LABORATORY SCIENCE

Predicting corneal cross-linking treatment efficacy with real-time assessment of corneal riboflavin concentration

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Purpose: To assess predictability of tissue biomechanical stiffening induced by UV-A light-mediated real-time assessment of riboflavin concentration during corneal crosslinking (CXL) of human donor tissues.

Setting: Studio Italiano di Oftalmologia, Rome, Italy.

Design: Laboratory study.

Methods: 20 sclerocorneal tissues were randomly stratified to undergo CXL with either the epithelium intact (n = 12) or removed (n = 8). Samples underwent corneal soaking with 0.22% riboflavin formulation (RitSight) with dosing time of t = 10 minutes and t = 20 minutes in epithelium-off and epithelium-on protocols, respectively. All tissues underwent 9-minute UV-A irradiance at 10 mW/cm^2 using theranostic device (C4V CHROMO4VIS). The device used controlled UV-A light irradiation to induce both imaging and treatment of the cornea, providing a real-time measure of corneal riboflavin concentration and treatment efficacy (ie, theranostic score) during surgery. Tissue biomechanics were assessed with an air-puff device (Corvis), which was performed before and after treatment. A 3-element viscoelastic model was developed to fit the corneal deformation response to air-puff excitation and to calculate the mean corneal stiffness parameter (k_c).

Results: Significant corneal tissue stiffening ($P < .05$) was induced by the theranostic UV-A device in either CXL treatment protocol. Significant correlation was found between the theranostic score and the increase in k_c ($R = 0.75$; $P = .003$). The score showed high accuracy (94%) and precision (94%) to predict correctly samples that had improved tissue biomechanical strengthening.

Conclusions: Real-time assessment of corneal riboflavin concentration provided a predictive and precise approach for significant improvement of tissue strength on individual corneas, regardless of CXL treatment protocol.

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In the last decade, a number of treatment protocols has been developed and tested with the aim to improve the efficacy of transepithelial corneal crosslinking (CXL) and avoid significant visual threatening complications of epithelium-off (epi-off) protocols. Methods and strategies for increasing the amount of stromal riboflavin diffusing across the intact epithelium included the development of dextran-free and/or hypotonic riboflavin ophthalmic formulations, the use of substances to increase the permeability of the epithelium to riboflavin (ethylenediaminetetraacetic acid, trometamol, vitamin E TPGS, NaCl, BAK, iodide, proparacaine, oxybuprocaine, etc), corneal iontophoresis, prolonged dosing time, etc.[1–5] In randomized controlled clinical trials, however, the epithelium-on (epi-on) or transepithelial CXL procedure still missed to confirm efficacy in the management of progressive keratoconus because of the large variability in outcomes among protocols.[6–10] Efficient transepithelial CXL treatment remains challenging because it lacks selectivity in understanding the amount of the therapeutic molecule penetrating into the corneal stroma through the intact epithelium.

Previous studies have demonstrated that the main mechanism of the CXL treatment is the direct interaction between riboflavin triplets and reactive groups of stromal proteins, which leads to the crosslinking of the proteins through radical reactions.[11] This notion implies that, in an ambient environment (ie, 21% partial pressure of oxygen), the amount of riboflavin into the stroma and the role of type-1 photochemical mechanism could be predominant

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for the formation of additional chemical bonds between stromal proteins during the CXL procedure.[11–13] A UV-A light-mediated theranostic approach has enabled an optical method to estimate the riboflavin concentration during CXL treatment by imaging the fluorescence emitted from the corneal tissue; the method has been used to monitor the corneal stromal diffusion and the UV-A light-mediated photodegradation of riboflavin during CXL, either with or without the epithelium intact.[14–16] The authors have previously assessed the reliability of the technology and have shown comparable riboflavin photodegradation kinetics among epi-off and epi-on CXL protocols using UV-A light irradiance in the 3 to 10 mW/cm^2 power density range (5.4 J/cm^2 energy dose).[14–17]

Recently, a UV-A medical device has been made available for the indication of use of treating keratoconus and corneal ectasia by theranostic-guided CXL (Thera-CXL). During surgery, the device provides the operator with a theranostic score predicting treatment efficacy; this is an imaging biomarker, which is calculated by the device through the estimation of the corneal riboflavin concentration and its UV-A light photodegradation in the cornea under treatment. The primary scope of this study was to assess the predictive performance of a theranostic UV-A medical device in improving the biomechanical strength of human eye bank donor tissues treated with either the corneal epithelium intact or removed.

