Influence of Corneal Collagen Crosslinking with Riboflavin and Ultraviolet-A Irradiation on Excimer Laser Surgery

Daniel Kampik, Bernhard Ralla, Sabine Keller, Markus Hirschberg, Peter Friedl, and Gerd Geerling

PURPOSE. Riboflavin/ultraviolet A (UVA) cross-linking (CXL) of corneal collagen is a novel method of stabilizing corneal mechanical properties and preventing progression of keratectasias. This study was conducted to investigate whether CXL influences ablation rate, flap thickness, and refractive results of excimer laser procedures ex vivo.

METHODS. Corneal epithelium was removed from enucleated porcine eyes, and CXL was performed with riboflavin 0.1% and UVA radiation (365 nm, 3 mW/cm²) for 30 minutes. Control eyes received epithelial abrasion only. Diffusion of riboflavin through the cornea was assessed by using infrared-excited, two-photon microscopy of riboflavin autofluorescence, combined with second-harmonic generation of fibrillar collagen. During phototherapeutic keratectomy, corneal thickness was measured by optical coherence pachymetry. During LASIK for myopia, the flap thickness of microkeratome cuts was measured and the induced refractive change assessed by Placido topography. Data were analyzed by Shapiro-Wilk test and Student’s t-test.

RESULTS. Multiphoton imaging showed a rapid (30-minute) and even distribution of riboflavin throughout the corneal stroma. No difference in ablation rate was measured in treated and untreated corneas (P = 0.90). Mean flap thickness was increased by 44% in cross-linked corneas (P < 0.01). After LASIK for myopia of 4 to 25 D, the mean corneal refractive change was reduced in CXL-treated eyes by 20.1% (P < 0.05). This effect was less pronounced in thinner flaps.

CONCLUSIONS. CXL reduces the amount of refractive change after LASIK for myopia. Although the laser ablation rate is unaffected, CXL results in an increased flap thickness. This study suggests the need for adjustment of microkeratome and laser parameters for LASIK after CXL and indirectly endorses the theory of a direct stiffening effect of CXL. (Invest Ophthalmol Vis Sci. 2010;51:3929–3934) DOI:10.1167/iovs.09-4524

Collagen cross-linking (CXL) with riboflavin and ultraviolet A (UVA) irradiation of the cornea is currently being evaluated in phase 3 clinical studies as a treatment to stop progression of keratectasias such as keratoconus. The method was first described in 1997, and the first clinical results were reported in 1998. The procedure includes an epithelial abrasion that allows a subsequently applied riboflavin (vitamin B₂) solution to penetrate the stroma and anterior chamber. There, riboflavin serves as a photosensitizer that is then activated by UVA radiation. The activation induces the release of oxygen radicals, leading to the formation of new covalent bonds between collagen fibrils and thereby stiffening the corneal stroma.

Stress-strain measurements demonstrate an increased stiffness of corneas after cross-linking treatment. Corneas after CXL show an increase in intermolecular spacing, as is also observed in collagen tissue cross-linked by age or glycation (Mee K, et al. IOVS 2008;49:ARVO E-Abstract 3913). Furthermore, an increased resistance against enzymatic digestion and a higher shrinkage temperature have been attributed to CXL treatment. The procedure also results in keratocyte apoptosis, as has been shown in an animal model and clinically by in vivo confocal microscopy. Safety parameters have been established in vitro and long-term clinical results endorse these analyses.

Although until now CXL has been the only therapy to stop the progression of keratoconus, it also induces some regression of keratectasia-associated refractive changes. However, these refractive effects are not sufficient to result in the visual rehabilitation of the patient. In those who do not benefit from spectacles or rigid gas-permeable contact lenses, surgical options have to be considered. Besides keratoplasty, only intra-corneal ring segments and phakic intraocular lenses are regarded as safe in keratoconus. Recently, corneal excimer laser surgery has been proposed as an alternative treatment for irregular astigmatism in selected patients with keratectasia. Phototherapeutic and photorefractive keratotomy have yielded especially promising results in eyes with keratoconus, but the fear of progressive corneal thinning and even keratolysis remains.

