also been confirmed with thermochemical studies on cross-linked rabbit corneas.

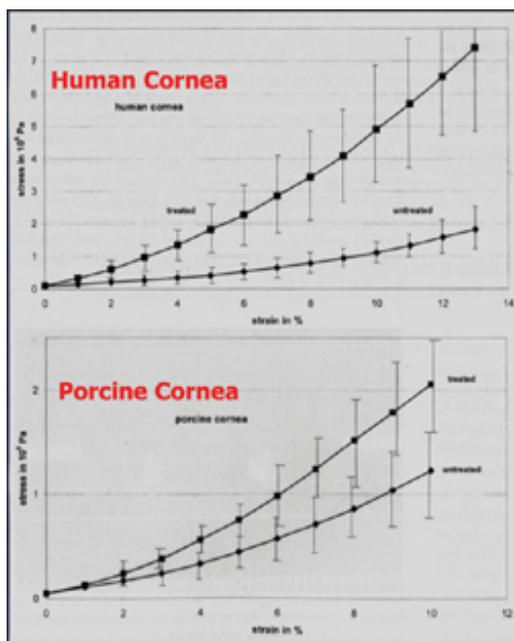


Figure 4. A 2003 study⁹ found that cross-linking produced a 328% increase in rigidity of the human cornea.

UV Concerns

A significant concern is the effect of the UV light on the cornea and other ocular structures. This has been studied in detail. The potential cytotoxicity of UVA light and the UVA/riboflavin exposure on keratocytes and endothelial cell function have been characterized in a series of *in vitro* experiments. In each of these, UVA exposure (370 nm, 30 minutes) and riboflavin (0.025% solution; equivalent to the corneal concentration after diffusion of a 0.1% solution) were administered to mimic conditions of clinical usage. Irradiance levels were varied to determine the irradiance threshold for cytotoxic effects. Keratocyte toxicity was evaluated in porcine keratocyte cell cultures after exposure to riboflavin alone, UVA light alone (irradiance range 2 to 9 mW/cm²), and UVA light plus riboflavin (irradiance range 0.4 to 1 mW/cm²).⁶

Riboflavin alone had no cytotoxic effect on keratocytes. The cytotoxic threshold for inducing cellular necrosis or apoptosis was 5 mW/cm² for UVA light alone and 0.5 mW/cm² for the UVA/riboflavin treatment. Using the Lambert-Beer equation, in human corneas the cytotoxic keratocyte UVA irradiance of 0.5 mW/cm² is reached at a stromal depth of 300 microns. The potential for endothelial cell toxicity was evaluated on endothelial cell cultures obtained from porcine cornea that were exposed to riboflavin alone and to various UVA irradiances (range 0.1 to 1.6 mW/cm²) with and without riboflavin.³ An abrupt cytotoxic threshold was observed at an irradiance of 4 mW/cm² for UVA light alone and was 10-fold lower with an irradiance threshold of 0.35 mW/cm² for the UVA/riboflavin treatment. No endothelial cell damage was observed in the cells treated with riboflavin alone.

Endothelial cell damage in the UVA groups is believed to be due to oxidative damage caused by the oxygen reactive-free radicals that are generated by the UV light.⁴ The lower cytotoxic thresholds observed for the UVA/riboflavin combination in the keratocyte and endothelial cell toxicity studies is consistent with the increase in UVA absorption in the presence of riboflavin.¹⁰ These studies suggest that this treatment is safe in corneas that have been adequately saturated with riboflavin and are at least 400 μ m in diameter (**Figure 5**).

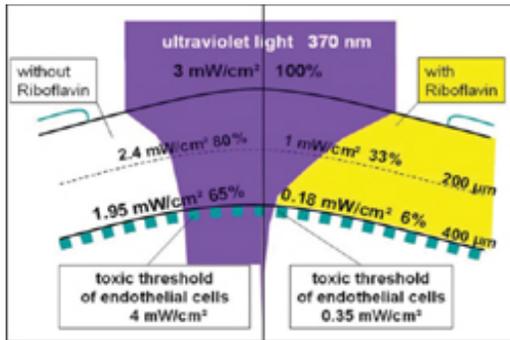


Figure 5. UVA radiant exposure of 5.4 mJ/cm² and the corresponding irradiance of 3 mW/cm² is below the damage thresholds of UVA for the corneal epithelium, lens and retina, noted Spoerl et al.¹⁰

