# Riboflavin, Oxygen and Light

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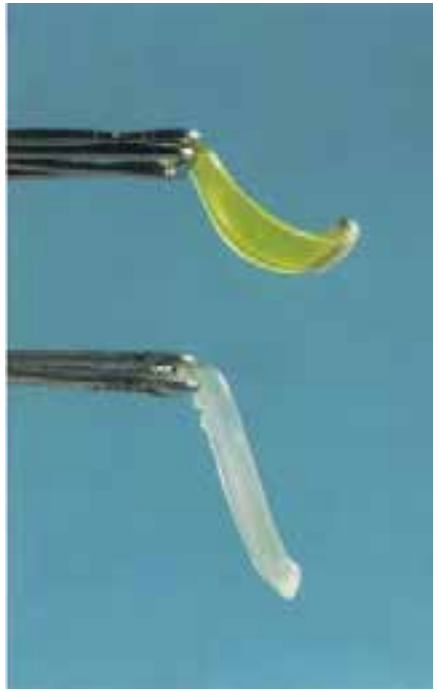
# Corneal Cross-linking

Increasing corneal stiffness to stop the progression of keratoconus

365 nm, 3mW/cm$^2$

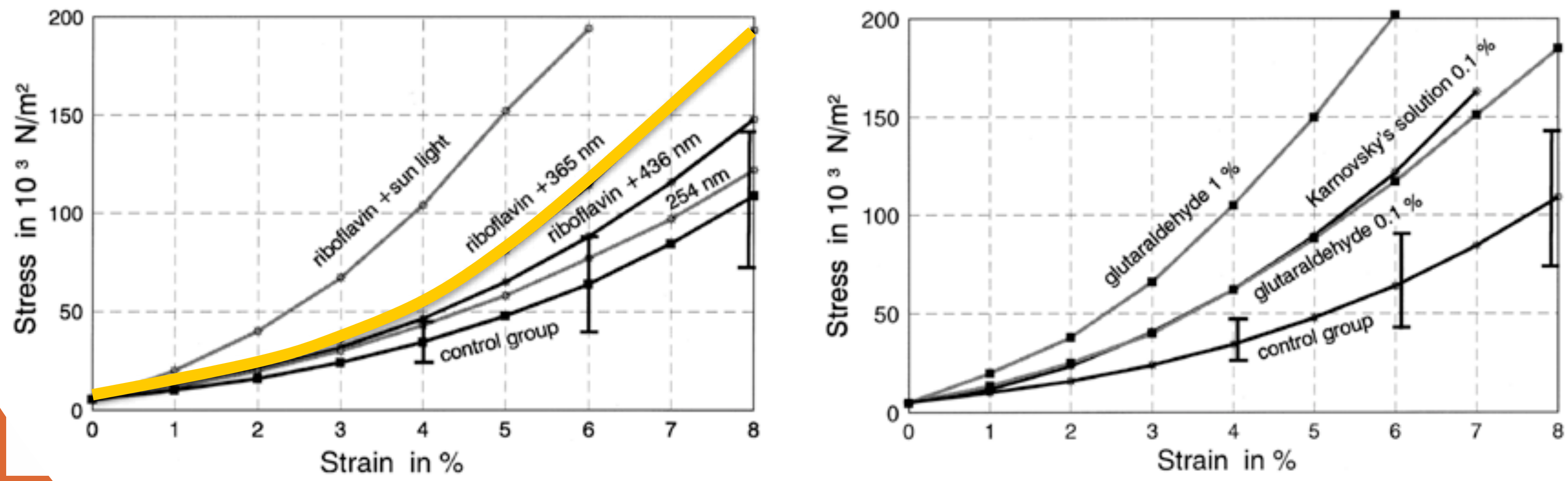



**de-epithelialization**


**Photosensitizer**
**30 min**

# Different techniques for cross-linking

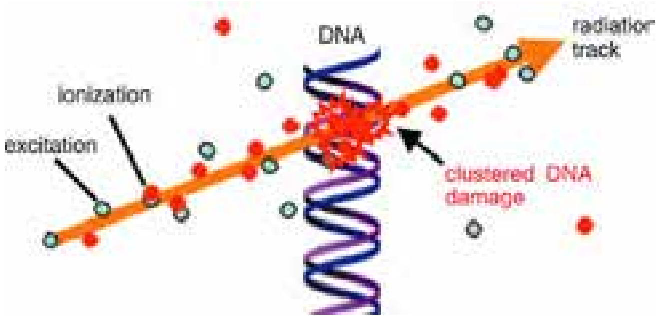
- chemically (e.g. glutaraldehyde)
- ionizing radiation (e.g. $\gamma$ ray, $e^-$ ray)
- photo-chemically (e.g. UV-light + photosensitizer)




*REF. Spoerl E. Exp Eye Res 1998*

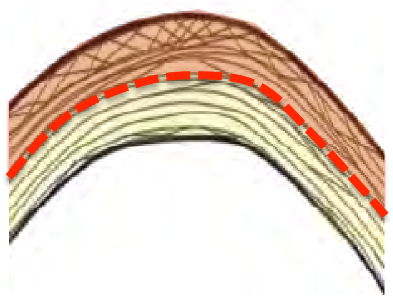
# Advantages of photo-chemically induced cross-linking

✗ no DNA mutations due to radiation




✗ no health hazard due to chemicals


✓ penetration depth is adjustable



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# Selection of the photosensitizer and wavelength

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# Selection of wavelength and photosensitizer

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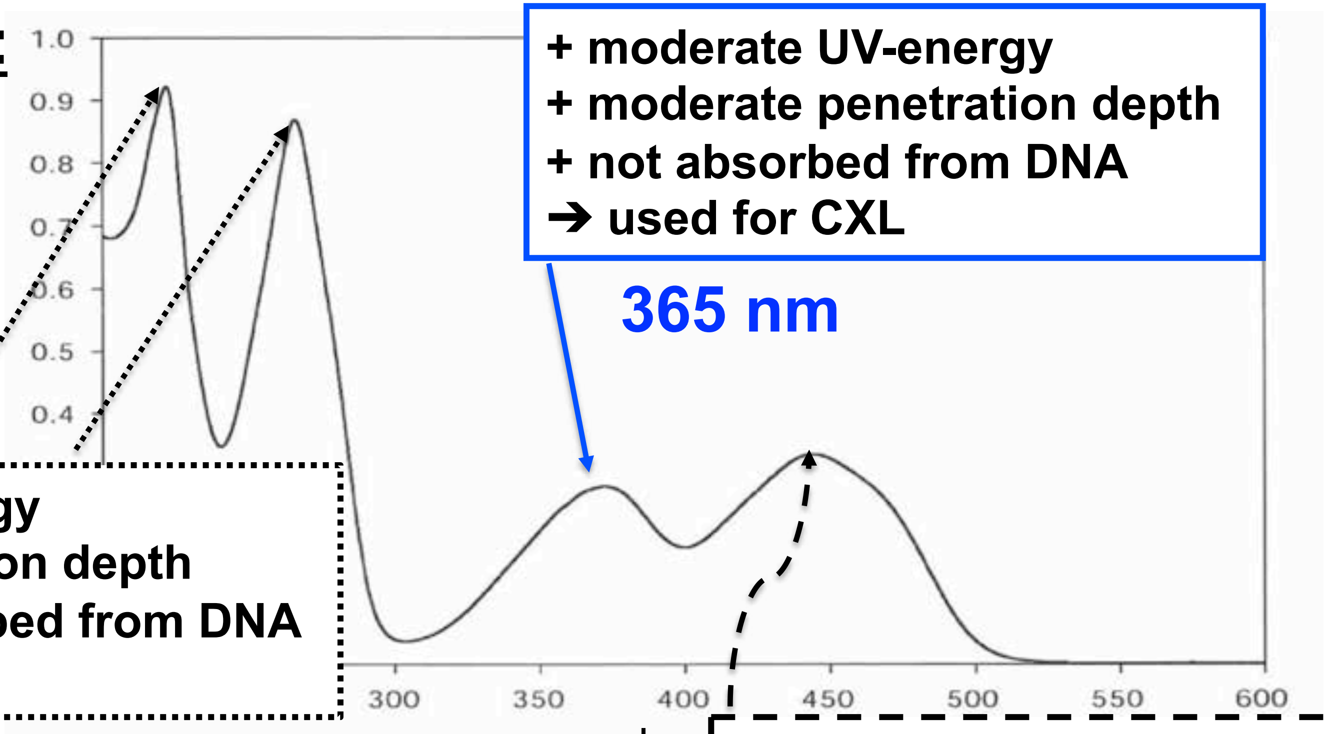
- **Increasing the absorption of UV-light**
- **Photosensitizer excitation**