METHODS

Human Donor Tissues

Twenty eye bank human donor tissues from different donors were obtained from the Veneto Eye Bank Foundation (Venezia Zelarino). The samples were shipped to the laboratory in 6% dextran-enriched corneal storage medium and were used for the experiment within 10 hours. Inclusion criteria included an endothelial cell density >1600 cells/mm^2; exclusion criteria included a history of corneal pathologies, traumas, or eye surgery. The study adhered to the tenets of Declaration of Helsinki for the use of human tissues.

Theranostic UV-A Medical Device and Procedure

CXL was performed using a new UV-A medical device (C4V CHROMO4VIS, software v. 2.0, Regensight srl). Full description of the medical device operation sequence was provided in previous reports (Figure 1).[15–18]

The sclerocorneal tissues were randomly stratified to receive either CXL treatment with the cornea de-epithelialized (epi-off, n = 8) or with the epithelium intact (epi-on, n = 12). Baseline data for each subgroup are summarized in Table 1. Each tissue was placed in an artificial anterior chamber (AAC, Coronet, Network Medical Products Ltd.) pressurized with the AAC filled with 0.9% sodium chloride; the AAC was connected, through tubing, to a column manometer to maintain intracameral pressure within physiological ranges during the experiment. The samples undergoing the epi-off protocol were de-epithelialized immediately before commencing the experiment using an Amoils brush (Innovative Excimer Solutions, Inc.). During the dosing phase, a 0.22% riboflavin ophthalmic solution (RitSight, Regensight srl) was applied every 20 seconds onto the cornea according to the instructions for use; the de-epithelialized samples underwent 10 minutes of dosing time, and the samples with intact epithelium underwent 20 minutes of dosing time. At the end of dosing phase, the corneal surface was gently washed with balanced salt solution only in samples with intact epithelium. All tissues underwent 10 mW/cm^2 UV-A irradiance for 9 minutes (5.4 J/cm^2 total UV-A energy) over an irradiation area of 7.00 mm diameter; no riboflavin was applied over the corneal surface during UV-A light irradiation in any case. During both the dosing and UV-A phototherapy phases, theranostic measurements were performed over a 3.0 mm central area of the cornea (Figure 1). At preset time intervals, the theranostic device irradiated the cornea under treatment with 3 mW/cm^2 UV-A power density for a few seconds; thereafter, it processed corneal fluorescence data and provided the operator with a real-time measure estimating the corneal riboflavin concentration (both during the dosing and UV phototherapy phases) and a measure estimating treatment efficacy, that is, the theranostic score (during the UV phototherapy phase only). Calculation of this imaging biomarker took into account the corneal riboflavin concentration before UV-A phototherapy and the amount of riboflavin photodegraded during UV-A phototherapy in the cornea under treatment.

Corneal Tissue Deformation Response

A dynamic tonometry device (Corvis, Oculus Optikgeräte GmbH) was used to assess the corneal tissue deformation response to an air-puff pulse before and 2 hours after CXL treatment. The maximum deformation amplitude (DA; expressed in micrometers), which is a biomechanical parameter that defines the highest concavity of corneal apex during movement backward induced by the air pulse jet, was recorded from the Corvis device and analyzed. In addition, the dynamics of corneal profile captured during the deformation event was exported from the Corvis software (.avi format; n. 140 frames of 200 × 576 pixels) and processed using custom software written in Matlab (Mathworks, Inc.).[18–20] The algorithm fitted the anterior and posterior curvature of the corneal tissue with an eighth-degree polynomial curve and calculated several parameters from corneal deformation data, as previously described.[18] A 3-element viscoelastic rheological model was developed to fit the corneal deformation to air-puff pulse and to calculate the mean corneal stiffness parameter (k_c; N/m), based on a modified differential equation, as follows[18,21–23] (Figure 2):