Research Results

Subsequent to Wollensak's initial report in 2003, several other studies have suggested the efficacy and safety of this procedure for treating patients with progressive keratoconus. Caporossi et al. reported a 3.6-line increase in uncorrected visual acuity, a 1.66 line improvement in BSCVA, a mean reduction in K-max of 2.1 D (± 0.13), and a 2.5 D reduction in MRSE at 3 months after cross-linking in a series of 10 eyes in 10 patients with progressive keratoconus. There were no changes in endothelial cell density.¹¹ Raiskup Wolf et al. reported on 7-year results at the University of Dresden. They noted a decrease in maximum keratometry of 2.7D at 1 year; 2.2D at 2 years and 4.8D at 3 years. Visual acuity improved by one line per year in 54% of patients in the first 3 years. Two patients had continued progression and had to undergo repeat cross linking procedures.¹²

In the only randomized prospective controlled clinical trial of collagen cross-linking in progressive keratoconus published to date, Wittig-Silva et al. reported on 66 eyes of 49 patients with documented progression of keratoconus. Interim analysis of treated eyes showed a flattening of the steepest simulated keratometry value (K-max) by an average of 0.74 D at 3 months, 0.92 D at 6 months, and 1.45 D at 12 months. A trend toward improvement in BSCVA was also observed. In the control eyes, mean K-max steepened by 0.60 D after 3 months, by 0.60 D after 6 months, and by 1.28 D after 12 months. BSCVA decreased by logMAR 0.003 over 3 months, 0.056 over 6 months, and 0.12 over 12 months. No statistically significant changes were found for spherical equivalent or endothelial cell density.¹³

Other potential applications for cross-linking have been reported. Chan et al. suggests that combining cross-linking with Intacs achieves a greater reduction in cylinder and steep K than by using Intacs alone for treating keratoconus.¹⁴ This is of particular interest to our group. We are planning a prospective study to determine if Intacs and cross-linking are more efficacious than cross-linking alone in young patients with progressive keratoconus.

Another report by Hafezi et al. cites 10 eyes treated with cross-linking for ectasia after LASIK with 2 years follow up and no progression of the ectasia.¹⁵ In yet another study Kannelopolous reports on PRK on patients with ectasia after they have been cross-linked with excellent results, suggesting that it might be safe to do PRK on these patients to correct their residual refractive error since the cornea is biomechanically stable after cross-linking.¹⁶

Laboratory studies by Spoerl et al. suggest that cross linking results in increased resistance vs. collagen-digesting enzymes such as collagenase. A later clinical report by Iseli demonstrates its efficacy in a patient treated with a corneal ulcer and in whom corneal melting was arrested.^{17,18} Other potential applications that have been discussed but not reported on include: cross-linking the cornea in place after orthokeratology and pretreating patients with cross-linking prior to PRK in those with keratoconus "suspect" topography. While the procedure appears to be safe and its many potential applications have generated excitement in the ophthalmic community, its efficacy in the treatment of progressive keratoconus needs to be interpreted with a certain level of caution. Currently there is only one prospective randomized clinical trial on which

preliminary data on only a small number of patients have been reported. An FDA clinical trial has begun but no data from this study is yet available.

All reports to date use a single K reading as an endpoint, which can be highly variable, particularly in keratoconus. The sum of multiple data points averaged over the apex as an initial data point and subsequent data points tracking progression over time will provide more accurate data to show lack of progression. Since keratoconus usually progresses between puberty and age 30, an analysis of patients in this age group who are progressing compared to age-matched controls are needed to demonstrate lack of progression. Patients treated in the older age groups are less likely to progress without treatment; if this group is included in the analysis, there is potential to skew the data in favour of no progression.

The Potential is There

In the United States this procedure is not FDA approved, but clinical trials are currently underway and are likely to generate additional useful data with regard to efficacy and safety. These hopefully will allow for FDA approval in the very near term. In the interim patients will be treated, studies will be performed and long-term data analysis will allow us to evaluate whether this will become a commonly accepted treatment for young patients with keratoconus and ultimately lead to a significant decrease in the number of penetrating keratoplasties performed.

Based on currently available data, cross-linking clearly has the potential to become an exciting new treatment for patients with keratoconus. Whether the treatment will be temporary or permanent will become evident as more data on young patients who have been treated in the progressive phase of the disease are tracked over time. **OM**

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