**Riboflavin:**



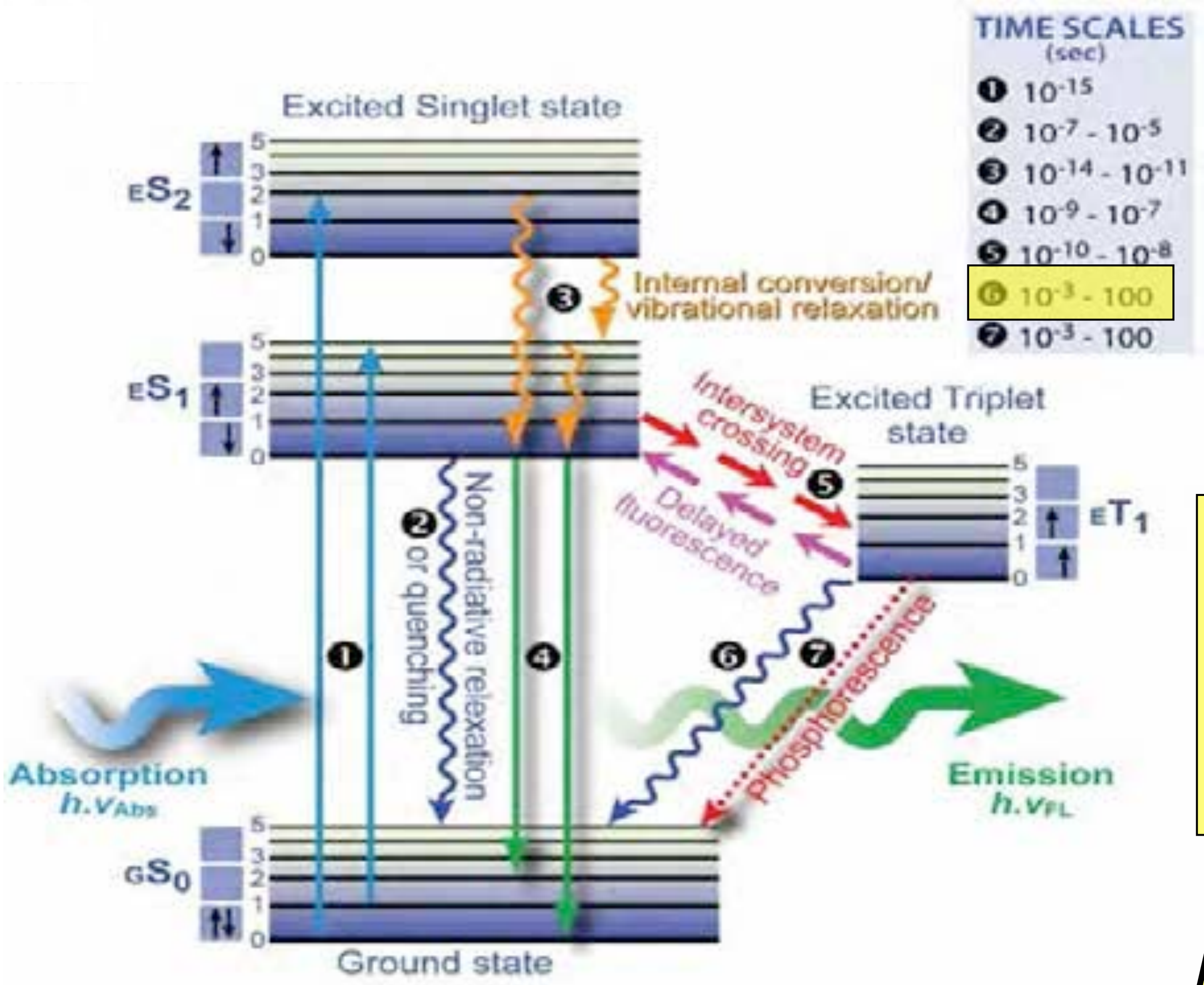

+ moderate UV-energy
+ moderate penetration depth
+ not absorbed from DNA
➔ used for CXL

**365 nm**

+ high UV-energy
- low penetration depth
- highly absorbed from DNA
➔ DNA damage

+ moderate penetration depth
- lower UV-energy
➔ less effective

# Photosensitizer




**Long-lived triplet state: allows for chemical reactions**

**generation of reactive oxygen species**

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# Reaction mechanism

## Type I mechanism:

$$Rf_3^* + SH \rightarrow (Rf^{-\cdot} + SH^{+\cdot}) \rightarrow \boxed{RfH^{\cdot} + S^{\cdot}}$$

$$2RfH^{\cdot} \rightarrow Rf + RfH_2$$

$$RfH_2 + O_2 \rightarrow Rf_{ox} + \boxed{H_2O_2}$$

radicals

**Little oxygen consumption.**

reactive oxygen species

## Type II mechanism:

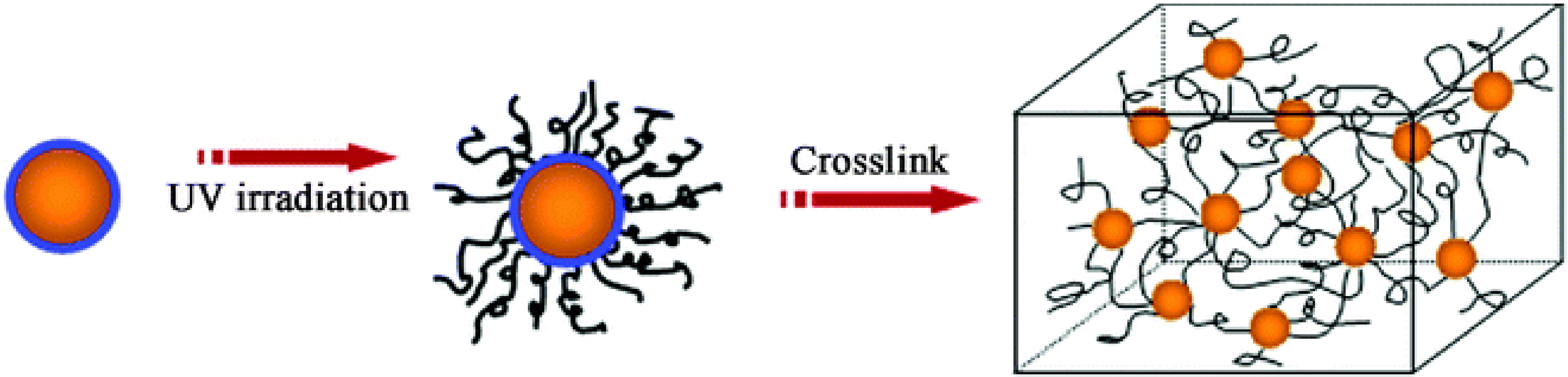
$$Rf_3^* + O_2 \rightarrow \boxed{{}^1O_2}$$