$$F_{air-puff} = k_c u_1(t) + k_g u_2(t) + \mu_g \frac{du_2(t)}{dt} \qquad (1)$$

The model takes into account both the effective corneal deflection, $u_1(t)$, and the response of the extracorneal tissue segments (ie, the sclera), $u_2(t)$. The sum $u(t) = u_1(t) + u_2(t)$ determines the global deformation of the corneal tissue; $k_c{*}u_1(t)$ is the force exerted onto the corneal tissue; $kg{*}u_2(t) + \mu_g{*}du_2(t)/dt$ is the force exerted on the extracorneal tissue, where k_g (N/m) represents the extracorneal tissue stiffness, μ_g (N*s/m) represents the extracorneal tissue viscosity, and k_c (N/m) represents the corneal stiffness; it can vary (nonlinear elastic response) as a function of the applied air-puff pressure exerted on the tissue, that is, $k_c = \beta e^{\alpha P_{air-puff}}$, which is the typical behavior for viscoelastic systems.[24] For this reason, the corneal stiffness was calculated as the mean of all k_c at the corresponding $P_{air-puff}$, describing accurately the corneal tissue stiffness increase as air pulse pressure varies.[22] Furthermore, the force applied by the air pulse was expressed as $F_{air-puff} = P_{air-puff} * Appl$, where Appl is the applanation area on the cornea, which was considered constant and equal to a circle with 2.5 mm diameter.[25] The modified differential equation of the 3-element model consisted in determining the mean corneal stiffness parameter, k_c, rather than the Young modulus (E) to improve the fit between the experimental dynamic tonometry data and the theoretical model.[26] The model was solved for each sample with a Levenberg-Marquardt least-square minimization algorithm.

Data Analysis

In this study, data were given as mean ± SD; the outcome measures included the concentration of riboflavin, which was expressed as μg/cm^3 (100 μg/cm^3 = 0.01%), the theranostic score, which is a dimensionless number (d.n.), and the k_c (N/m) and DA (μm) parameters.

