Fluence and corneal thickness

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Definitions

- Irradiance: E=power/area [mW/cm²]

  (light intensity)

  light power per area

  example: 3 mW/cm² to 30 mW/cm²

- Irradiation dose: H=E*t [J/cm²]

  (light fluence)

  t=irradiation time

  light energy per unit area

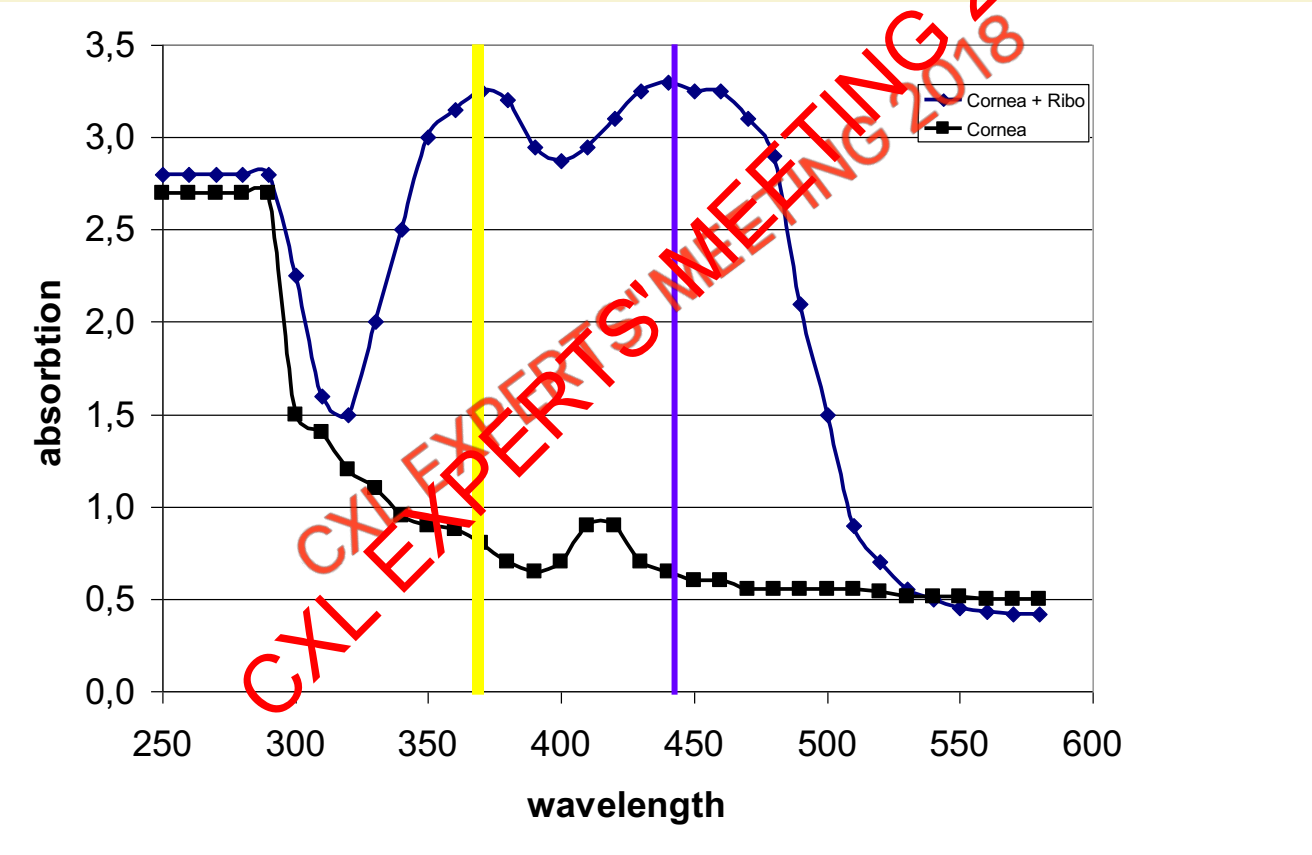
  example: 5.4J/cm² to 10J/cm²

Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

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

Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye res. 1998; 66: 97-103

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

Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

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

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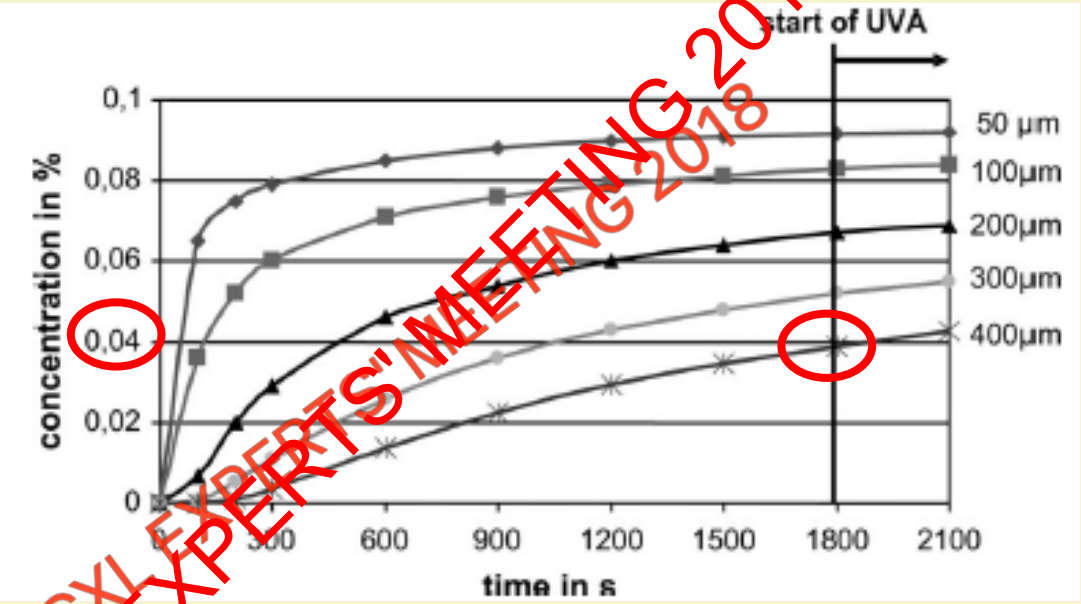
Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye res. 1998; 66: 97-103

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

Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

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

Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

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

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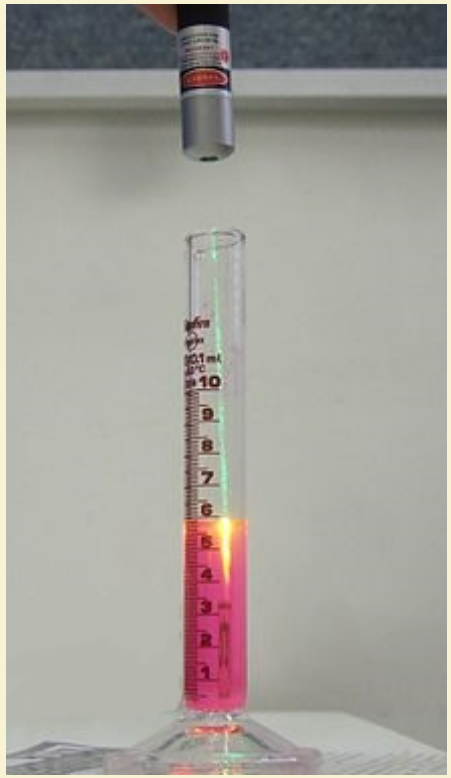
Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

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**



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Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

Riboflavin shielding

TABLE 1. Absorption Coefficients of Different Corneas (unpublished data)

Cornea Type	Absorption Coefficient/cm^{-1}	
	Without Riboflavin	With Riboflavin 0.1%
Porcine	13.6	59
Rabbit	13.8	63
Human	14	70

- **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/cm^2 at the endothelial level

Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-Riboflavin cross-linking of the cornea. Cornea 2007; 26: 385-389

