Determination of the Excimer Laser Ablation Rate in Previously Cross-linked Corneas

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ABSTRACT

PURPOSE: To evaluate the need for and quantify the extent of nomogram adjustments to compensate for potential changes in the amount of effective corneal stroma ablated in previously cross-linked corneas.

METHODS: Ex vivo porcine corneas were divided into two groups (the corneal cross-linking [CXL] group, n = 30; and the control group, n = 3): these experimental corneas underwent CXL including deepithelialization, instillation of riboflavin solution for 25 minutes, and ultraviolet-A irradiation at 9 mW/cm^2 for 10 minutes. The control group was deepithelialized only. Four consecutive excimer laser ablations of 50 μm each were performed (AMARIS 750S; SCHWIND eye-tech-solutions, Kleinostheim Germany), and stromal bed thickness was measured with a built-in optical coherence pachymeter. To determine the potential influence of riboflavin, a third group (the riboflavin group, n = 12) underwent deepithelialization and instillation of riboflavin, but no ultraviolet-A irradiation.

RESULTS: The mean individual ablation depth across the four ablations was significantly smaller in cross-linked corneas (-17%) when compared to untreated control corneas ($P < .001$). A consistent reduction of 12% was observed via a cumulative analysis when assessing the relative isolated effect of CXL on the ablation rate. There was no significant effect from riboflavin in the deeper ablations, except for the first ablation (68.6 ± 1.1 mm [range: 1 to 50 μm]). This may be due to a measurement error in pachymetric readings due to the thin film of riboflavin on the surface that resists even extensive rinsing.

CONCLUSIONS: CXL reduces the corneal ablation depth of excimer lasers in the anterior 200 μm of the porcine cornea by approximately 12%. Further clinical studies are needed to validate these findings in human corneas.

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Corneal cross-linking (CXL) with riboflavin and ultraviolet-A is a technique to treat keratoconus in children, adolescents, and adults,[1-4] as well as postoperative ectasia.[5-8] In clinical use since 1999, CXL increases corneal biomechanics by more than 300% in the human cornea[3,9,10] and halts progression of ectasia. Current limitations of the technique include reduced efficacy in extremely thin corneas and during pregnancy.[11-13]

The improvement in the biomechanical state of the cornea opens up new opportunities for visual rehabilitation in irregular astigmatism: customized photorefractive keratectomy may be used to regularize the corneal surface, combining a depth-limited surface ablation with a subsequent CXL either in the same procedure or sequentially (months to years after CXL).[14-19]

The photopolymerization process that occurs during CXL follows the Lambert–Beer law of photophysics, which describes the gradual light energy absorption with depth.[19] Similarly, the riboflavin concentration in the stroma also decreases with depth.[20] CXL changes several properties of the corneal stroma, including the packing density of stromal collagen, its ability to swell, and the permeability to pharmacological substances.[21-23] One might speculate whether CXL might also influence the excimer laser ablation rate of the stroma. If this were the case, then nomograms would be needed to adjust the fluence per pulse (or the number of pulses for constant fluence) delivered by the excimer laser to ablate a defined amount of tissue.

In the current study, we quantified the amount of excimer laser ablation in cross-linked corneas at various stromal depths and compared it to untreated controls, and to a cohort of nonirradiated but riboflavin-soaked specimens.

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MATERIALS AND METHODS

CXL

Freshly enucleated porcine eyes were obtained from a local abattoir. All eyes were stored at 5°C and prepared for the experiments within less than 6 hours after harvest. Only eyes displaying corneas with an intact epithelium, lack of focal stromal edema, and a corneal thickness of 700 ± 900 µm (range: 800 to 61 mm) as measured by ultrasound pachymetry (SP-2000; Tomey Corporation, Nagoya, Japan) were used. A 23-gauge needle was inserted into the globe at the pars plana and eyeballs were inflated using balanced salt solution at a height of 20 cm.

Corneas were treated by conventional epithelium-off CXL described previously.[24] In brief, corneas were deepithelialized mechanically on a diameter of 8 mm. Isoosmolaric 0.1% riboflavin solution containing 20% dextran (MedioCross D solution; Peschke Meditrade GmbH, Hünenberg, Switzerland) was instilled every 2 minutes for 25 minutes. The group undergoing CXL prior to excimer laser ablation represents the experimental group (CXL group, n = 30). Control corneas were deepithelialized but not soaked with riboflavin and were not irradiated prior to excimer laser ablation (control group, n = 30); however, the same 25 plus 10 minute regimen was followed as a waiting time. Eyes from each group were treated in an alternating manner. To investigate the potential effect of riboflavin on excimer laser ablation, a third group of corneas was deepithelialized and soaked with riboflavin, but not irradiated prior to excimer laser ablation (riboflavin group, n = 12).