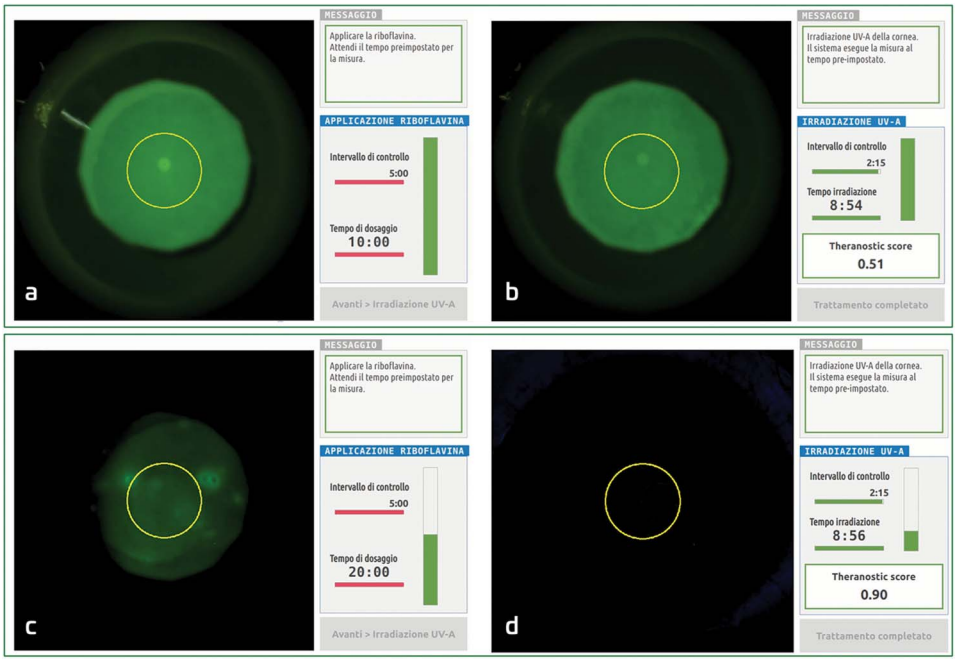

Figure 1. Operational sequence of Thera-CXL epi-off (*upper row*) and epi-on (*lower row*) protocols. Corneal riboflavin concentration is estimated at preset monitoring time intervals both during the dosing (*a* and *c*) and UV-A phototherapy (*b* and *d*) phases. During the UV-A phototherapy phase, the device tracks the photodegradation of corneal riboflavin and further calculates the theranostic score providing an estimation of treatment efficacy in real time.

A minimum sample of 7 tissues from the positive group (ie, theranostic score predicting increased corneal stiffness correctly) and 7 tissues from the negative group achieved 88% power to detect a difference of 0.30 between the area under the receiving operating characteristics curve under the null hypothesis of 0.50 and an area under the receiving operating characteristics curve under the alternative hypothesis of 0.80 using a 2-sided *z*-test at a significance level of 0.05.

Efficacy of treatment, expressed by k_c and DA parameters, was assessed with the paired *t* test. The correlation between treatment variables (riboflavin concentration and theranostic score) and between changes of biomechanical parameters induced by treatment ($k_{c\ post}/k_{c\ pre}$ and DA_{post}/DA_{pre}) was expressed with the Pearson correlation coefficient (*R*).

A second-order polynomial regression analysis was performed to correlate the increment of mean corneal stiffness parameter, $Y = k_{c\ post}/k_{c\ pre}$, as a function of the theranostic score,

$$Y = b_0 + b_1{*}\text{therascore} + b_2{*}\text{therascore}^2 \qquad (2)$$

where b_0, b_1, and b_2 are the coefficients of regression. The accuracy and precision of the regression model incorporating the theranostic score for predicting the increase in mean corneal stiffness parameter were determined by calculating the proportion of correctly classified samples and the positive predictive value, respectively.[27] The false-negative rate, or miss rate, was also calculated to determine the rate of predicted condition negative.

Statistical analysis was performed using SPSS statistical software (v. 17, SPSS, Inc.), and $P \leq .05$ was considered as statistically significant.

RESULTS

Corneal tissue parameters and intracameral pressure were stable during experiments, as summarized in Table 1. Corneal riboflavin concentration increased with application time in both epi-on and epi-off soaking protocols; Table 2 summarizes the average riboflavin concentration during the soaking phase for each protocol. At the end of treatment, the average corneal riboflavin concentration decreased by 48% ± 10% and 38% ± 8% in Thera-CXL epi-on and epi-off protocols, respectively. The average theranostic score was 0.8 ± 0.3 d.n.; the higher the stromal riboflavin concentration at the end of dosing phase, the greater the theranostic score at the end of UV-A phototherapy

Table 1. Donor and corneal parameters in the study groups

Parameter	Donor age (y)	Postmortem time interval (h)	ECD (cells/mm²)	CCT (µm)		CC (radius; mm)		IOP (mm Hg)	
				Baseline	After Thera-CXL*	Baseline	After Thera-CXL*	Baseline	After Thera-CXL*
All tissues	68 ± 7	15.1 ± 4.1	2040 ± 500	574 ± 84	579 ± 58	7.7 ± 0.3	7.7 ± 0.4	11.6 ± 3.3	12.3 ± 3.1
Thera-CXL epi-on protocol	72 ± 3	15.6 ± 4.3	1990 ± 400	578 ± 77	591 ± 66	7.7 ± 0.3	7.7 ± 0.4	12.1 ± 3.3	12.7 ± 3.3
Thera-CXL epi-off protocol	64 ± 6	14.4 ± 4.0	2100 ± 500	571 ± 99	569 ± 47	7.7 ± 0.3	7.6 ± 0.3	11.1 ± 3.4	11.4 ± 2.7

