Fluence and corneal thickness

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Definitions

• **Irradiance**: \( E = \text{power/area} \ [\text{mW/cm}^2] \)
  (light intensity)
  light power per area
  example: 3 mW/cm\(^2\) to 30 mW/cm\(^2\)

• **Irradiation dose**: \( H = E \times t \ [\text{J/cm}^2] \)
  (light fluence)
  \( t = \) irradiation time
  light energy per unit area
  example: 5.4 J/cm\(^2\) to 10 J/cm\(^2\)
Corneal cross-linking

- **UV light** causes an effect only where it is absorbed
- the selection of the **wavelength** of the used **UV light** that corresponds to one of the **absorption maxima** of the riboflavin
- riboflavin acts as a photomediator, creating free radicals to induce new chemical bonds
Absorption spectrum of riboflavin

According to the absorption peaks the wavelength of 365-370 nm was chosen.

UV light with a wavelength of 370 ± 5nm

- < 300 nm is not acceptable because of potential DNA damages
- > 400 nm may be dangerous because of the blue light hazard to the retina

Corneal cross-linking

• the cornea is exposed to UV light with a wavelength of 370 nm and irradiance of 3 mW/cm² for a total time of 30 min.

• this corresponds to a total irradiation dose (fluence) of 5.4 J/cm² to the cornea

Riboflavin diffusion into the eye

- both, the time course of the diffusion process and the concentration of the superficially applied riboflavin solution are relevant for the absorption of the riboflavin into the cornea
- applied riboflavin must diffuse into the corneal stroma
- this process requires a certain time

Riboflavin diffusion

- The concentration of the riboflavin can be calculated for each stromal depth because it depends on elapsed time.

- After approx. 30 min., a concentration of 0.04% is achieved 400μm deep in the stroma.

Riboflavin in anterior chamber

• the aqueous humor without riboflavin does not have any relevant absorption at 370 nm
• it starts clinically to stain after ~5 min. of surface exposure to riboflavin
• 30 min. after riboflavin application, an absorption coefficient of 0.7 cm\(^{-1}\) was measured, corresponding to a concentration of 0.002% (rabbit study)
• this leads to a further reduction of the UV light by 20%
• the shielding effect caused by riboflavin in the AC is not significant
• the yellow staining of the AC serves more as a safety feature, indicating that the riboflavin has penetrated the cornea

Riboflavin shielding

- Lambert-Beer law yields a reduction of the irradiance caused by absorption in a 400μm-thick layer by a factor of 5.5
- because of the additional riboflavin shielding effect, all structures behind the corneal stroma, including corneal endothelium, anterior chamber, iris, lens and retina are exposed to a residual UV radiant exposure that is less than 1 J/cm²
• the riboflavin imbibed in the corneal stroma enhances the absorption coefficient by a factor of ~ 5, which limits the UV irradiance through a 400μm-thick stroma to 0.18mW/cm² at the endothelial level

Direct UV damage

- the damage mechanism from the UV light depends on its wavelength, its irradiance and the irradiation time
- without the presence of a photosensitizer, light at wavelengths 350 nm and higher and an irradiance of 3 mW/cm² will not cause damage to the endothelium
- in rabbits, the threshold radiant exposure for damage has been shown to be 70 J/cm² for the lens and 42 J/cm² for the cornea
- comparing these thresholds with the UV irradiance and dose used during the CXL procedure (3mW/cm², 5.4J/cm²), it is not expected any damage to the corneal endothelium, the lens or the retina
Guidelines on limits of exposure to UV radiation of wavelengths between 180nm and 400nm

• for longer UV irradiation times, the limiting radiant exposure of 1 J/cm² should not be exceeded

Matthes R. Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incoherent optical radiation). Health Phys. 2004; 87: 171-186
Calculated irradiance in human cornea

Light-emitting diodes with 370 nm

Homogeneous illumination

- clinically used light source must guarantee a perfect homogeneity of the irradiance
- hot spots may cause localized endothelium cell damage, especially in thin corneas
Irradiation and distance

- important is the fluence at the corneal surface.
- adjust the recommended distance
Endothelial cell damage after riboflavin–ultraviolet-A (UVA) treatment in the rabbit

Gregor Wollensak, MD, Eberhard Spoerl, PhD, Michaela Wikel, PhD, Theo Seiler, MD, PhD

Purpose: To evaluate the possible cytotoxic effect of combined riboflavin–ultraviolet-A (UVA) treatment on the corneal endothelium.

Conclusions: In rabbit corneas with a corneal thickness less than 400 μm, the endothelial UVA dose reached a cytotoxic level of \( \geq 0.65 \text{ J/cm}^2 \) (0.36 mW/cm\(^2\)) using the standard surface UVA dose of 5.4 J/cm\(^2\) (3 mW/cm\(^2\)). Pachymetry should be routinely performed before riboflavin–UVA treatment; in thinner corneas, irradiation should not be done because of the cytotoxic risk to the endothelium.

Endothelial UVA dose of 0.9 J/cm² (0.5 mW/cm²) and of 0.65 J/cm² (0.36 mW/cm²) were twice as high as the therapeutic endothelial dose in humans of 0.32 J/cm² (0.18 mW/cm²).
UVA cytotoxicity for human endothelium...

- **Purpose:** to evaluate endothelial cytotoxicity after exposure of human corneas to UVA in an experimental ex vivo corneal CXL
- **Methods:** by *irradiating from the endothelial side*, the UVA dosage exceeded eight times the cytotoxic threshold established in animal models
- **Conclusion:** despite direct irradiation of human donor endothelium using the clinical dosage for CXL, *equivalence in endothelial cell counts was observed* between irradiated tissues and controls...
- **Human endothelial cells seem to be much more resistant to the cytotoxic effect of UVA than previously assumed**

Riboflavin concentration during corneal crosslinking at the endothelial level

• riboflavin concentration is decreasing in the posterior stroma down to 0.03%, reaching only about 0.01% just anterior of the endothelium...

• this implicates new, higher safety thresholds for human application of clinical CXL...

Seiler TG, Batista A, Frueh B et al. Riboflavin concentration during corneal crosslinking (CXL) at the endothelial level. Der Ophthalmologe 2018; Suppl 1 (Presentation to DOG 2018)
Evaluation of Corneal Stromal Demarcation Line Depth Following Standard and a Modified-Accelerated Collagen Cross-linking Protocol

GEORGE D. KYMIONIS, Konstantinos I. TSOULNARAS, Michael A. Grentzelos, Dimitrios A. Liakopoulos, Nikolaos G. Tsakalis, Styliani V. Blazaki, Theodoros A. Paraskevopoulos, and Miltiadis K. Tsilimbaris

There was no significant difference in the ECD preoperatively and postoperatively between the 2 groups.

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

Sabine Kling, PhD, Farhad Hafezi, MD, PhD

Purpose: To assess whether full biomechanical stiffening can be achieved with corneal crosslinking (CXL) when applying 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 hypoisomolar riboflavin 0.1% solution for 30 minutes. Slow low-irradiance CXL (30 minutes at 1.5 mW/cm², fluence 2.7 J/cm²) was compared with standard CXL (30 minutes at 3 mW/cm², fluence 5.4 J/cm²). 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 (66.9 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 ≤ .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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„Customized“ fluence

For thin corneas the fluence can be reduced according the stromal thickness.

Eberhard Spoerl: unpublished data