$$SH + {}^1O_2 {}^{\rightarrow S_{ox}}$$

**High oxygen consumption.**

*REF. Kamaev P.*
*IOVS 2012*

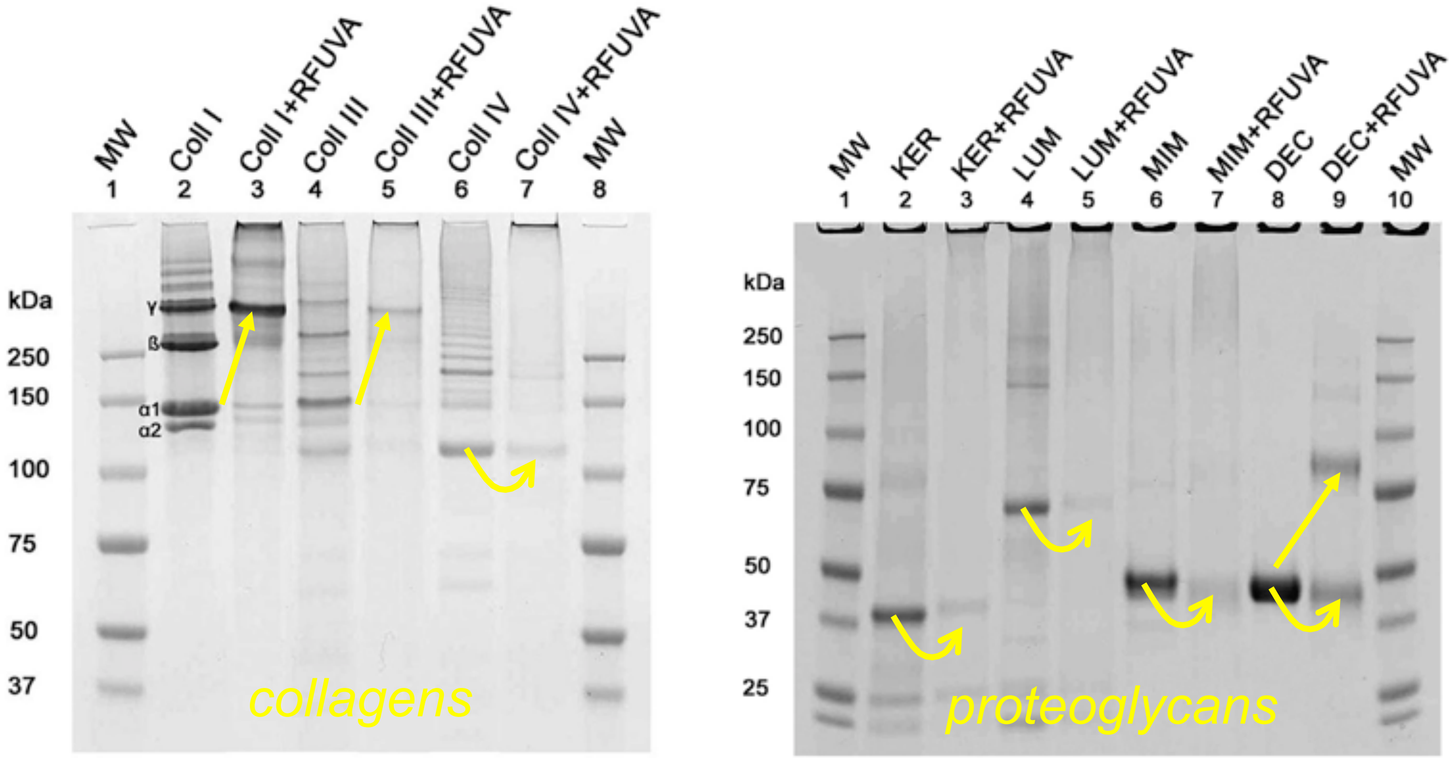
# Oxidation of the extracellular matrix




➔ **Formation of new cross-links**

➔ **Increased mechanical stiffness of the corneal tissue**

# Cross-linking of collagens and / or proteoglycans?




*REF. Zhang Y. J Biol Chem 2011*

**cross-links:**
- **among collagens**
- **among proteoglycans**
- **between collagens and proteoglycans**

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# Riboflavin

- concentration
- osmolality
- application modes
- photo-degradation

# Function of riboflavin

- Generation of radicals in the presence of UV and oxygen

- Protection of the endothelium and crystalline lens from UV damage

# Safety of CXL treatment

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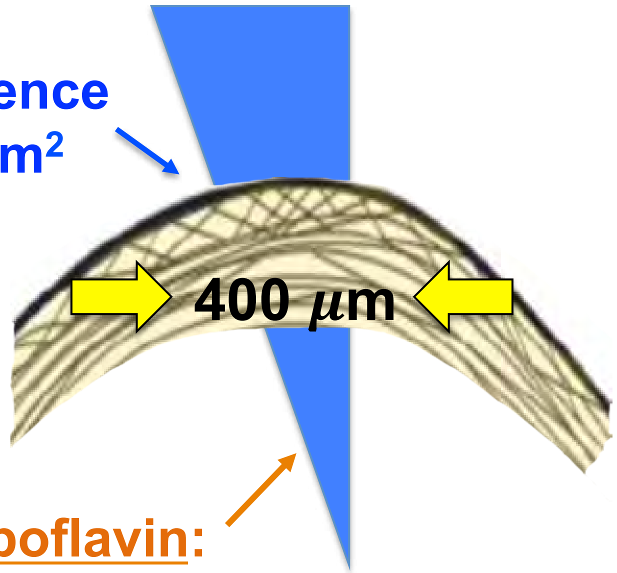
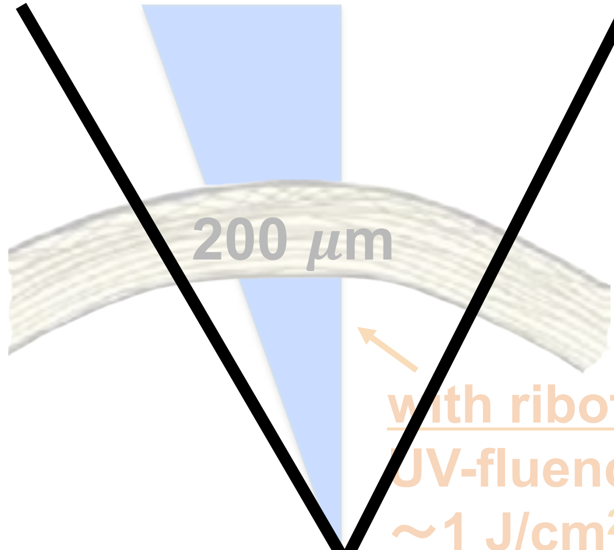
UV-fluence
5.4 J/cm$^2$