Stiffening the cornea by CXL before excimer laser ablation could be effective in overcoming these problems. Kanellopoulos and Binder reported a case of phototherapeutic keratectomy 1 year after CXL for early keratoconus with a good refractive outcome and no adverse effects. A series of 45 keratoconic eyes with excimer surface ablation immediately after CXL showed effective visual rehabilitation, but one eye had to undergo penetrating keratoplasty (Ewald M, et al. IOVS 2008;49:ARVO E-Abstract 4338).
The objective of this study was to examine whether CXL has an impact on the excimer laser ablation rate, on tissue response to a microkeratome lamellar cut, and on the refractive results of myopic LASIK procedures.

**METHODS**

Freshly enucleated porcine eyes were retrieved from an abattoir and used within 2 to 9 hours after death. To avoid a systematic error due to hydration of the cornea, we selected pairs of eyes (CXL-treated and untreated control) for all experiments so that each pair was treated at approximately the same time after death. Both CXL-treated and untreated control groups were therefore subject to the same error caused by corneal swelling. Intraocular pressure was maintained hydrostatically at 20 mm Hg throughout the experiments, by adjusting the height of an intravitreal infusion of isotonic saline. Initial measurements for reference pressure were performed with a Perkins tonometer (Clement Clarke International Ltd., Essex, UK). Throughout all measurements, the eyes were mounted on a newly developed fixation device that maintains the natural shape of the globe without distortion and allows hydrostatic intraocular pressure control (Fig. 1). After it was mounted, the eye remained in the fixation device for all experimental procedures and measurements.

**Corneal CXL**

The treatment group received corneal cross-linking with riboflavin and UVA irradiation, according to the method in the first publication of its clinical use by Wollensak et al.5 The epithelium was completely removed with a D15 blade. Riboflavin was applied to the cornea as a 0.1% solution: 0.3 mL riboflavin-5-phosphate (5 mg/mL; Streuli Pharma, Uznach, Switzerland) was diluted in 0.25 g dextran (20%, molecular mass, 500 kDa; Carl Roth, Karlsruhe, Germany) in 0.7 mL 0.9% saline. Fifteen minutes before and during irradiation, riboflavin was administered drop-wise every 5 minutes to cover the cornea. The resulting riboflavin penetration was visible as a yellow flare in the corneal stroma and anterior chamber by slit lamp examination. UV irradiation was performed with a radiation device also used clinically (UV-X Corneal Cross-linking System; developed at the Institute for Refractive and Ophthalmic Surgery [IROC], Zurich, Switzerland). It is precalibrated to provide a 365-nm UVA beam of 3 mW/cm². A circular area with a diameter of 11.5 mm was irradiated for 30 minutes, resulting in a total UVA surface dose of 5.4 J/cm².

The control group received corneal abrasion only. The eyes were kept in a moist chamber at room temperature for a period equal to that of the CXL procedure to prevent dehydration, without receiving radiation or riboflavin.

**Multiphoton Imaging**

The penetration and intracorneal location of riboflavin during the diffusion phase was obtained by two-photon microscopy (TrimScope; LaVision, Bielefeld, Germany) with a high-resolution, long-distance, water-immersion objective (20x, NA 0.95; Olympus, Tokyo, Japan), as described elsewhere.22 Riboflavin autofluorescence was excited at 880 nm and detected as green emission (band-pass filter ranging from 505 to 555 nm, BP 530/50). Second-harmonic generation by collagen fibers was simultaneously detected in the blue channel (band-pass filter ranging from 420 to 460 nm, BP 440/40), as described elsewhere.23 Z-stacks were acquired, and the riboflavin autofluorescence was measured densitometrically for each section (Imspecor software, ver., 1.3; LaVision Biotech) and represented specific fluorescence after subtraction of the corneal background autofluorescence. The depth-associated loss of signal caused by scattering of both laser light and emission was used to normalize the measured riboflavin signal by determining the intensity loss in the second-harmonic generation by collagen fibers.24

**Excimer Laser Ablation and Microkeratome Cut**

Excimer laser ablations were performed with a flying-spot laser (Isiris; Schwind, Kleinostheim, Germany) after standard laser calibration. To assess the impact of CXL on laser ablation rate, we performed a phototherapeutic kerectomy (PTK) procedure with a diameter of 6 mm until the anterior chamber was perforated. The ablation zone was centered manually over the corneal apex. Central corneal thickness was measured before PTK and after every 50 μm of intended ablation by optical coherence pachymetry (OCP; Heidelberg Engineering, Heidelberg, Germany), which was integrated into this specific excimer laser system to measure corneal thickness in a coaxial alignment with the fixation beam used for centration.