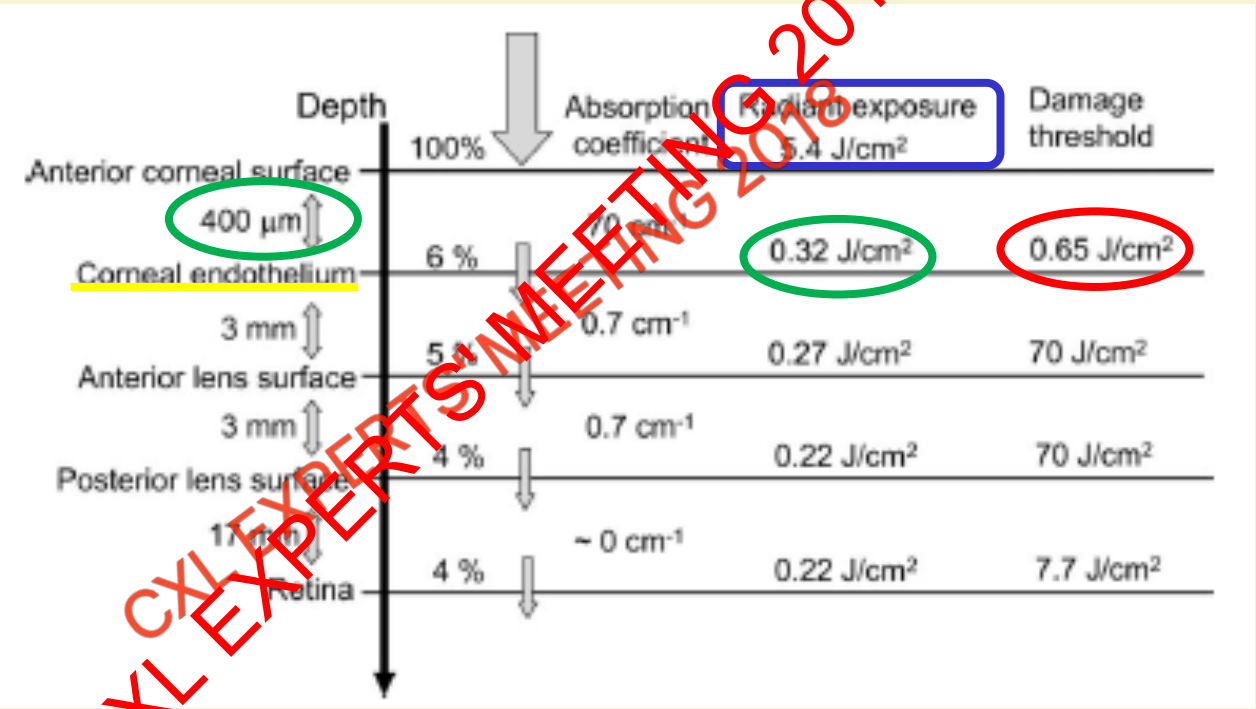
Direct UV damage

- 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/cm^2 **will not cause damage to the endothelium**
- **in rabbits, the threshold radiant exposure for damage** has been shown to be 70 J/cm^2 for the lens and **42 J/cm^2 for the cornea**
- comparing **these thresholds with the UV irradiance** and dose used **during the CXL procedure** (3mW/cm^2, 5.4J/cm^2), **it is not expected any damage to the corneal endothelium**, the lens or the retina

Ringvold A, Davanger M, Olsen EG. Changes of the cornea endothelium after ultraviolet radiation. Acta Ophthalmol. 1982; 60: 41-53

Pitts DG. The ocular effects of ultraviolet radiation. Am J Optom Physiol Opt. 1978; 55: 19-35