with riboflavin:
UV-fluence
$\sim$0.18 J/cm$^2$

**Normal cornea:**

- high UV absorption
➔ protection of the endothelium and crystalline lens




with riboflavin:
UV-fluence
$\sim$1 J/cm$^2$

**Thin cornea:**

- low UV absorption
- higher UV dose at the endothelium

➔ risk of endothelial damage
➔ risk of cataract

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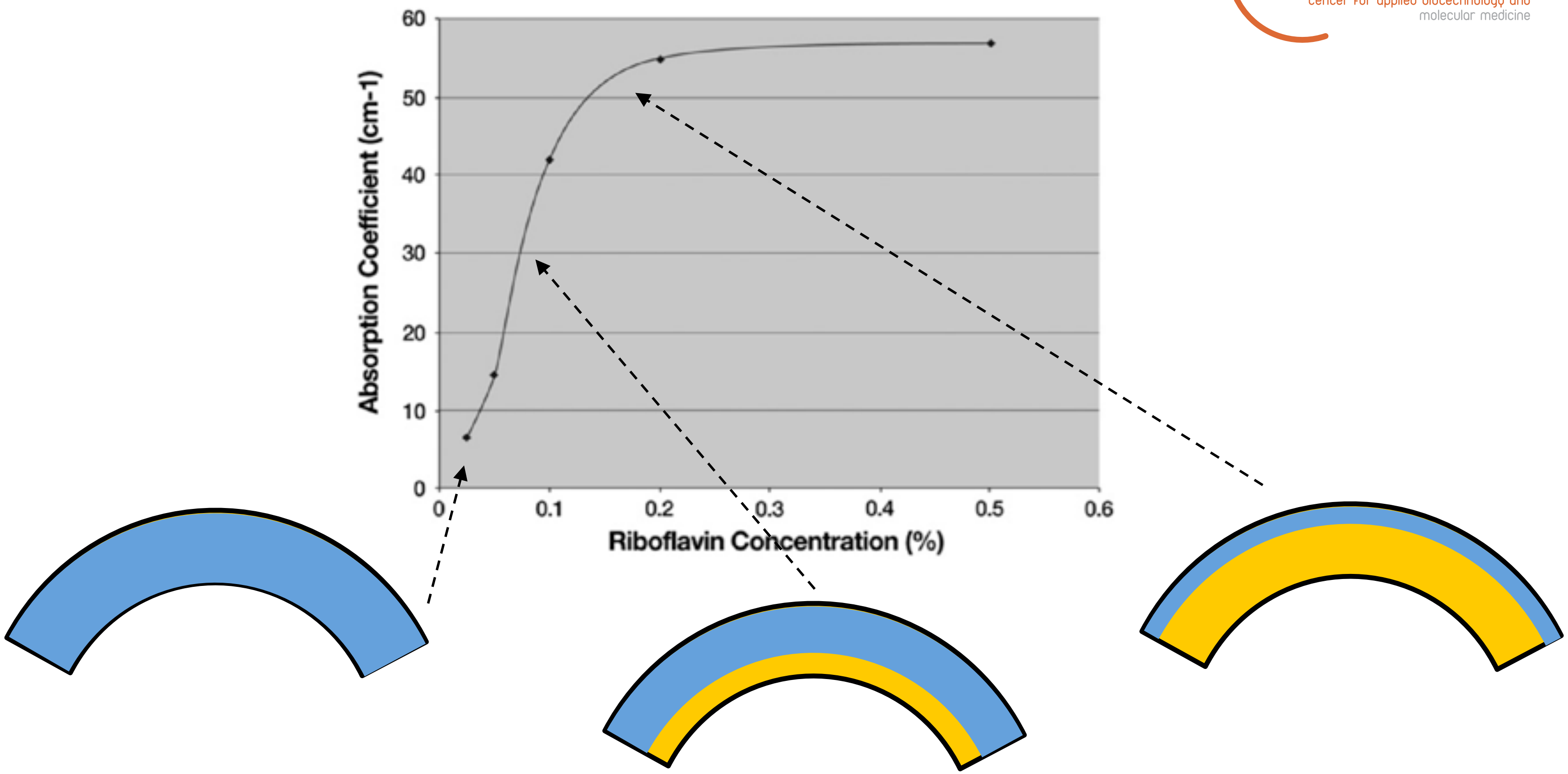
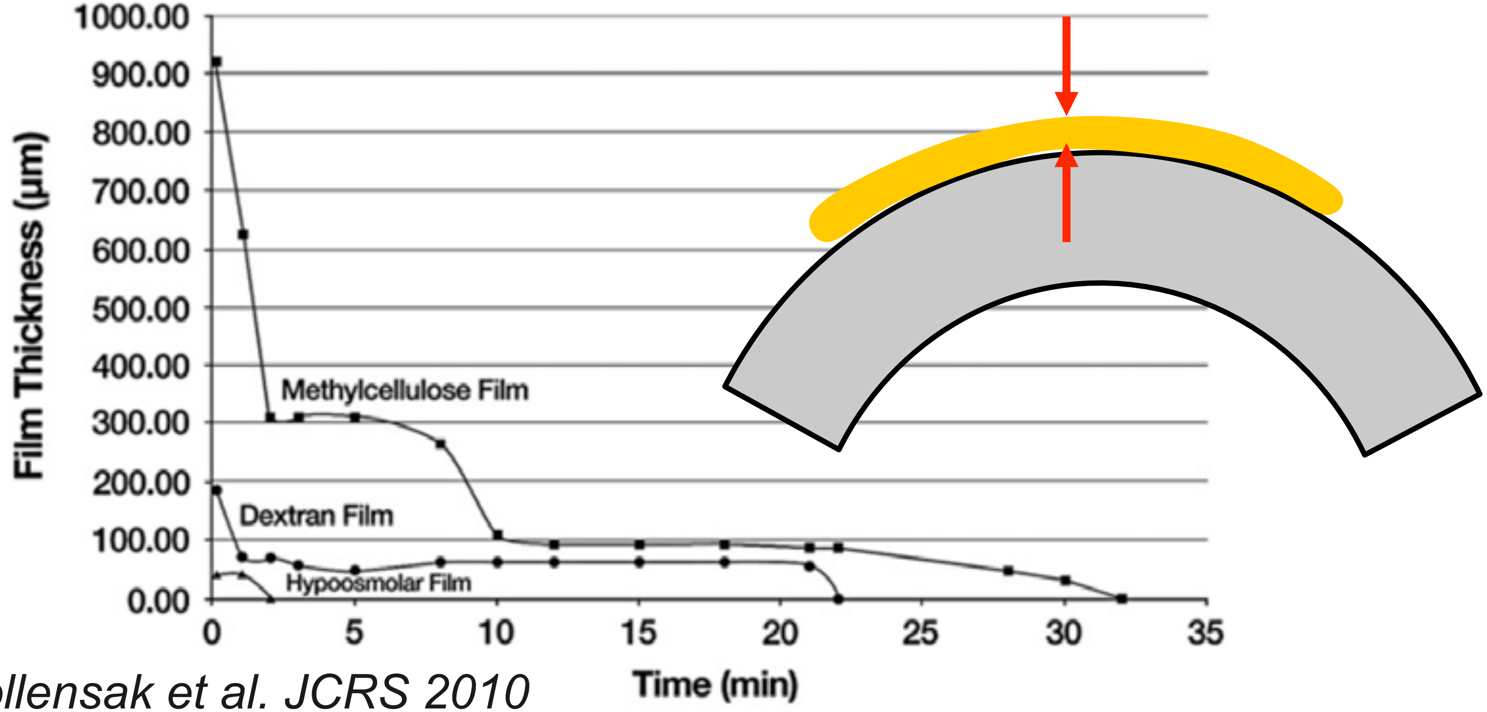
# UV absorption of riboflavin




- The higher the riboflavin concentration, the stronger the UV absorption.
- The stronger the UV absorption, the less penetration into deeper stromal layers.

# Viscosity of the riboflavin solution and dropping frequency




*Wollensak et al. JCRS 2010*

- The higher the viscosity, the thicker the riboflavin film on the corneal surface.
- The thicker the riboflavin film, the less UV energy reaches the corneal stroma.

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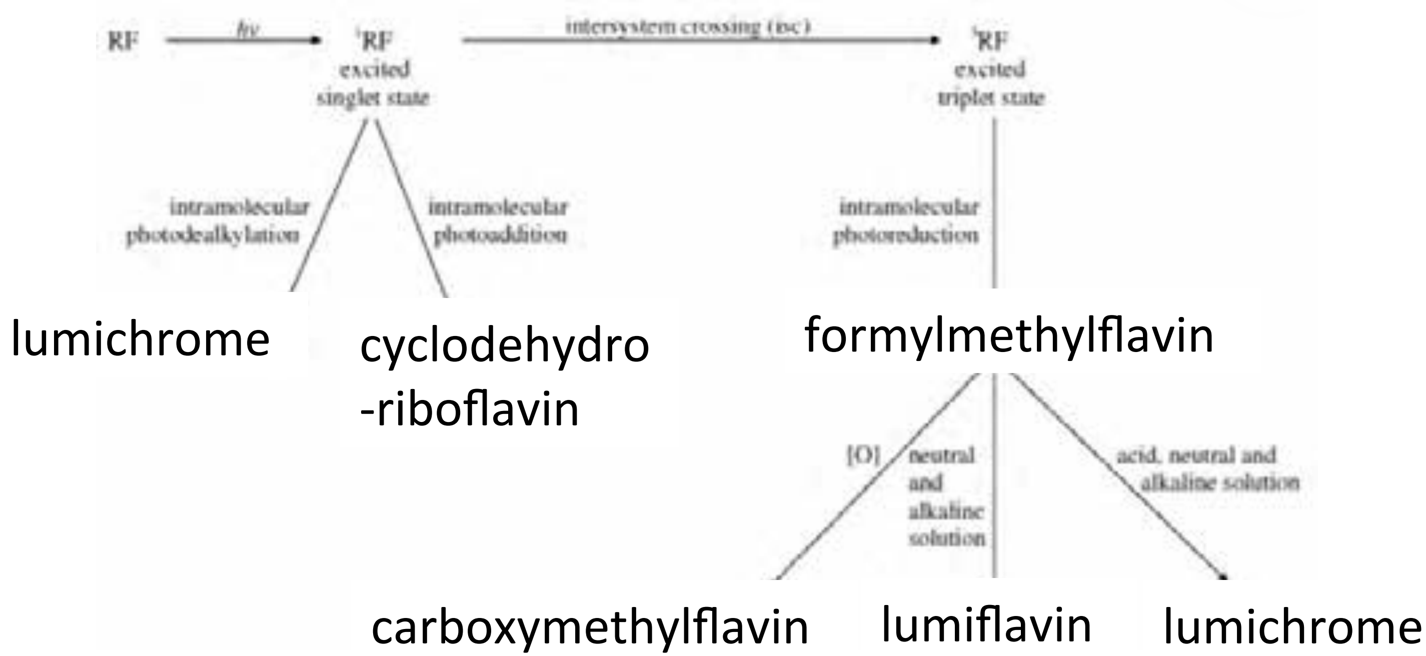
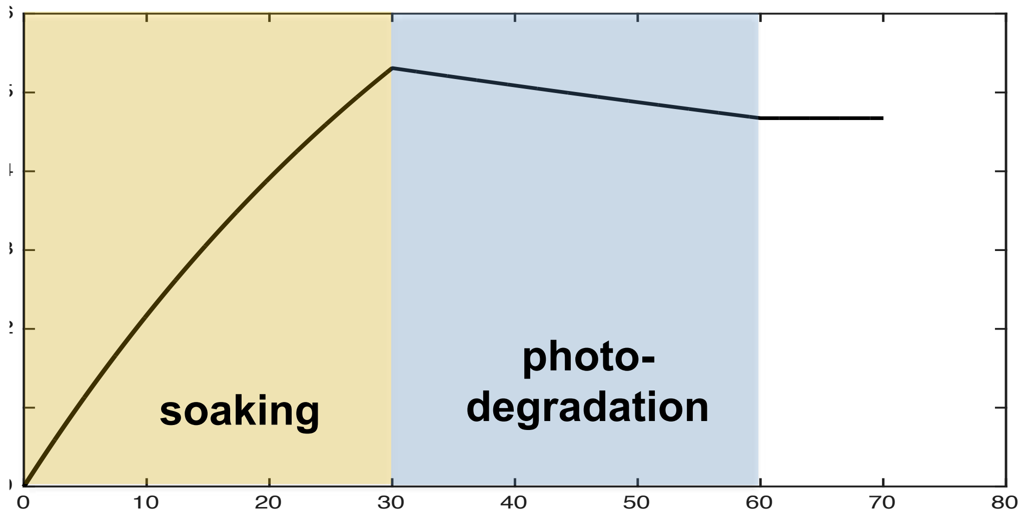
# Photodegradation of riboflavin