To assess the impact on the microkeratome cut, we created lamellar flaps with standard cutting heads for an intended flap thickness of 130 and 150 μm in human eyes (Carriazo-Pendular; Schwind). A new blade had to be used for each cut, to maintain comparable results. Flap thickness was measured by OCP and mechanically by a manual micrometer caliper (Käfer, Villingen-Schwenningen, Germany). This device applied a constant measurement pressure to the flap between two flat feelers of 6.35-mm diameter, minimizing any expression of water from the tissue. Preliminary repeatability tests were accurate to ±5 μm, and average values were therefore rounded to multiples of 5 μm.

To assess the impact of CXL on the refractive outcome of a LASIK procedure, we performed a microkeratome cut and subsequent excimer laser ablation after CXL (treatment group) or corneal abrasion only (control). We used a spherical myopic LASIK profile of an intended 2 to 24.5 D with various optical zones between 4 and 8 mm. For proof of principal, excessive ablation depths and small diameters were also tested. Corneal refraction was measured before and after ablation by Placido-based topography (Optikon Keratron Scout; Schwind). The flap was left in place during measurement and artificial tears (polyvinyl alcohol 14 mg/mL; Liquifilm; Pharm-Allergan, Ettlingen, Germany) were used to improve the Placido disc reflection while the eye was mounted in front of the topography device (Fig. 1B). Keratometric...
power within the central 3-mm radius was calculated as the arithmetic mean of the steep and flat mean radius of curvature (best fit sphere mode of the Keratron Scout; Schwind).

Statistical Analysis

Normal distribution of the data was verified by Shapiro-Wilk test. Student’s t-test was used to compare data from cross-linked and untreated corneas. P < 0.05 was considered significant (SPSS ver. 16.1; SPSS, Inc., Chicago, IL).

RESULTS

Riboflavin Penetration

Because of the differences between porcine and human corneas, the homogeneous and depth-efficient distribution of riboflavin in the tissue was measured as two-photon excitation autofluorescence from the intact eye after corneal abrasion. Densitometric analysis of both riboflavin emission and collagen structure revealed that riboflavin penetrated the entire corneal stroma in a homogeneous fashion regarding the x-y axis and showed an exponential decay with depth (Fig. 2A, z-axis). The corneas were exposed to riboflavin for different durations, and the riboflavin autofluorescence was measured densitometrically. After 30 to 45 minutes of penetration, diffusion reached near-homogeneity level (Figs. 2B, 2C). Regions of dense collagen bundles were equally saturated with riboflavin, as tissue gaps and spaces that arguably served as diffusion and convection tracks (Fig. 2A, arrowheads).

Excimer Laser Ablation Rate

Central corneal thickness was measured during PTK treatment in steps of 50 μm of intended ablation until perforation of the anterior chamber in 10 eyes each of the cross-linked and control corneas. In the cross-linked corneas, each intended 50-μm step resulted in an actual mean ablation of 45.3 ± 12.5 μm. In the control group receiving corneal abrasion, a mean 45.2 ± 11.0 μm was ablated per step (Fig. 3). No ablation step showed a statistically significant difference between the CXL-treated and control corneas (P = 0.90, Student’s t-test). In both groups, ablation rates tended to be lower toward the deeper stroma, but comparison of the superficial three ablation steps with the three deepest steps revealed no significant difference (P = 0.053 for CXL treated, P = 0.138 for control eyes, Student’s t-test).

Flap Thickness

Microkeratome cuts in the porcine eyes after corneal abrasion with or without CXL (n = 60 eyes in each group) resulted in homogeneous flaps with smooth cutting edges and regular

FIGURE 2. Two-photon microscopy of riboflavin penetration into porcine cornea. (A) Fluorescence and second-harmonic generation of riboflavin and fibrillar collagen after 60 minutes of incubation. Images represent the vertical reconstruction (y–z) from deep z-stacks that encompass the complete cornea. Both diffusion direction of riboflavin and imaging were from the anterior to the posterior corneal surface. (B) Time-resolved diffusion of riboflavin into the porcine cornea. (C) Densitometric quantification of riboflavin autofluorescence in relation to the penetration depth from the posterior corneal interface. Riboflavin drops every 5 minutes were applied for 5, 17, 31, 45, and 60 minutes. The intensity was normalized to the intensity at the entry section and corrected for light absorption/scattering by using the intensity loss of the second-harmonic signal with increasing penetration depth.