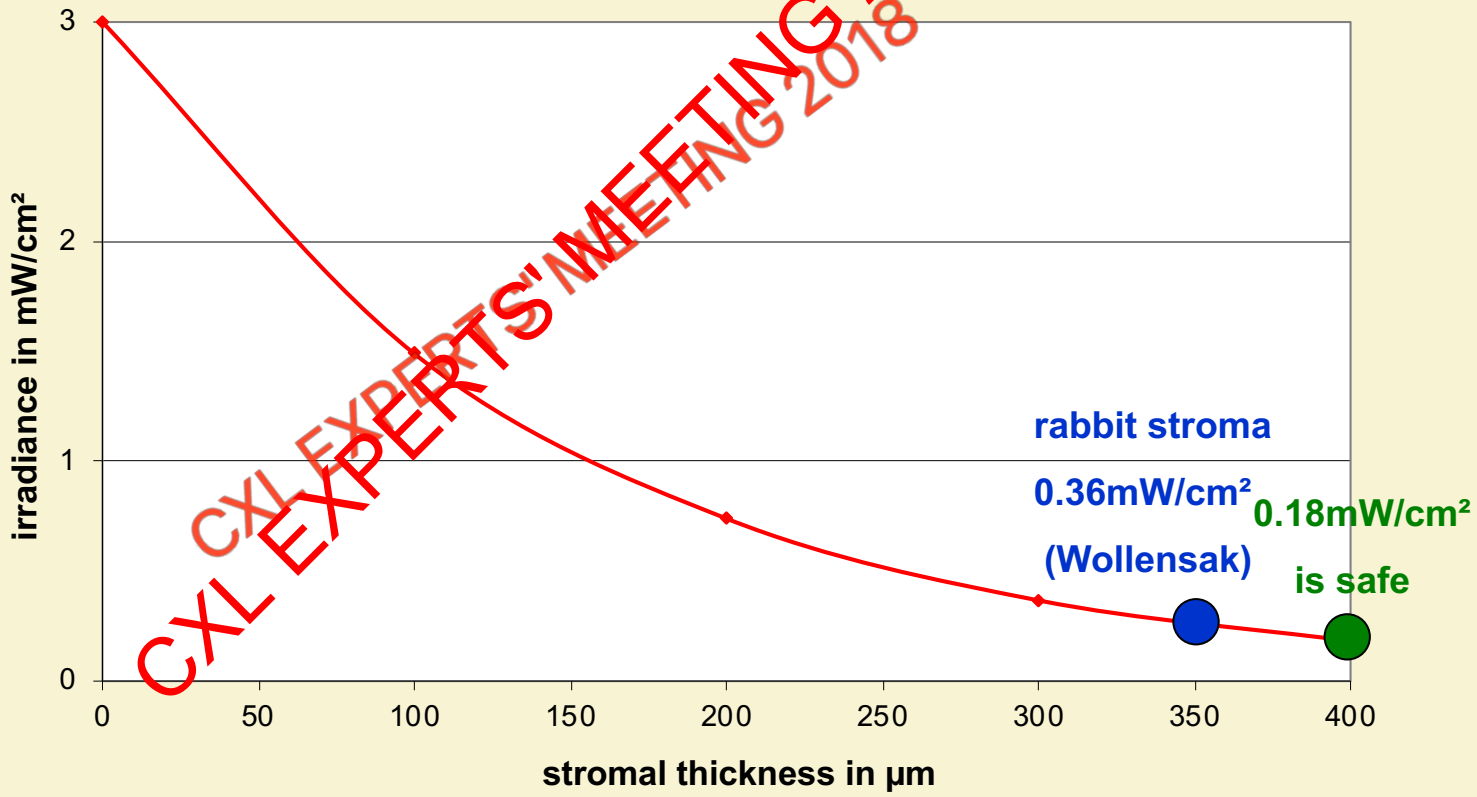
„Guidelines on limits of exposure to UV radiation of wavelengths between 180nm and 400nm“



- for longer UV irradiation times, the limiting radiant exposure of 1 J/cm^2 should not be exceeded

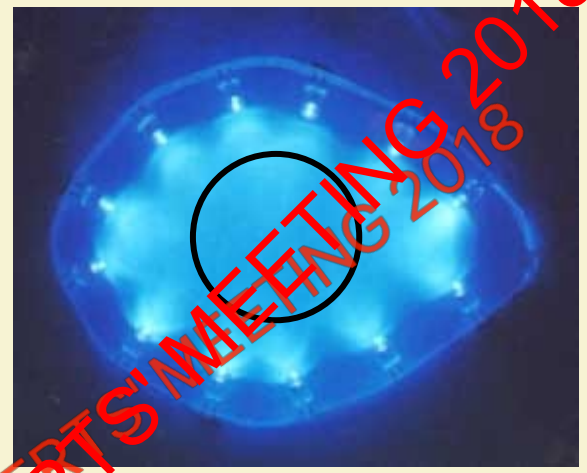
Matthes R. Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incoherent optical radiation). Health Phys. 2004; 87: 171-186

Calculated irradiance in human cornea



Iseli HP, Popp M, Seiler T, Spoerl E, Mrochen M.Laboratory measurement of the absorption coefficien of riboflavin for ultraviolet light (365nm). J Refract Surg. 2011; 27:195-201

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 treatment in the rabbit

Gregor Wollensak, MD, Eberhard Spoerl, PhD, Michaela Wilsch, 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 ≥0.65 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.