CC = corneal curvature; CCT = central corneal thickness; ECD = endothelial cell density; Thera-CXL = theranostic-guided corneal crosslinking
*Statistically significant (paired *t* test)

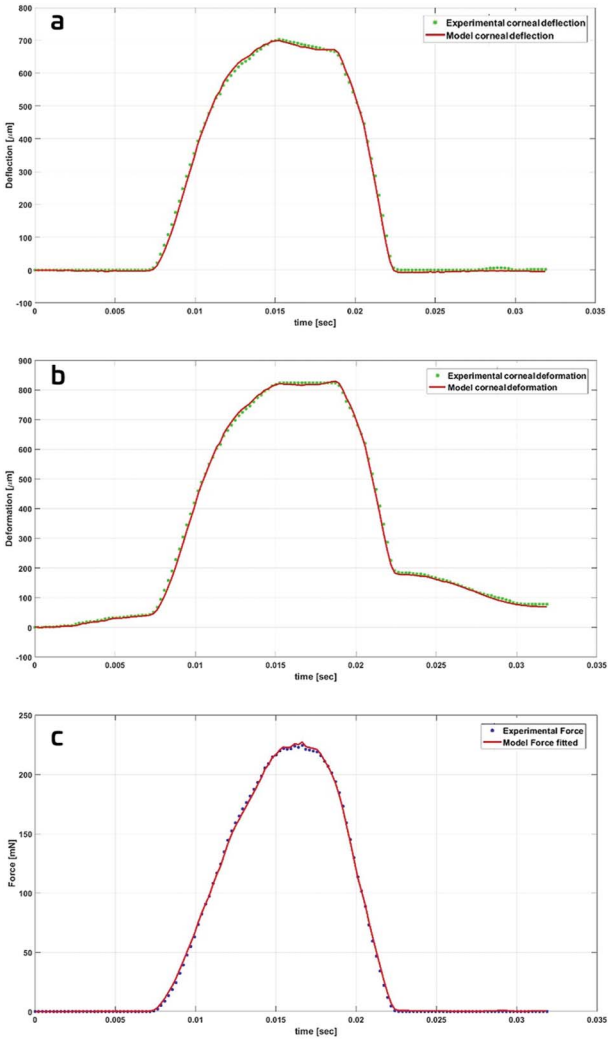

Figure 2. The 3-element viscoelastic model fitted the corneal dynamic deformation to air-puff excitation with high accuracy. In the panels, the experimental data and the model fit are represented by the green dashed curve and the red curve, respectively. In (*a*), corneal deflection; in (*b*), global deformation of the corneal tissue; and in (*c*), air pulse force applied to the corneal surface.

phase ($R = 0.81$; $P < .001$) (Table 2 summarizes data of both CXL protocols). The mean corneal stiffness parameter, k_c, increased from 38.2 ± 10.7 N/m to 46.2 ± 10.5 N/m, and significant tissue stiffening was induced by both Thera-CXL protocols, as summarized in Table 2. The result of the regression model ($Y = -1.255x^2 + 3.971x - 1.716$) was highly statistically significant ($R = 0.75$ and $P = .003$; Figure 3). The accuracy and precision of the model incorporating the theranostic score as a predictor of treatment efficacy were both 94%. Based on the results of corneal dynamic tonometry testing, the theranostic score was successful to detect a true-negative case but failed to detect 1 false negative case in the Thera-CXL epi-on protocol (miss rate = 6%); samples that underwent Thera-CXL epi-off were all predicted correctly, except for 1 false-positive. The presence of corneal epithelium did not influence the predictive ability of the imaging biomarker to define correctly samples that had improved biomechanical strengthening after CXL.

