Riboflavin, Oxygen and Light

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Corneal Cross-linking

Increasing corneal stiffness to stop the progression of keratoconus

de-epithelialization

365 nm, 3mW/cm²

30 min UV-light

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)

Advantages of photo-chemically induced cross-linking

- no DNA mutations due to radiation
- no health hazard due to chemicals
- penetration depth is adjustable
Selection of the photosensitizer and wavelength
Selection of wavelength and photosensitizer

- Increasing the absorption of UV-light
- Photosensitizer excitation

**Riboflavin:**

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

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

365 nm

+ moderate penetration depth
- lower UV-energy
→ less effective
Photosensitizer

Long-lived triplet state: allows for chemical reactions

generation of reactive oxygen species
Reaction mechanism

**Type I mechanism:**

\[ Rf_3^* + SH \rightarrow (Rf^- + SH^+) \rightarrow RfH^+ + S \]

\[ 2RfH^- \rightarrow Rf + RfH_2 \]

\[ RfH_2 + O_2 \rightarrow Rfox + H_2O_2 \]

Little oxygen consumption.

**Type II mechanism:**

\[ Rf_3^* + O_2 \rightarrow ^1O_2 \]

\[ SH + ^1O_2 \rightarrow ^{ox}S \]

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?

Cross-links: • among collagens
• among proteoglycans
• between collagens and proteoglycans

REF. Zhang Y. J Biol Chem 2011
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

UV-fluence 5.4 J/cm²

with riboflavin:
UV-fluence ~0.18 J/cm²

Normal cornea:
• high UV absorption ➔ protection of the endothelium and crystalline lens

Thin cornea:
• low UV absorption
• higher UV dose at the endothelium ➔ risk of endothelial damage ➔ risk of cataract
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.
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.

Wollensak et al. JCRS 2010
Photodegradation of riboflavin

- lumichrome
- cyclodehydro-riboflavin
- formylmethylflavin
- carboxymethylflavin
- lumiflavin
- lumichrome

Diagram showing the photodegradation process:
- RF (riboflavin) absorbs light (hv).
- Excited state intersystem crossing.
- Triplet state.
- Various photodegradation pathways:
  - Intramolecular photodealkylation
  - Intramolecular photoaddition
  - Intramolecular photoreduction

Graph showing:
- Soaking and photodegradation over time.

Universität Zürich
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% B₂-riboflavin-5-phosphate, 0.9% sodium chloride

**EPI-ON**

- **Accelerated:**
  - 0.25% Riboflavin, HPMC, BAC (Paracel)

- **Iontophoresis:**
  - 0.1% Riboflavin + enhancers (RICROLIN®TE)
Osmolality

\[ \text{osmolality} = \sum \varphi_i n_i C_i \]

\[ \text{osmotic coefficient} \]
\[ \text{solute molar concentration} \]
\[ \text{number of particles into which a molecule dissociates} \]

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

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

- Fast oxygen depletion (UV on):
  10-15 seconds (3mW/cm²)
  2-5 seconds (30mW/cm²)

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

REF. Kamaev P. IOVS 2012

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

REF. Richoz O. IOVS 2013
Oxygen diffusion into the corneal stroma

Fick’s law of diffusion:

\[
\frac{\partial \text{concentration}}{\partial \text{time}} = -\text{diffusion coefficient} \cdot \frac{\partial^2 \text{concentration}}{\partial \text{distance}^2}
\]

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

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

REF. Kling S. JRS 2015
3 essential components for CXL:

- UV-light absorption
- excitation of riboflavin
- oxidation of the extracellular matrix

\[
\begin{align*}
\text{Riboflavin} & \quad + \quad \text{UV-light} & \quad + \quad \text{Oxygen} \\
\text{RF} & \quad \rightarrow \quad 1 \text{RF}^+ & \quad \rightarrow \quad 3 \text{RF}^+ & \quad \rightarrow \quad 3 \text{O}_2 & \quad \rightarrow \quad 1 \text{O}_2 & \quad + \quad \text{EM} & \quad \rightarrow \quad \text{EM}_{\text{ox}} \approx \text{CXL} \\
& \quad \quad \quad \quad k_{\text{qRibo}} & \quad \quad \quad \quad \Phi_{\text{ISC}} & \quad \quad \quad \quad k_{\text{degRibo}} & \quad \quad \quad \quad k_{\text{qRibo}} & \quad \quad \quad \quad \Phi_{\text{Soxy}} & \quad \quad \quad \quad k_{\text{degSoxy}} & \quad \quad \quad \quad k_{\text{EMox}} \\
& \quad \quad \quad \quad \text{LC} & \quad \quad \quad \quad \text{CDRF} & \quad \quad \quad \quad \text{RFH}^+ & \quad \quad \quad \quad \text{O}_2 & \quad \quad \quad \quad \text{O}_2 & \quad \quad \quad \quad \text{EM} \\
& \quad \quad \quad \quad k_{\text{qRadical}} & \quad \quad \quad \quad \text{RFH}_2 & \quad \quad \quad \quad \text{O}_2 & \quad \quad \quad \quad \text{H}_2\text{O}_2 & \quad \quad \quad \quad \text{CMF} \\
\end{align*}
\]
Clinical implications
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 (?)
Accelerated CXL

Idea: Shorter irradiation time & higher UV irradiance.

Clinical outcome:
• efficiently stops keratoconus progression
• no difference to standard CXL

Experimental quantification:
• less efficient

REF. Wernli J. IOVS 2013
REF. Hammer A. IOVS 2014

REF. Tomita M. JCRS 2014
Oxygen availability

Pulsed CXL

Idea: Interrupt UV-irradiation periodically to allow oxygen diffusion into the cornea

→ Increase the effect of CXL

Clinical outcome:
• Efficiently stops keratoconus progression
• No difference to standard CXL

Experimental quantification:
• Less efficient

REF. Mazzotta C. J Ophthalmol 2014

REF. Kling S. ESCRS 2015
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.

however:
no stiffening effect in the epithelium

➔ comparable to standard CXL in a deeper stromal layer

REF. Kolozsvari L. IOVS (2002)
CXL efficacy as a function of corneal thickness

- Higher oxygen availability
- Larger proportion of the stroma is cross-linked

→ Stronger CXL in thinner corneas
Complications of CXL in thin corneas

100 μm murine cornea treated with 5.4 J/cm²

404 μm human cornea treated with 5.4 J/cm²

REF. Kling S. CXL congress (2014)

A model to predict the stiffening effect of CXL

Patient-specific adaptation of CXL treatment parameters
Conclusions

• CXL requires 3 main components: photosensitizer (riboflavin), UV-light (365nm, 5.4J/cm²) 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