J Cataract Refract Surg 2003; 29:1786–1790 © 2003 ASCRS and ESCRS

Endothelial cell damage after riboflavin–ultraviolet-A treatment in the rabbit

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

Group	Number of Animals	Surface Epithelial UVA Irradiance (mW/cm^2)	Endothelial UVA Irradiance (mW/cm^2)	Endothelial Cytotoxicity
1	3	4.0	0.50 ± 0.07	+++
2	6	[illegible]	0.36 ± 0.04	+++
3	5	[illegible]	0.32 ± 0.04	0
4	3	2.25	0.27 ± 0.03	0
5	3	1.88	0.23 ± 0.03	0
6	5	1.5	0.18 ± 0.02	0
7	3	0.75	0.09 ± 0.01	0

Endothelial UVA dose of 0.9 J/cm^2 (0.5 mW/cm^2) and of 0.65 J/cm^2 (0.36 mW/cm^2) were twice as high as the therapeutic endothelial dose in humans of 0.32 J/cm^2 (0.18 mW/cm^2)

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

Mooren P, Gobin L, Bostan N et al. Evaluation of UVA cytotoxicity for human endothelium in an ex vivo corneal cross-linking experimental setting. J Refract Surg. 2016; 32(1): 41-46

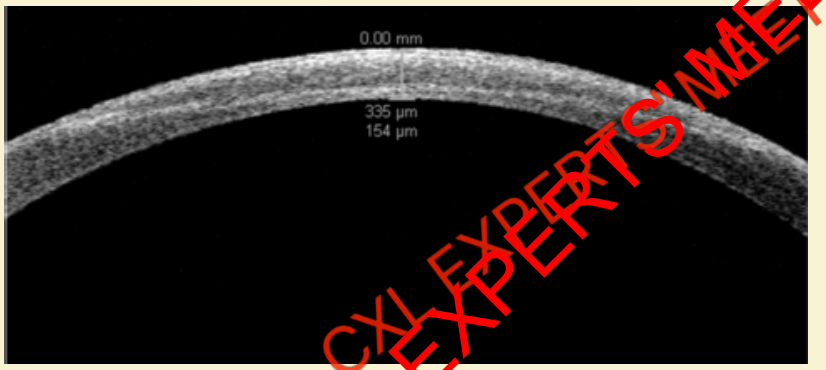
Riboflavin concentration during corneal crosslinking at the endothelial level

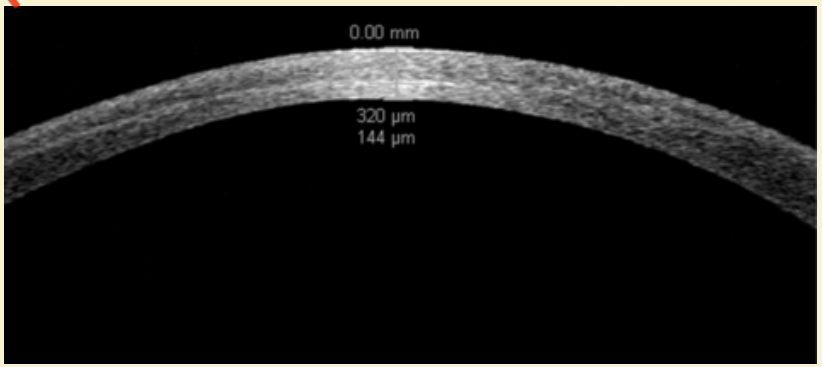
- **riboflavin concentration is decreasing in the posterior stroma** down to 0.03%, reaching only about 0.01% just anterior of the endothelium…
- this implicates new, **higher safety thresholds for human applicaton of clinical CXL**…

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





3.0 mW/cm² for 30 min → 5.4J/cm²

9.0 mW/cm² for 14 min → 7.5J/cm²

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

Am J Ophthalmol 2014; 158: 671-675

LABORATORY SCIENCE

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 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.