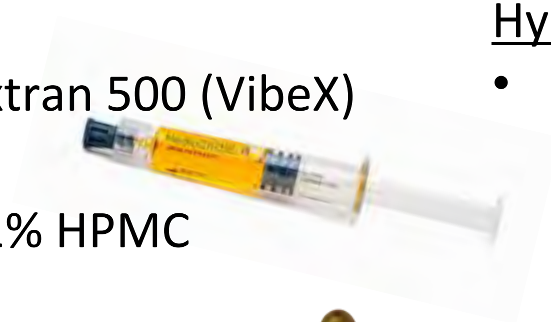



# Different riboflavin solutions

## EPI-OFF

Hypertonic:

- riboflavin 0.1 g, dextran 500 (VibeX)



Isotonic:

- 0.1% Riboflavin, 1.1% HPMC (MedioCROSS D)

Hypoosmolar / hypotonic:

- 0.1% Riboflavin, 1.1% HPMC (MedioCROSS M)
- 0.1% $B_2$-riboflavin-5-phosphate, 0.9% sodium chloride

Accelerated:

- riboflavin 0.1 g, HPMC (VibeX Rapid)

## EPI-ON

Hypoosmolar:

- 0.25% Riboflavin, 1.2% HPMC, 0.01% Benzalkonium Chloride (MedioCROSS TE)

Accelerated:

•0.25% Riboflavin, HPMC, BAC (Paracel)

Iontophoresis:

•0.1% Riboflavin + enhancers (RICROLIN®TE)

# Osmolality

**=> considers total concentration of penetrating solutes *and* non-penetrating solutes**

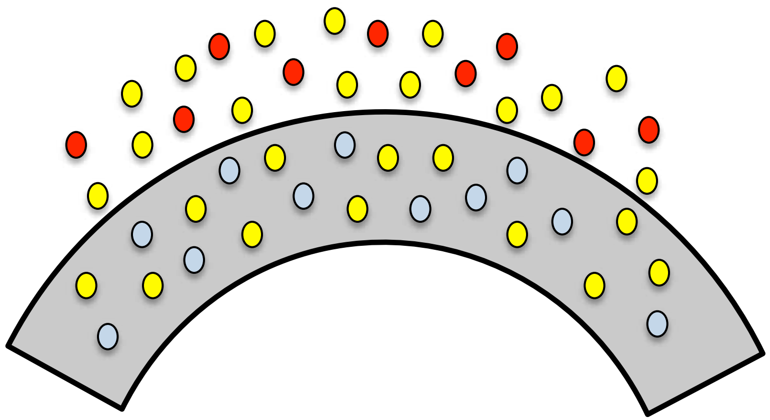
*Osmolality is a property of a particular solution and is independent of any membrane.*

$$\text{osmolarity} = \sum_i \varphi_i \, n_i C_i$$

osmotic coefficient

solute molar concentration

number of particles into which a molecule dissociates



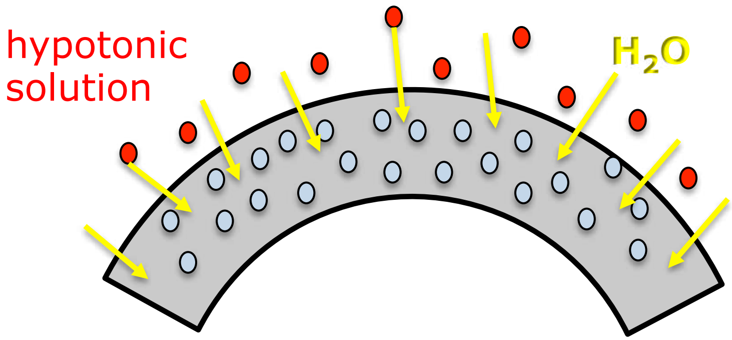
**human cornea = 300 mOsm/L**

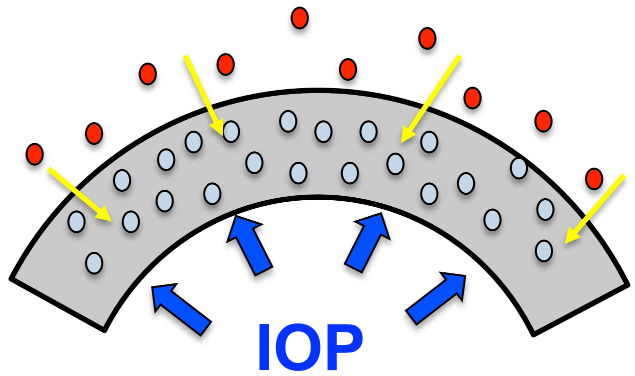
# Tonicity

**=> considers total concentration of *only* non-penetrating solutes**

**=> considers external pressure difference (unilateral pressure)**

*Tonicity is a property of a solution in reference to a particular membrane.*



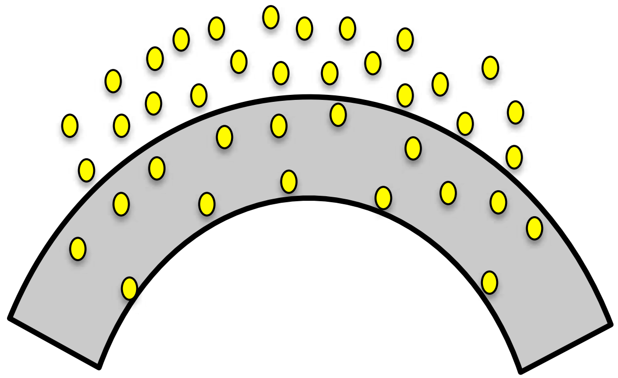

Dextran T500 cannot penetrate the denuded stroma. If prepared as hyperosmolar solution => hypertonic => stromal dehydration

Riboflavin can penetrate the denuded stroma. If prepared as hyposomolar solution => hypotonic => stromal swelling