Optical coherence pachymetry (a single point time-domain simplification of optical coherence tomography) was performed with a modified firmware, changing the scanning range to allow measuring of corneas thicker than the average human cornea. Measurements were performed immediately prior to irradiation. All eyes showed a stromal thickness within 5% of the values measured prior to riboflavin instillation. Ultraviolet-A irradiation was performed at 365 nm with a fluence of 5.4 J/cm^2 (irradiance of 9 mW/cm^2 for 10 minutes), using a commercially available device (CXL-365; SCHWIND eye-tech-solutions, Kleinostheim, Germany).

Corneal Thickness and Excimer Laser Ablation

Prior to excimer laser ablation, central corneal thickness was measured using the inbuilt optical coherence pachymetry of the AMARIS 750S (SCHWIND eye-tech-solutions). Three consecutive measurements were taken and the mean value was calculated. Only corneas with a central corneal thickness between 700 and 900 µm and intact epithelium were used. Excimer laser ablation was performed as a constant-depth phototherapeutic keratectomy in a 4-mm optical zone. Four consecutive ablations were performed with an intended ablation depth of 50 µm each. The interval between consecutive ablations was 3 minutes and corneas remained in the same alignment. After each ablation, central corneal thickness was measured three times with the optical coherence pachymetry and the effective ablation depth was calculated from the mean of the three measurements.

TABLE 1

Average Individual Stromal Ablation Depth

Nominal Ablation Depth (µm)	Achieved Ablation Depth (µm)[a] CXL Group (µm)	Control Group (µm)	Difference (%)[b]	P[c]
50	57.5 ± 1.6	60.9 ± 0.8	-5.6	.03
100	43.0 ± 0.8	53.8 ± 0.7	-20.1	< .001
150	43.8 ± 0.7	52.9 ± 0.9	-17.3	< .001
200	44.3 ± 0.7	51.5 ± 0.9	-14.0	< .001

CXL = corneal collagen cross-linking

[a]*Data are expressed as mean ± standard deviation in 30 eyes (no CXL control and CXL-treated) and 12 eyes (riboflavin control).*

[b]*Difference between CXL-treated and control groups. Control eyes were set at 100%.*

[c]*Student's t test.*

Statistics

Data analysis was performed using SPSS software (version 22.0.0.0; SPSS, Inc., Chicago, IL). All data were expressed as the mean ± standard error. Student's *t* test was performed to analyze the individual and cumulative differences across experimental groups and across consecutive ablations. Confidence levels were set to 95%.

RESULTS

In the first step, we analyzed the individual 50-µm ablation steps and compared CXL to the control and riboflavin groups. The average ablation depths of the individual ablations are shown in **Table 1** and a graphic representation is shown in **Figure 1**. When we omitted the results for the first ablation (range: 1 to 50 µm), which were potentially distorted by the presence of riboflavin, we observed a 20.1% reduction of ablation depth in the CXL group from 51 to 100 µm, a 17.3% reduction from 101 to 150 µm, and a 14.0% reduction from 151 to 200 µm. The group that received epithelial abrasion and riboflavin instillation but no ultraviolet-A irradiation (riboflavin group, **Figure 1**) showed an achieved ablation of 68.6 ± 1.1 µm (range: 1 to 50 µm) for the first ablation, 48.7 ± 1.6 µm (51 to 100 µm) for the second ablation, 47.1 ± 1.8 µm (range: 101 to 150