The CXL treatment significantly decreased the DA parameter from 654 ± 108 µm preoperatively to 586 ± 92 µm postoperatively ($P = .003$); a significant decrease in average DA max parameter was induced by both Thera-CXL protocols (Table 2; Figure 4). The decrease of DA (DA_{post}/DA_{pre}) induced by CXL was significantly correlated with the increase of mean corneal stiffness, $k_{c\ post}/k_{c\ pre}$ ($R = -0.55$; $P = .03$).

DISCUSSION

In this experimental study, the ability of a new technology, based on theranostics, for inducing a predictive corneal stiffening effect with the riboflavin/UV-A CXL procedure was assessed in human donor eye bank tissues. Twenty samples were randomly stratified to undergo 2 different riboflavin soaking protocols, that is, with or without the epithelium intact, and same UV-A irradiation at 10 mW/cm^2 for 9 minutes (5.4 J/cm^2 energy dose). Treatment efficacy was assessed by analysis of corneal biomechanical parameters extracted by a commercially available dynamic tonometry device, including the maximum deformation

Table 2. Average values of corneal riboflavin concentration (±SD) during the dosing phase of Thera-CXL, mean corneal stiffness parameter (k_c, ±SD), maximum deformation amplitude (DA, ±SD) before and after Thera-CXL protocols, and average theranostic score in both protocols (±SD)

Parameter	Time (min)	Thera-CXL epi-on protocol (n = 12)	Thera-CXL epi-off protocol (n = 8)
Corneal riboflavin concentration (µg/cm^3)	5	50 ± 19	344 ± 146
	10	73 ± 36	558 ± 235
	15	118 ± 68	—
	20	145 ± 77	—
Mean corneal stiffness (k_c, N/m)	Baseline k_c	41.4 ± 12.7	34.0 ± 5.0
	After treatment k_c	49.9 ± 11.2 ($P = .03$)*	41.7 ± 7.0 ($P = .04$)*
Maximum deformation amplitude (DA, µm)	Baseline DA max	635 ± 118	673 ± 86
	After treatment DA max	561 ± 104 ($P = .03$)*	610 ± 76 ($P = .04$)*
Theranostic score (d.n.)		0.7 ± 0.3	1.0 ± 0.2

d.n. = dimensionless number; Thera-CXL = theranostic-guided corneal crosslinking
*Statistically significant (paired *t* test)

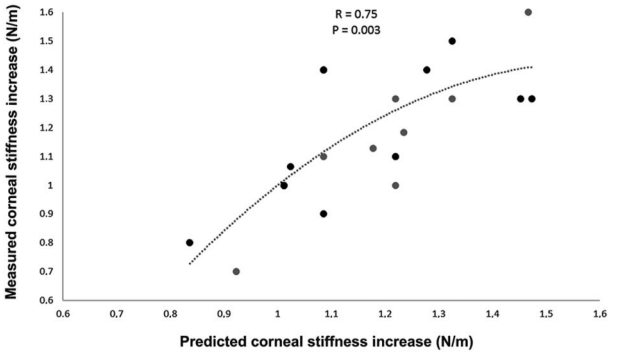

Figure 3. Correlation between the increase of mean corneal stiffness, $k_{c\ post}/k_{c\ pre}$, induced by corneal crosslinking treatment and that predicted by a second-order polynomial regression model incorporating the theranostic score as a predictor. Each symbol (*black dots* for the Thera-CXL epi-on protocol and *gray dots* for the epi-off protocol, respectively) represents a human donor corneal tissue (n = 20; sample dots 3 and 5 and 16 and 17 are overlapped).