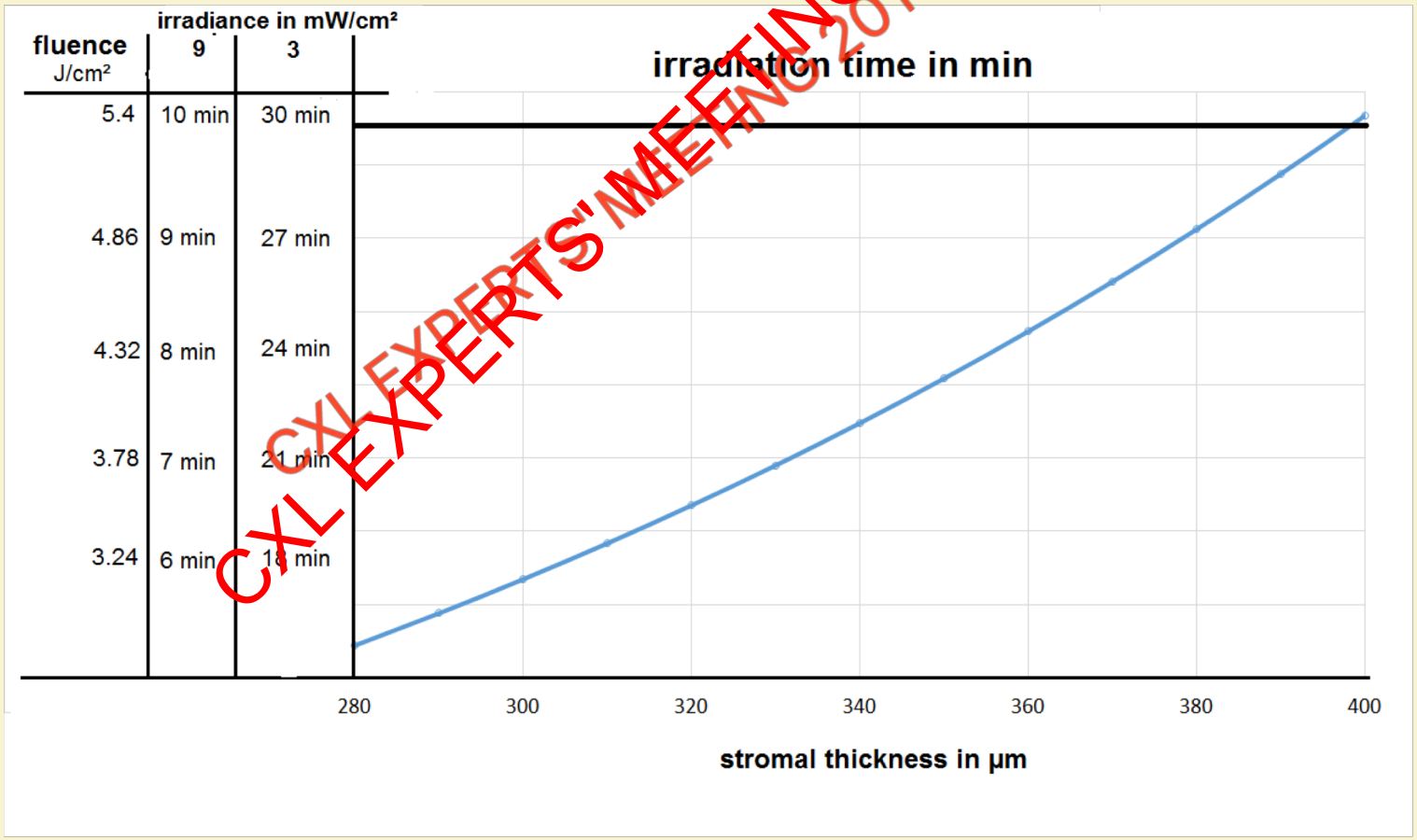
Results: Corneas crosslinked with low and standard UV irradiances had a significantly higher mean elastic modulus (65.9 MPa ± 15.7 [SD] and 67.1 ± 15.6 MPa, respectively) than controls (52.4 ± 12.3 MPa) ($P < .001$). Also, the remaining stress after 120 seconds of stress–relaxation was significantly 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.

J Cataract Refract Surg 2017; 43:975–979 © 2017 ASCRS and ESCRS

„Customized“ fluence

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



Eberhard Spoerl: unpublished data