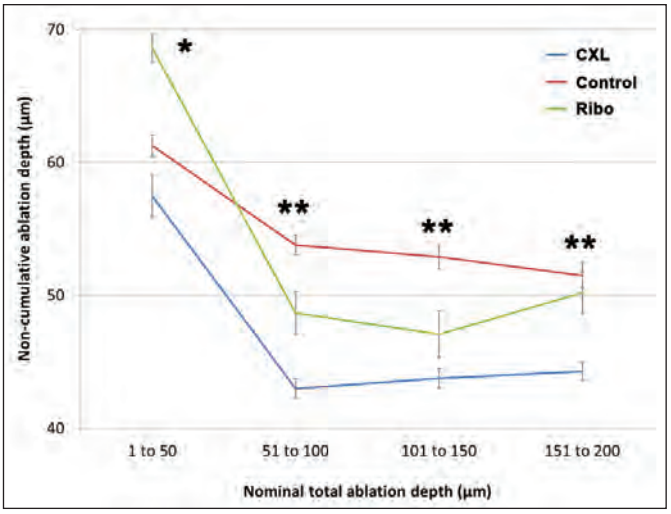

Figure 1. Excimer laser ablation rate at various depths in the corneal collagen cross-linking (CXL) group (n = 30), control group (control, n = 30), and riboflavin (ribo) group (n = 12). The ablation depth in the CXL group was significantly lower. Error bars represent the standard error. * = significant ($P < .05$); ** = highly significant ($P < .01$)

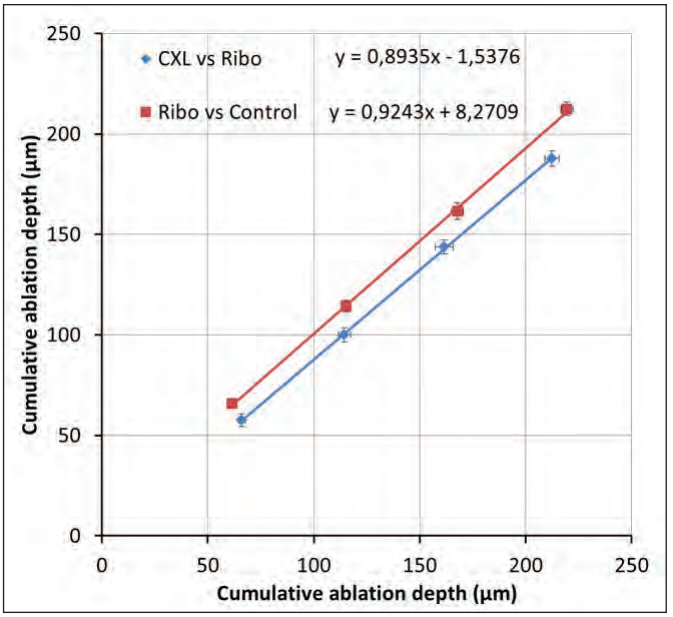

Figure 2. Correlations between cumulative ablation rates in the corneal collagen cross-linking–treated (CXL) group (n = 30) versus the riboflavin (ribo) group (n = 12) (in red), and the riboflavin group (n = 12) versus the control group (control, n = 30) (in blue). The decoupled effect of CXL on the ablation rate was a -11% reduction, whereas the decoupled effect of riboflavin soaked on ablation rate was a -8% reduction. Error bars represent two standard errors (ie, 95% confidence interval).

µm) for the third ablation, and 50.2 ± 1.6 µm (range: 151 to 200 µm) for the fourth ablation.

In the second step, to decouple and isolate the relative contributions of riboflavin and CXL to the global ablation rate, we analyzed the cumulative effect of the individual ablations using the later introduced riboflavin group as "enjambment" or "staging post" (**Figure 2**). In this way and instead of comparing the CXL group to the control group, we correlated the CXL group to the riboflavin group to obtain the relative isolated effect of CXL on the ablation rate (-11%) and the riboflavin cumulative ablation depth to the reference cumulative ablation depth of the controls to obtain the relative isolated effect of riboflavin on the ablation rate (-8%).

DISCUSSION

When riboflavin is instilled in the corneal tissue, it absorbs the ultraviolet light according to the Lambert–Beer law. The Lambert–Beer law states that the amount of light that penetrates through a substance is reduced along the distance the light travels due to the absorption of the material (described by the extinction coefficient).[25,26] Consequently, the CXL effect is diminished in the posterior cornea when compared to the anterior cornea.[10,27,28] Because of this, the stiffness gradient along the corneal thickness might be an important consideration when performing excimer ablation on previously cross-linked corneas.