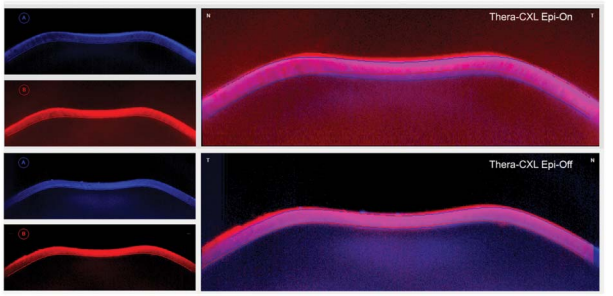

Figure 4. Image frames captured by Corvis during the air-puff event in 2 representative cases treated by epi-on (*upper row*) and epi-off (*lower row*) Thera-CXL protocols. Two-image frames (*A*: pretreatment and *B*: after treatment), overlapped at maximum deformation, are shown for both study cases. The treated cornea is pseudocolored red, and the pretreatment cornea is pseudocolored blue. Both treated eyes have less deformation at the same intracameral pressure indicating a stiffer response, that is, more resistance to load.

amplitude parameter, DA, and the mean corneal stiffness parameter, k_c. The former biomechanical parameter was recorded from the air-puff device, while the latter was the biomechanical parameter specifically developed for determining the increased tissue elasticity related to the change of tissue strength induced by CXL.[18,24,28]

The predictive ability of the theranostic score, which is the imaging biomarker calculated by the UV-A device during treatment, was assessed by measuring the coefficients of accuracy and precision. A second-order polynomial regression model incorporating the score as a predictor of treatment efficacy had 94% accuracy and 94% precision in predicting the tissue biomechanical strengthening induced by CXL. The UV-A light-mediated theranostic strategy for CXL has shown that significant tissue mechanical stiffening requires for a minimum concentration of riboflavin to achieve into the stroma before UV-A light irradiation and that this concentration differs between epi-on and epi-off protocols.[14–16,29] The scope of technology development was to solve the need for more effective and predictable CXL treatment with the corneal epithelium intact. Although the epi-on CXL procedure has shown improved safety in comparison with the epi-off CXL procedure, its clinical use is still under discussion because of variable clinical outcomes on efficacy in halting keratoconus progression. Much debate has pointed out 2 limiting variables in the epi-on CXL procedure, including (1) the lower amount of riboflavin availability into the corneal stroma than the epi-off CXL procedure and (2) the UV filtering effect of the intact corneal epithelium. However, these notions do not take into account the selective biophoton interaction among the concentration of riboflavin and UV-A light into the corneal tissue.[13] To solve these limits, real-time analysis of corneal riboflavin concentration during surgery enables the operator to know (1) that an adequate amount of riboflavin must be reached, through the intact epithelium, before UV-A therapeutic photoirradiation and (2) that the UV-A light-mediated photodegradation of riboflavin, at 10 mW/cm^2 irradiance for total 5.4 J/cm^2 energy dose, was not limited by the presence of intact epithelium. Monitoring of the UV-A light-mediated photodegradation of riboflavin during treatment allows the operator to understand any possible absorbing influence caused by the presence of epithelium or an excess of the riboflavin film onto the corneal stroma in EpiOn or EpiOff protocols, respectively.[13] Under real-time monitoring of corneal riboflavin concentration during both the dosing and UV-A phototherapy phases, in this study, a comparable stiffening effect on human tissues was found between epi-on and epi-off CXL protocols.

The results from the epi-off protocol were in strict agreement with previous work, thus confirming the theranostic-guided CXL procedure reproducibility.[17] In addition, they were in agreement with previous reports showing, in the 3 to 10 mW/cm^2 range of power densities for 5.4 J/cm^2 energy dose, that UV-A light-mediated photodegradation of riboflavin in the corneal stroma is only energy-dependent in accordance with the Bunsen-Roscoe law and that the transepithelial irradiation with 10 mW/cm^2 for 9 minutes is effective in photodegrading intrastromal riboflavin as the conventional direct stromal irradiation by 3 mW/cm^2 for 30 minutes.[11,14–16,29–31] This evidence could not be extrapolated for UV-A power density or energy dose higher than 10 mW/cm^2 or 5.4 J/cm^2, respectively.[14] In addition, the results achieved with the hypotonic, viscoelastic-free, 0.22% riboflavin ophthalmic solution used in this work could not be directly extrapolated to other riboflavin formulations, which include different substances, riboflavin concentration, and formulation stability.