# Administration modalities

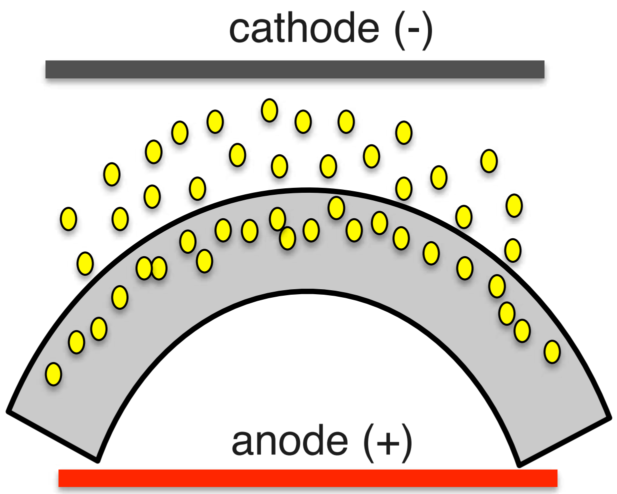
**Dropping in intervals of 3-5 min on the corneal surface**

- Distribution determined by the Fick's law of diffusion



**Iontophoresis-assisted application**

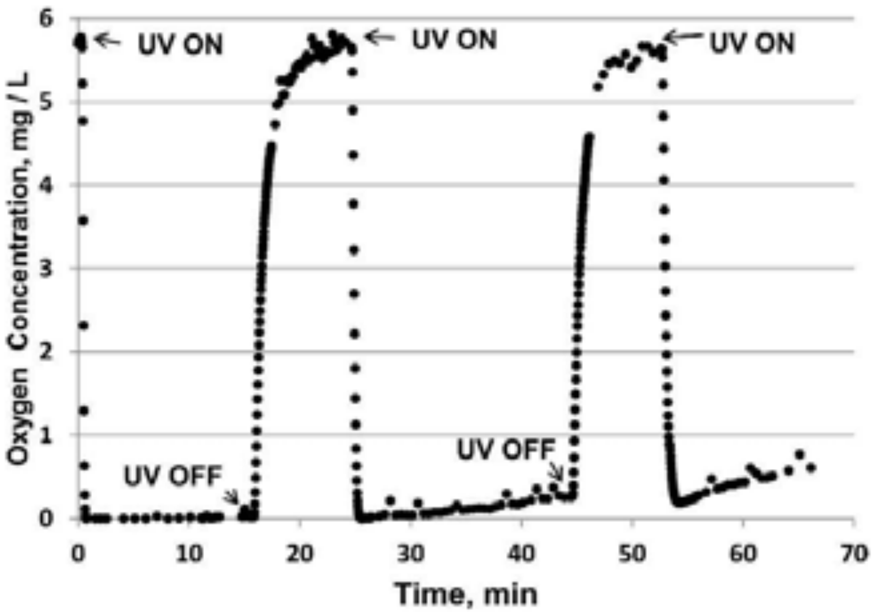
- Distribution determined by the applied voltage



# Oxygen

- availability
- consumption

# Oxygen dependency




*REF. Kamaev P. IOVS 2012*

- **Fast oxygen depletion (UV on):**
  **10-15 seconds (3mW/cm$^2$)**
  **2-5 seconds (30mW/cm$^2$)**

- **Slow oxygen replenishment (UV off):**
  **3-4 minutes**

**No stiffening effect of cross-linking in the absence of oxygen.**

*REF. Richoz O. IOVS 2013*

# Oxygen diffusion into the corneal stroma

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**Fick's law of diffusion:**

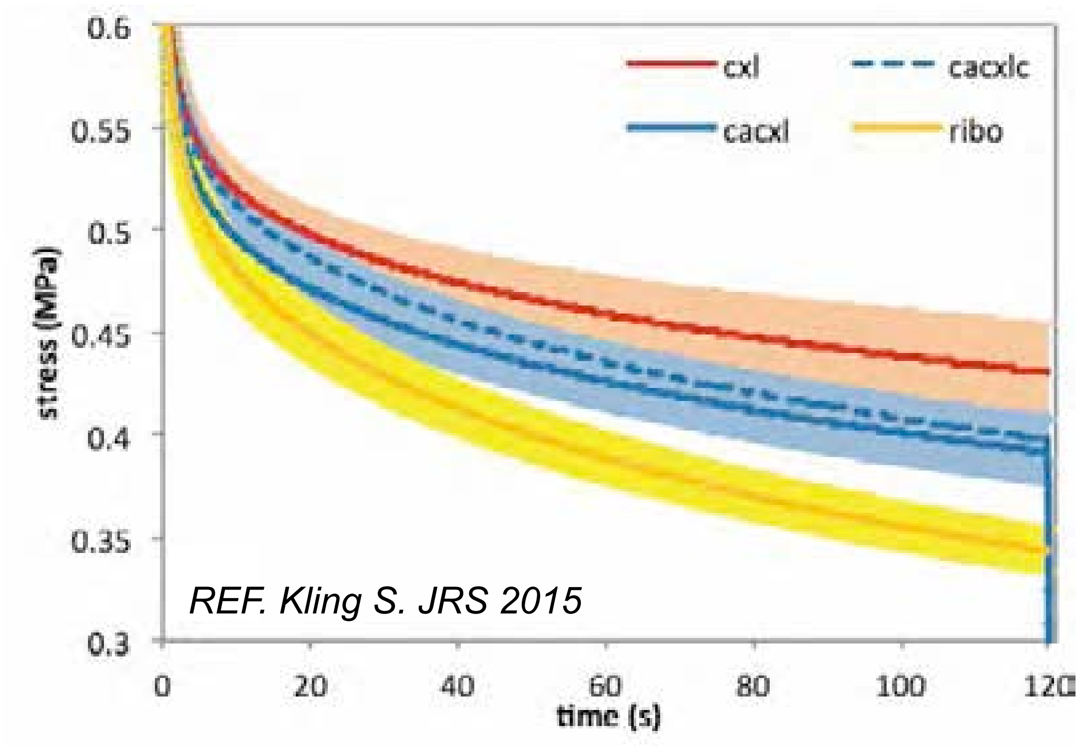
$$\frac{\partial_{concentration}}{\partial_{time}} = -diffusion\ coefficient \cdot \frac{\partial^{2}_{concentration}}{\partial_{distance^{2}}}$$

corneal thickness

Depends on the partial pressure of oxygen in the air and could be modified by increasing/decreasing:

**- atmospheric pressure**
**- percentage of oxygen**

# The effect of oxygen reduction on biomechanical stiffening




*REF. Kling S. JRS 2015*

**Oxygen reduction by -50% leads to a decreased stiffening effect by -50%.**

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# 3 essential components for CXL:

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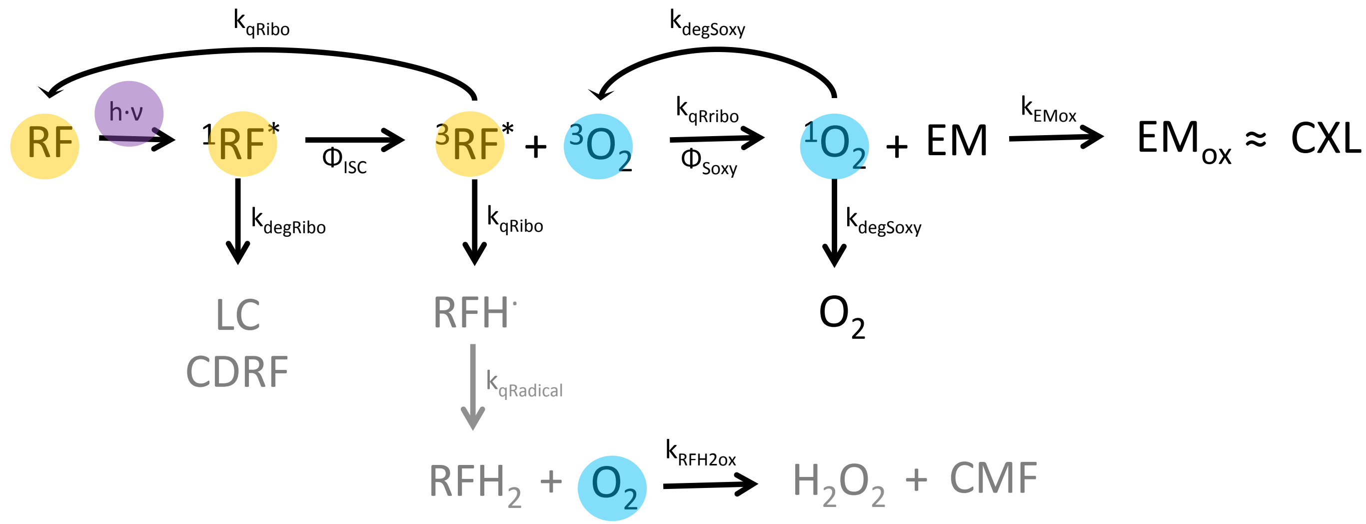
RiboflavinA ✚ UV-lightA ✚ OxygenA

