LABORATORY SCIENCE

Biomechanical stiffening: Slow low-irradiance corneal crosslinking versus the standard Dresden protocol

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Purpose: To assess whether full biomechanical stiffening can be achieved with corneal crosslinking (CXL) when applying a reduced ultraviolet (UV) fluence during the standard irradiation time.

Setting: Laboratory of Ocular Cell Biology, Center for Applied Biotechnology and Molecular Medicine, University of Zurich, Zurich, Switzerland.

Design: Experimental study.

Methods: Thirty-four freshly enucleated porcine corneas were deepithelialized and soaked with hypoosmolar riboflavin 0.1% solution for 30 minutes. Slow low-irradiance CXL (30 minutes at 1.5 mW/cm^2, fluence 2.7 J/cm^2) was compared with standard CXL (30 minutes at 3 mW/cm^2, fluence 5.4 J/cm^2). The controls were soaked with riboflavin but not exposed to UV light. Elastic (stress–strain) and viscoelastic (stress–relaxation) 2-dimensional testing was performed with a commercial stress–strain extensometer to quantify the biomechanical stiffening.

Results: Corneas crosslinked with low and standard UV irradiances had a significantly higher mean elastic modulus (65.9 MPa ± 15.7 [SD] and 67.1 ± 15.6 MPa, respectively) than controls (52.4 ± 12.3 MPa) ($P < .001$). Also, the remaining stress after 120 seconds of stress–relaxation was significantly higher after CXL with low and standard UV irradiances (159 ± 21 kPa and 158 ± 25 kPa, respectively) compared with controls (135 ± 20 kPa) ($P \leq .013$). No difference was observed in low and standard irradiances between CXL conditions ($P = .64$).

Conclusions: The UV fluence for CXL might be reduced while maintaining the biomechanical efficacy by using a lower UV irradiance and the same irradiation duration. This might open avenues in the treatment of extremely thin corneas.

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Since its first application for keratoconus in 2003,[1,2] the protocol for corneal crosslinking (CXL) has been modified several times. Although the first efforts were aimed at adapting the CXL protocol to corneas thinner than 400 µm,[3,4] the latest efforts strive to accelerate treatment duration.[5–7]

According to the Bunsen-Roscoe law of reciprocity, the photobiologic effect should only depend on the total administered ultraviolet (UV) energy (fluence), independent of the duration of administration and irradiation. However, studies[8,9] reported that this law cannot readily be applied to CXL; when analyzing the CXL effect of several clinical protocols sharing the same fluence but different time/irradiance combinations, they observed significant decreases in the CXL effect at high intensities. A potential factor responsible for this is oxygen. Ex vivo studies of porcine eyes[8,10] have shown that oxygen is essential to the biomechanical stiffening effect of CXL; Kamaev et al.[11] found that oxygen is rapidly consumed during CXL. The more oxygen available, the stronger the CXL-induced stiffening effect.[10] Oxygen availability has an inverse relationship with corneal thickness, and thinner corneas should theoretically experience stronger stiffening after CXL than thicker corneas, even if the UV dose is adapted so that the relative UV absorption along the corneal stroma is the same. This hypothesis was recently verified experimentally.[10]

Thus, CXL protocols at present are mainly limited by oxygen diffusion and therefore by irradiation duration, which means that the UV irradiance could be decreased without reducing the stiffening effect. In the field of ophthalmology, irradiance is sometimes incorrectly referred to as intensity. In physics, intensity is used to

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describe the UV power of the light source (per steradian) and irradiance, to describe the UV power at the target surface (per square meter).

Few clinical studies[12,13,A] as well as our recently presented model (created to predict CXL efficacy as a function of UV, riboflavin, and oxygen availability[14,B]) suggest that the efficacy of CXL should remain constant when using a protocol with only one half of the irradiance, and hence one half of its fluence, while maintaining the same irradiation duration. In this study, we tested this hypothesis by comparing a slow low-irradiance CXL setting (1.5 mW/cm^2 for 30 minutes; fluence 2.7 J/cm^2) with the standard CXL setting (3 mW/cm^2 for 30 minutes; fluence 5.4 J/cm^2).

MATERIALS AND METHODS

Theoretical Model Estimates

Our recently published model[14] estimates the extent of corneal stiffening for different CXL protocols and is based on extracellular matrix (ECM) oxidation induced by singlet oxygen. It has been suggested that singlet oxygen–induced ECM oxidation correlates with the biomechanical stiffening effect on the cornea.[15] In the current study, this model was applied to predict whether the UV irradiance might be reduced to 1.5 mW/cm^2 in the standard CXL protocol without reducing the biomechanical stiffening effect. A recently introduced similar approach using reduced UV irradiance for CXL treatment has shown promising results.[12,13,A] According to the model, CXL with 3 mW/cm^2 for 30 minutes induces a concentration of additional crosslinks similar to CXL with 1.5 mW/cm^2 for 30 minutes (0.3235 mol/m^3 versus 0.3197 mol/m^3), whereas both conditions are predicted to have a nearly identical distribution throughout the stroma. For comparison, CXL with 9 mW/cm^2 for 10 minutes is predicted to induce a concentration of 0.2220 mol/m^3 and CXL with 18 mW/cm^2 for 5 minutes, a concentration of 0.1710 mol/m^3, whereas both conditions reach a lower penetration into the stroma than standard CXL.