FIGURE 3. Intended versus achieved stromal excimer ablation in cross-linked and control eyes (n = 10 each). PTK treatments were performed in 50-μm steps until perforation of the anterior chamber. Data show no significant difference in ablation rate between cross-linked and untreated corneas (P = 0.90, Student’s t-test). Ablation tended to be lower toward the deeper stroma in both groups, but the difference was not statistically significant.
Refractive Change

Mean central corneal refraction was calculated by topography measurements before and after LASIK for myopia, with a fixed central ablation depth of 170 μm and a variable optical zone of 4, 6, or 8 mm, resulting in a theoretical myopic correction of 24.5, 10.4, and 8.0 D, respectively. A 150-μm microkeratome head was used for flap creation, and 20 eyes were studied in each group (120 eyes in total). Data are summarized in Table 2. In these extremely deep ablations, a statistically significant reduction (10%-22%) in the refractive outcome of the LASIK procedures was measured in the cross-linked corneas compared with the control corneas.

To determine to what extent myopic LASIK outcomes are influenced by corneal cross-linking, we reduced the intended refractive change in steps of 2 D until no significant difference was measured between the cross-linked and control eyes. With the 150-μm microkeratome head, differences in refractive outcome were significantly reduced in the CXL-treated corneas, down to an intended myopic correction of 4 D (3.0 D was achieved correction in CXL eyes versus 4.1 D in control eyes, P = 0.058). At 2 D intended refractive change, the cross-linked corneas showed a reduced refractive outcome that was not statistically significant (P = 0.057, Table 3). With the 130-μm microkeratome head, the refractive outcome of a 6 D intended correction was significantly reduced in the cross-linked corneas (4.6 D achieved correction in CXL-treated eyes versus 5.5 D in control eyes, P = 0.016), whereas a 4 D correction showed a reduced refractive outcome that was not statistically significant (3.0 D vs. 3.5 D, P = 0.324, Table 3). Thus, the cross-linked corneas respond to LASIK with a significantly lower change in corneal curvature than did the untreated corneas; this effect was more pronounced in the thicker flaps.

### Table 1. Flap Thickness Of Microkeratome Cuts (Intended Flap Thickness of 150 μm) in Eyes Immediately after CXL and in Control Eyes

<table>
<thead>
<tr>
<th>Measurement Technique</th>
<th>CXL-Treated</th>
<th>Control</th>
<th>Difference†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual caliper</td>
<td>165 ± 15</td>
<td>155 ± 20</td>
<td>30 (+22.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OCP</td>
<td>135 ± 25</td>
<td>130 ± 25</td>
<td>30 (+22.7%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean micrometers of 60 eyes per group ± SD, rounded to multiples of 5 μm, according to the accuracy of measurement.
† Differences between CXL-treated and control eyes are shown in micrometers (percent). Control eyes were set at 100%.
‡ Statistically significant differences according to Student’s t-test.

### Table 2. Refractive Change after LASIK Procedures for Extreme Ablations

<table>
<thead>
<tr>
<th>Intended Refractive Change (D)</th>
<th>CXL-Treated</th>
<th>Control</th>
<th>Difference†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.5</td>
<td>17.9 ± 2.2</td>
<td>19.9 ± 2.5</td>
<td>2.0 (-10%)</td>
<td>0.01</td>
</tr>
<tr>
<td>10.4</td>
<td>9.7 ± 2.1</td>
<td>11.3 ± 2.3</td>
<td>1.6 (-14.2%)</td>
<td>0.03</td>
</tr>
<tr>
<td>8.0</td>
<td>4.9 ± 1.3</td>
<td>6.3 ± 1.8</td>
<td>1.4 (-22.2%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Data are expressed as mean diopters in 20 eyes per group ± SD.
† Differences between CXL-treated and control eyes are shown in diopters (percent). Control eye were set at 100%.
‡ Statistically significant differences according to Student’s t-test.