- UV-light absorptionA
- excitation of oxygenA

- excitation of riboflavinA

- oxidation of the extracellular matrixA



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# Clinical implications

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# Accelerating CXL treatment

**Bunsen-Roscoe law of reciprocity**:

*A certain biological effect is*

*directly proportional to the total energy dose,*

*irrespective of the administered regime.*

**corneal stiffening ~ UV fluence (?)**

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irradiation time

# Accelerated CXL

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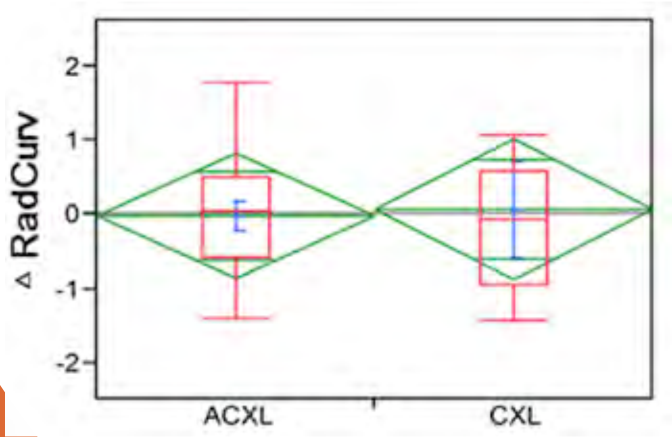
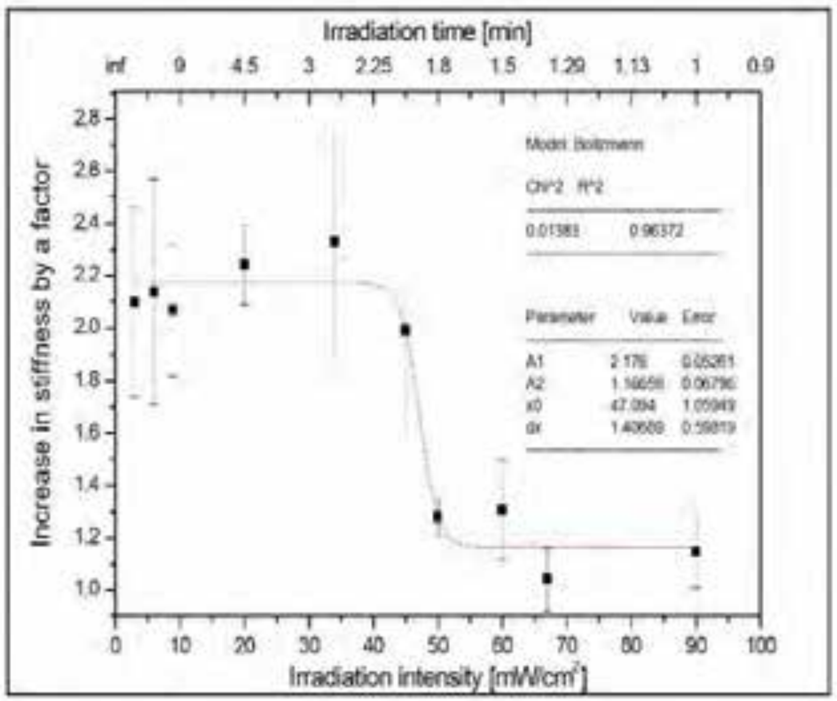
**Idea:** Shorter irradiation time & higher UV irradiance.

**<u>Clinical outcome</u>:**
•efficiently stops keratoconus progression
•*no difference to standard CXL*




*REF. Tomita M. JCRS 2014*

**<u>Experimental quantification</u>:**
•less efficient




*REF. Wernli J. IOVS 2013*

*REF. Hammer A. IOVS 2014*

## oxygen availability

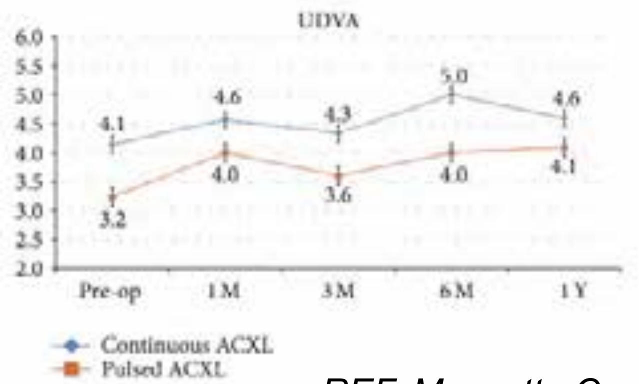
# Pulsed CXL

**<u>Idea:</u> Interrupt UV-irradiation periodically to allow oxygen diffusion into the cornea**

⇨ **Increase the effect of CXL**

**<u>Clinical outcome</u>:**

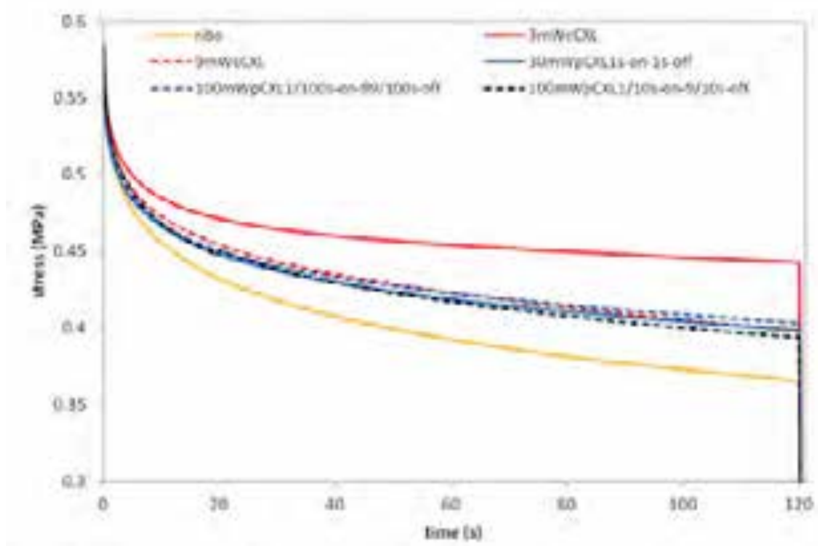
- efficiently stops keratoconus progression
- *no difference to standard CXL*




*REF. Mazzotta C. J Ophthalmol 2014*

**<u>Experimental quantification</u>:**

- less efficient




*REF. Kling S. ESCRS 2015*

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oxygen availability

# Trans-epithelial CXL

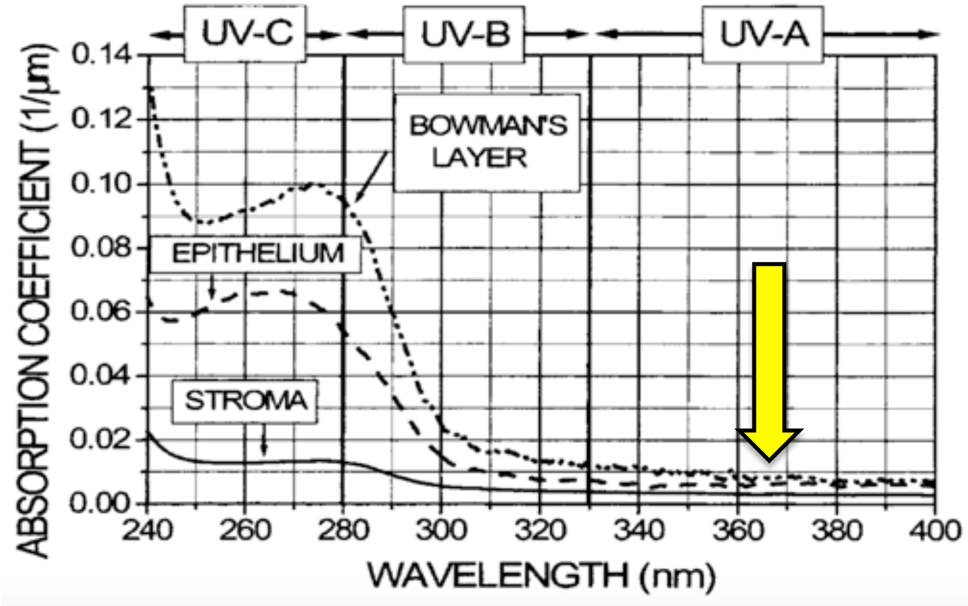
- **is less effective than standard CXL**