It is known from literature that the ablation rate is strongly dependent on the hydration state of the corneal stroma, the latter being depth dependent.[29-31] Accordingly, we found a decrease in ablation depth between the anterior and posterior stroma not only for controls, but also for corneas after CXL (**Figure 2**). We also noticed a distinctly higher ablation rate in the first 50 µm in the CXL group and suspected these might represent measurement errors due to a thin film of riboflavin on the corneal surface. To analyze the potential influence of riboflavin on this first ablation from 1 to 50 µm, we included another group that underwent epithelium abrasion and riboflavin instillation, but no ultraviolet-A exposure prior to excimer laser ablation (riboflavin group). The riboflavin–dextran solution seems to artificially increase the depth of the first ablation. This might be an artifact because dextran tightly adheres to corneal tissue: even when rinsing off the riboflavin at the end of the instillation, residual riboflavin might have led to a higher corneal thickness reading in the measurement before ablation.

We observed the design of the protocol to follow the same timing scheme for all groups (eventually interleaving waiting periods for the control and riboflavin groups) to exclude time as a major confounding factor in our series.

In the current study, we used optical coherence pachymetry to determine corneal thickness. When compared to the ultrasound pachymeter, the optical coherence pachymetry is not affected by the increase of the speed of sound after CXL.[32] Thus, it was not nec-

essary to correct our corneal thickness values in the treatment group.

It can be argued that eventual synergistic effects in the ablation rate of the combined instillation of riboflavin and irradiation with ultraviolet-A are not accounted for, in the sense that both contributions may not be strictly independent. However, we think the used approach for the analysis represents a closer look at the effects of CXL alone rather than the crude comparison of CXL-treated to control corneas. Ablation on CXL-treated corneas in the clinical setting occurs months after CXL treatment, so no presence of soaked riboflavin is expected and the isolate effect of CXL on the ablation rate represents the boost factor for those treatments.

The question may be raised why the ablation rate is consistently reduced by 11% over the entire depth from 1 to 200 µm in the CXL group. This might be explained by the fact that CXL significantly reduces the stromal swelling capacity, reducing the depth-dependent differences in the hydration state of the cornea.[23,33] Another explanation might be the creation of additional cross-links between proteoglycans and collagen in the anterior stroma during CXL.[34,35] To break these additional bonds, additional energy is needed.

Kampik et al. reported on the excimer laser ablation rate following CXL in ex vivo porcine corneas.[36] Although they found no differences in the ablation rate between cross-linked and non-cross–linked corneas, they observed that CXL reduces the amount of refractive change after LASIK for myopia by 20%. Chen et al. investigated the efficacy of excimer laser ablation of cross-linked porcine cornea.[37] In contrast to our results, they reported an overall ablation depth of 9%. Although Chen et al. performed their experiments on a different excimer laser platform, they observed a reduction in stromal ablation similar to the one observed in our study, indicating that our results may be independent of the technical platform used and rather reflect the biological response of the tissue. The slightly lower percentage reported by Chen et al. may be caused by the presence of residual riboflavin on the corneal surface during the first ablation, artificially reducing the overall ablation rate. Alternatively, the observed difference might come from the fact that Chen et al. used the standard "Dresden protocol" settings (3 mW/cm^2 for 30 minutes) to provide a fluence of 5.4 J/cm^2, whereas we irradiated with 9 mW/cm^2 for 10 minutes. Although the overall fluence remains the same in both cases, we recently showed that the increase in the biomechanical response is different.[24]

A limitation of this study might be that all experiments were performed in porcine corneas: earlier studies have shown that the increase in corneal stiffness after CXL is higher in human than in porcine corneas (328.9% vs 71.9%), which suggests that the excimer ablation rate after CXL may be even more affected in humans.[10] We are planning on performing a follow-up study on corneal donor eyes unsuitable for transplantation to support this hypothesis.

CXL paired with photorefractive keratectomy holds promise for patients with keratoconus because it not only stabilizes disease progression, but can also help increase the quality of the optical image by making a highly irregular surface less irregular. A remaining question is whether CXL should be performed prior to or simultaneously with photorefractive keratectomy. In some patients with keratoconus, corneal curvature can decrease over years and up to 7 diopters following CXL.[38,39] Therefore, it may be reasonable to perform CXL first and await stable corneal curvature prior to performing surface ablation. To do so, and also to perform customized photorefractive keratectomy on patients who underwent CXL, our results might help in establishing nomograms for the correct ablation of corneal stroma in a previously cross-linked cornea.

AUTHOR CONTRIBUTIONS

Study concept and design (OR, SAM, TM, FH); data collection (OR, AH, MMB, FH); analysis and interpretation of data (OR, SAM, SK, AH, TM, MMB, FH); drafting of the manuscript (OR, SK, FH); critical revision of the manuscript (SAM, SK, AH, TM, MMB, FH); supervision (FH)

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