### Table 3. Refractive Change after LASIK Procedures for Low Ablations

<table>
<thead>
<tr>
<th>Intended Flap Thickness (μm)</th>
<th>Intended Refractive Change</th>
<th>CXL-Treated</th>
<th>Control</th>
<th>Difference†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>6</td>
<td>4.0 ± 1.8</td>
<td>5.5 ± 1.6</td>
<td>1.5 (-27.3%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.0 ± 1.2</td>
<td>4.1 ± 2.0</td>
<td>1.1 (-26.8%)</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.7 ± 1.1</td>
<td>2.5 ± 1.8</td>
<td>0.8 (-52.0%)</td>
<td>0.057</td>
</tr>
<tr>
<td>130</td>
<td>6</td>
<td>4.6 ± 0.9</td>
<td>5.5 ± 1.4</td>
<td>0.9 (-16.4%)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.0 ± 1.5</td>
<td>3.5 ± 1.3</td>
<td>0.5 (-13.3%)</td>
<td>0.324</td>
</tr>
</tbody>
</table>

* Data are expressed as mean diopters in 20 eyes per group ± SD.
† Differences between CXL-treated and control eyes are shown in diopters (percent). Control eyes were set at 100%.
‡ Student’s t-test.

### Discussion

To first stabilize a keratoconic cornea with UVA/riboflavin collagen cross-linking and then employ excimer laser and/or microkeratome procedures is a tempting treatment concept. The precision of the laser and keratome could be used to reduce corneal surface irregularities, correct refractive errors, or prepare the recipient cornea for lamellar or penetrating corneal transplantation. Furthermore, acceptance of CXL is becoming more and more widespread, with expanded indications including forme frust keratocous or corneal infections. We investigated the effects of CXL on various aspects of therapeutic and refractive excimer laser surgery in porcine eyes ex vivo.

The quality of collagen cross-linking by riboflavin depends on the homogeneous distribution of riboflavin within the corneal stroma. The central corneal thickness of porcine eyes is considerably higher (600–1000 μm) than in human corneas, and the corneal structure is inhomogeneous and varies between densely packed collagen bundles. For these reasons, we first examined the distribution characteristics of riboflavin in the porcine cornea. Two-photon excitation autofluorescence microscopy demonstrated that the porcine corneal stroma is porous enough to allow complete saturation with the photosensitizer.

Collagen cross-linking leads to alterations in the chemical and ultrastructural characteristics of the stroma. In immunofluorescence confocal microscopy, a more compact organization of collagen fibers in the cross-linked stromal tissue is observed. We hypothesized that, due to increased stiffness, the central corneal thickness of porcine eyes was on average 30 μm thinner than those in the untreated eyes, as calculated by caliper or OCP measurements (Table 1), corresponding to a 22% increase. This result was equally true of the thinner flaps. A 130-μm microkeratome cut was 165 μm and a variable optical zone of 150 μm microkeratome cut was measured between the cross-linked and control eyes. With the 130-μm microkeratome head, differences in refractive outcome of the LASIK procedures were measured in the cross-linked corneas compared with the control corneas.

To determine to what extent myopic LASIK outcomes are influenced by corneal cross-linking, we reduced the intended refractive change in steps of 2 D until no significant difference was measured between the cross-linked and control eyes. With the 150-μm microkeratome head, differences in refractive outcome were significantly reduced in the CXL-treated corneas, down to an intended myopic correction of 4 D (3.0 D was achieved correction in CXL eyes versus 4.1 D in control eyes, P = 0.058). At 2 D intended refractive change, the cross-linked corneas showed a reduced refractive outcome that was not statistically significant (P = 0.057, Table 3). With the 130-μm microkeratome head, the refractive outcome of a 6 D intended correction was significantly reduced in the cross-linked corneas (4.6 D achieved correction in CXL-treated eyes versus 5.5 D in control eyes, P = 0.016), whereas a 4 D correction showed a reduced refractive outcome that was not statistically significant (3.0 D vs. 3.5 D, P = 0.324, Table 3). Thus, the cross-linked corneas respond to LASIK with a significantly lower change in corneal curvature than did the untreated corneas; this effect was more pronounced in the thicker flaps.
increased resistance against enzymatic digestion, and a higher shrinkage temperature, the CXL-treated cornea would show an altered response to excimer laser and/or microkeratome surgery. Our study demonstrated the rapid and homogeneous penetration of riboflavin through the entire corneal stroma, yet no significant difference in ablation rate in cross-linked compared to control eyes, irrespective of the ablation depth. Both cross-linked and control corneas revealed an equal tendency toward lower ablation efficiency in the posterior compared to the anterior stroma. This difference is consistent with the increased hydration of the deeper stroma, resulting in a lower ablation rate. The equal response to excimer laser ablation in the cross-linked and control eyes in all stromal layers indicates that PTK after CXL does not necessitate alteration of laser parameters.