*REF. Leccisotti A. JRS (2010)*

**Similar UV absorption and oxygen consumption in epithelial cells and stroma.**

**<u>however</u>:**

**no stiffening effect in the epithelium**




*REF. Kolozsvari L. IOVS (2002)*

**➔ comparable to standard CXL in a deeper stromal layer**

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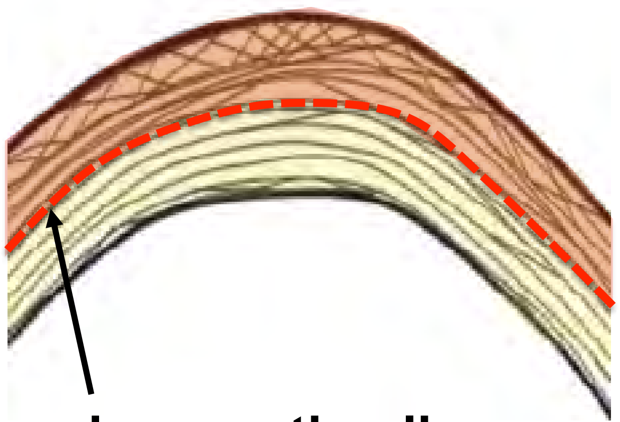
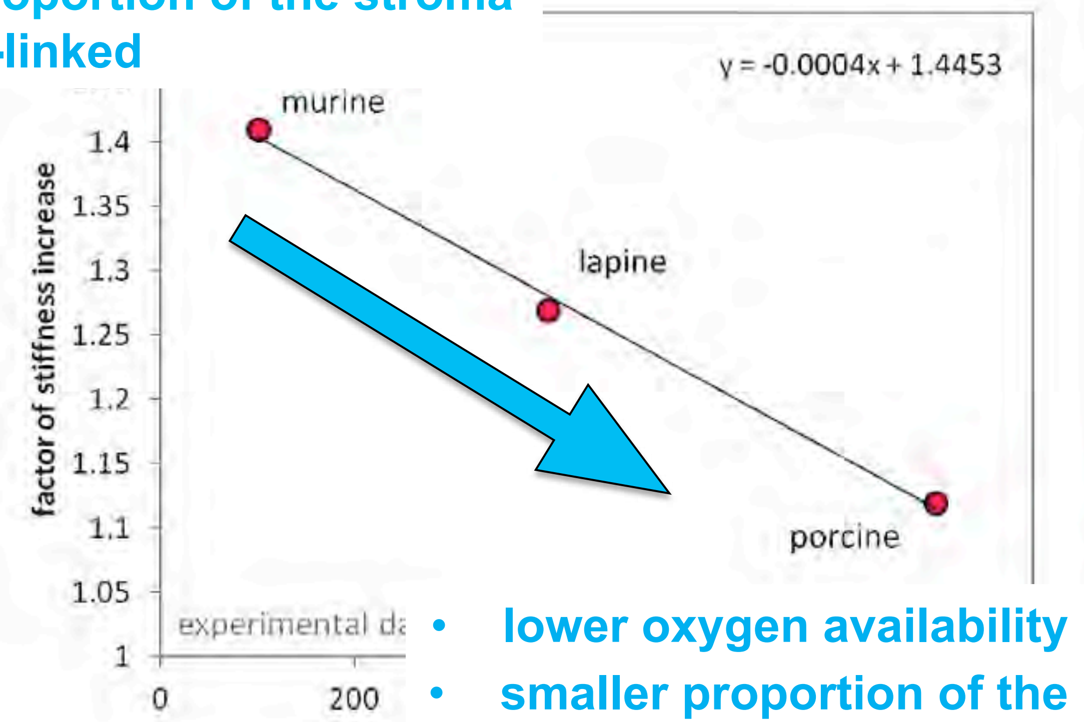
# CXL efficacy as a function of corneal thickness

- higher oxygen availability
- larger proportion of the stroma is cross-linked



demarcation line




- lower oxygen availability
- smaller proportion of the stroma is cross-linked

➔ **stronger CXL in thinner corneas**

UV fluence

# Complications of CXL in thin corneas

**100 $\mu$m murine cornea**
treated with 5.4 J/cm$^2$

*REF. Kling S.*
*CXL congress (2014)*

**404$\mu$m human cornea**
treated with 5.4 J/cm$^2$

*REF. Soeters N. J Pediatr*
*Ophthalmol Strabismus (2011)*

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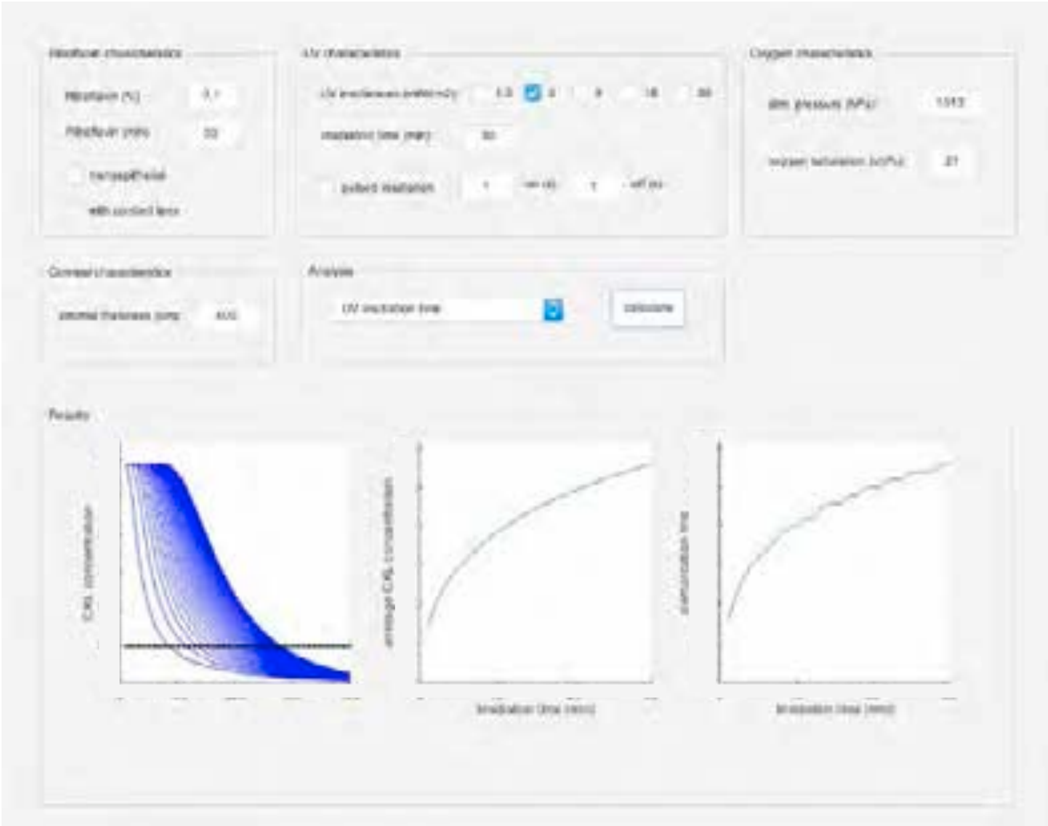
# A model to predict the stiffening effect of CXL

Tomorrow



Patient-specific adaptation of CXL treatment parameters

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# Conclusions

- CXL requires 3 main components: photosensitizer (riboflavin), UV-light (365nm, 5.4J/cm$^2$) and oxygen.
- Riboflavin is required to initiate the generation of additional cross-links – but also to protect the endothelium and crystalline lens from damage.
- Oxygen is rapidly consumed during CXL. Current protocols are limited by the speed of oxygen diffusion into the stroma.
- CXL protocols may be *clinically* effective, even if they have a reduced *experimental* stiffening effect.

# Thank you

# for your attention

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