In this work, CXL efficacy was determined by modeling the corneal response to the Corvis ST air-puff pulse event. The corneal tissue parameters, such as corneal curvature, corneal thickness, and intraocular pressure, which could influence corneal deformation to air-puff excitation, were

strictly monitored during experiments (Table 1).[32,33,36–38] No change in any of these parameters was found throughout the experiments. The mean corneal stiffness parameter, k_c, was chosen to determine the effect of CXL on tissue biomechanical strengthening, and the DA parameter was analyzed to validate our approach with an established biomechanical index calculated by a commercially available air-puff device. The mean DA value was significantly lower after treatment; CXL had a significant effect on the DA parameter because a stiffer cornea exhibited greater resistance to deformation (at matched intracameral pressure); accordingly, the decrease of DA parameter induced by CXL was significantly correlated with the increased mean corneal stiffness parameter. The present finding was in fair agreement with previous laboratory and clinical studies.[32,33] It was of note that the range of DA values in pretreatment corneal tissues was in agreement with previous report, where the authors compared the deformation response of the cornea with the Corvis air-puff jet between whole eye globes and AAC-mounted sclerocorneal tissues.[34] The k_c parameter represents the resistance to deformation under load and is a strain-dependent value that changes with strain (or corneal deformation).[17] It has been previously validated through analysis of several corneal conditions, including response to CXL treatment.[17,24,25,28,39,40] The authors have shown that during the air-puff event, due to the high loading magnitude (greater than 45 mm Hg) and fast rate (shorter than 20 milliseconds), the cornea is not able to undergo any viscous deformation; for this reason, a pure elastic material model, as performed in this study, could be selected for biomechanical modeling of cornea in dynamic tonometry tests because neglecting a corneal viscoelastic behavior has been shown not to significantly affect the results of the study.[25,28,40] Limitations of the model included the lack of tissue anisotropic properties and that it did not segregate the sclera contribution to the deformation amplitude; using finite element analysis, the authors have hypothesized that the corneal deformation may differ under different ex vivo boundary conditions of fixed vs flexible limbus showing that the sclera could play a role in assessing corneal biomechanics.[41]

A limit of this work included that we did not measure endothelial cell density at the end of treatment, on the other hand, several clinical studies have shown safety of CXL procedure for treating patients with keratoconus in children and adults using 10 mW/cm^2 power irradiance (5.4 J/cm^2 energy dose).[42–46]

In conclusion, this laboratory study provided evidence on the highly predictive corneal stiffening effect induced by theranostic-guided CXL, regardless of the riboflavin application protocol. The amount of riboflavin into the corneal stroma before UV-A light irradiation has been confirmed to be the main variable leading to effective CXL.[11–18,30,31] Providing personalized patient care through the noninvasive measurement of corneal riboflavin during treatment could be advantageous to minimize the occurrence of crosslinking failure.[6,9] The theranostic-guided CXL approach is currently object of an ongoing randomized clinical trial (NCT05457647), whose aim is to validate the predictive ability of the theranostic score for treating patients with progressive keratoconus with either epi-off or epi-on treatment protocols.[35]

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WHAT WAS KNOWN

- Epithelial removal was considered a fundamental prerequisite to attain certain therapeutic efficacy of CXL procedure. Establishing efficacy of transepithelial CXL was challenging because the procedure lacks selectivity in understanding the amount of the therapeutic molecule penetrating into the corneal stroma through the intact epithelium.

WHAT THE PAPER ADDS

- Theranostic-guided CXL provides a predictive and precise surgical approach for significant improvement of corneal tissue strength on individual corneas, regardless of the treatment protocol. The procedure enables the operator to precisely monitor the tissue permeation and UV-A light-mediated photodegradation of riboflavin in individual corneas and provides estimates of treatment efficacy in real time.

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