Collagen cross-linking however, led to increased flap thickness after 130- and 150-μm microkeratome cuts. This change was observed independent of the measurement technique: both OCP, which is dependent on the refractive index of the cornea, and the manual caliper, which is independent of this parameter, showed the same increase in flap thickness after CXL. Although CXL could have changed the refractive index of the cornea and therefore could bias the OCP values after CXL, this potential error seems negligible.

The increase in flap thickness after CXL can be attributed to the stiffening effect of CXL in the anterior 200 μm of the stroma, resulting in deeper penetration of the microkeratome blade. A stiffer cornea with its dome-shaped configuration exerts a higher resistance against the microkeratome, therefore, presumably forcing a thicker slice of tissue between blade and keratome. Since microkeratomes could also be used to prepare the recipient cornea in lamellar keratoplasty, the surgeon has to be aware of the altered tissue response when operating on a cross-linked cornea.

CXL-treated corneas respond to a myopic LASIK with a significantly lower change in corneal curvature (i.e., a reduction in refractive correction of ~20% compared with untreated eyes). This effect was observed, not only in extreme ablations but also in clinically relevant corrections of ~4 D and was more pronounced in thicker flaps. We assume that this result is due to the increased rigidity of both the cross-linked flap and the residual stromal bed. According to the mechanism proposed by Dupps and Roberts, PTK results in the severing of collagen lamellae which are responsible for the cornea’s tensile strength. The retraction of the lamellae leads to central flattening and midperipheral steepening, accounting for a hyperopic shift. This phenomenon is important in any central keratectomy, such as photorefractive keratectomy (PRK) and LASIK for myopia. The same biomechanical mechanism applies to the flap cut: An isolated microkeratome incision without laser ablation in a normal cornea results in central flattening and midperipheral steepening. Collagen cross-linking reportedly augments corneal stiffness by an increased interweaving of collagen fibers, thereby reducing the biomechanical mechanisms normally taking place after excimer laser ablation. Consistent with this theory we found that CXL-treated corneas showed a reduced central flattening and therefore a reduced refractive effect after myopic LASIK. Indirectly, these findings provide further evidence for a direct stiffening effect of CXL.

Furthermore, thicker cross-linked flaps (150 μm) reduced the refractive effect of LASIK more than thinner cross-linked flaps (130 μm). This result could be due to a lack of alignment of the thicker, more rigid flap to the stromal bed, thereby masking the altered curvature of the underlying stroma. This explanation is corroborated by the findings of Kohlihaas et al. who showed a macroscopically visible stiffening effect in cross-linked flaps.

We can only speculate on the effect of CXL on PRK by extrapolating our findings in LASIK and applying the model of Dupps and Roberts: By stiffening of the cornea, the reduced biomechanical response after CXL should reduce the refractive effect of PRK for myopia. Whether the CXL-mediated reduced effect is also true of hyperopic excimer laser treatment is subject to further investigation. Certainly, we do not advocate at present performing LASIK after collagen cross-linking in keratoconus. PRK avoids the additional destabilizing effect of the flap cut and should give more predictable results not altered by a cross-linked flap. Therefore PRK could be applied after an early keratoconus is first stabilized with CXL. We are awaiting further clinical results of PRK in this situation. The indications for CXL are expanding, and our results provide a further basic understanding of the biomechanical properties of the cornea after collagen cross-linking with riboflavin and UVA light.

Acknowledgments
The authors thank Johann Schmidl (Schwind Eye Tech Solutions) for excellent technical support and Jörg Haßpacher (University Hospital Würzburg Technical Services) for assembling the fixation device for the porcine eyes.

References