Corneas

Thirty-four freshly enucleated porcine eyes were obtained from a local slaughterhouse (Schlachthof Zürich, Zurich, Switzerland) within 4 hours postmortem. The epithelium was removed, and hypoosmolar riboflavin 0.1% (in phosphate-buffered saline) was applied for 30 minutes. The eyes were then divided into 3 groups as follows: Group 1 (n = 12) was irradiated with 1.5 mW/cm^2 for 30 minutes (fluence 2.7 J/cm^2), Group 2 (n = 12) was irradiated with 3 mW/cm^2 for 30 minutes (fluence 5.4 J/cm^2), and Group 3 (n = 10) served as the control and was not exposed to UV light.

Immediately after UV irradiation, the corneas were excised and mounted circumferentially on a 2-dimensional customized holder (diameter 10.0 mm) for biomechanical characterization. Elastic and viscoelastic material properties were measured with a commercial stress–strain extensometer (Zwicki line, Zwick GmbH & Co. KG) as described previously.[10] Briefly, the testing procedure consisted of 2 cycles of preconditioning from 1.2 to 12.5 N followed by stress–relaxation at 12.5 N for 120 seconds. The tensile elastic modulus was determined during the first loading cycle to characterize the elastic material properties, and the remaining stress after stress–relaxation was taken as a measure of the viscous properties. The higher the elastic modulus and the higher the remaining stress, the stiffer and more stable the tissue over time.

Statistical Analysis

Statistical significance was determined with a 2-sided Student *t* test and a 95% confidence interval using Excel for Mac software (version 15.26, Microsoft Corp.).

RESULTS

The stress–strain analysis showed that corneal stiffness increased significantly with both CXL treatment protocols. The mean tangent elastic modulus between 5% and 15% of strain was 65.9 MPa ± 15.7 (SD) in Group 1 (CXL at 1.5 mW/cm^2), 67.1 ± 15.6 MPa in Group 2 (CXL at 3 mW/cm^2), and 52.4 ± 12.3 MPa in Group 3 (controls) (Figure 1, *A*). Table 1 shows the local elastic moduli at 5%, 10%, and 15%. Significant differences were observed between crosslinked corneas and control corneas, although not between the slow low-irradiance CXL and the standard CXL protocols (Table 2).

Stress–relaxation tests showed that the stress remaining after 120 seconds was significantly higher in crosslinked corneas, both with 1.5 mW/cm^2 (159 ± 21 kPa) and with 3 mW/cm^2 (158 ± 25 kPa), than in control corneas (135 ± 20 kPa) ($P \leq .013$), but not between the 2 CXL protocols ($P = .92$) (Table 2 and Figure 1, *B*).

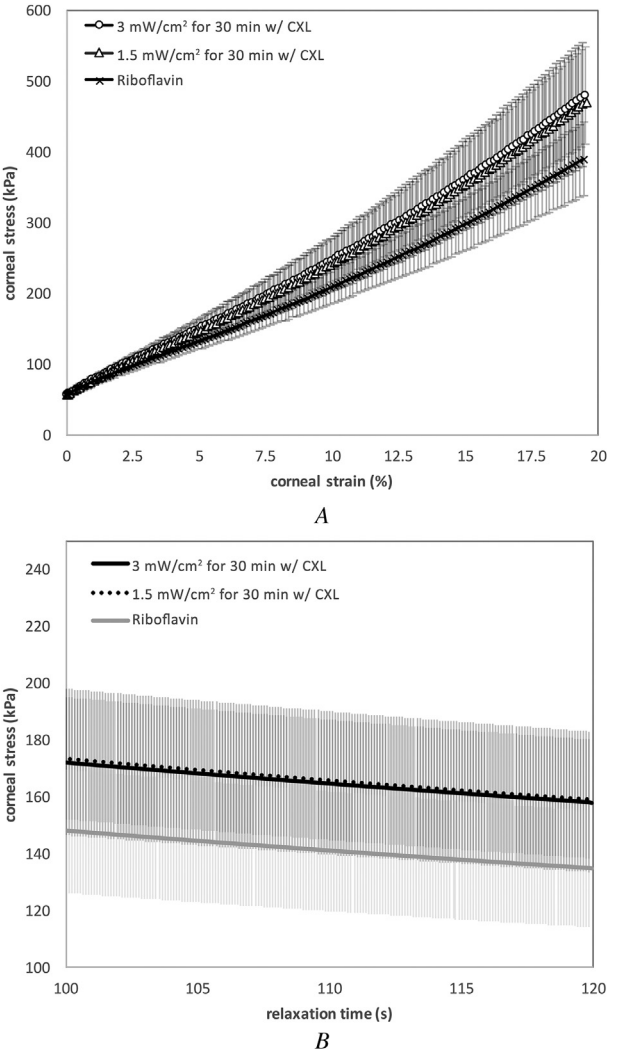

Figure 1. Stress–strain diagram of porcine corneas soaked with riboflavin 0.1% solution. Comparison between normal UV intensity (3 mW/cm^2; 30 minutes), low UV intensity (1.5 mW/cm^2; 30 minutes), and no UV light. *A*: Corneal strain (%). *B*: Relaxation time (seconds) (CXL = corneal crosslinking).