Basics of cross-linking
Fluence, illumination, beam profile

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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)
  light energy per unit area
  example: 5.4J/cm\(^2\) to 10J/cm\(^2\)

- Continuous irradiation

- Pulsed irradiation:
  example: 1s on/1s off; 10s on 10s off
Absorption spectrum of Riboflavin (Vitamin B2)

According to the absorption peak the wavelength was chosen to 365-370 nm
Light-Emitting Diodes with 370 nm

Homogeneous illumination

A clinically used light source must guarantee a perfect homogeneity of the irradiance.

Hot spots may cause localized endothelium cell damage, especially in thin corneas.
Limited area of irradiation

- Only the cornea is irradiated, sclera and limbus are not treated.
- Due to the fluorescence the irradiated area is visible.
## Irradiation of Limbus?

<table>
<thead>
<tr>
<th>safe</th>
<th>damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>rabbit limbal epithelial cells</td>
<td>human limbal epithelial cells</td>
</tr>
<tr>
<td>double-standard fluence 10.8 J/cm²</td>
<td>reduced cell expansion</td>
</tr>
<tr>
<td>no changed regenerative capacity</td>
<td>reduced regenerative capacity</td>
</tr>
<tr>
<td>eccentric CXL may be performed safely in PMD</td>
<td>induced apoptosis,</td>
</tr>
</tbody>
</table>

*Richoz O et al. The effect of standard and high-fluence CXL on cornea and limbus. IOVS 2014;55:5783-7*


Avoid riboflavin+UVA irradiation on the limbus during CXL.
Irradiation and distance

- Important is the fluence at the corneal surface.
- Adjust the recommended distance
Accelerated CXL

• Accelerated CXL = shorter treatment time (no information about irradiance)

• Increase UVA intensity and reduce irradiation time while maintaining the total amount of fluence (5.4 J/cm²)

• Optimize the beam profile according to the corneal thickness distribution
Different types of CXL treatments regarding the irradiation devices

Aim: reduced time and increased efficiency

<table>
<thead>
<tr>
<th>Low fluence &lt;5.4J/cm²</th>
<th>Standard fluence 5.4 J/cm²</th>
<th>high fluence &gt;5.4 J/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>low fluence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2J/cm² - 5.4J/cm²</td>
<td>Standard CXL</td>
<td>high fluence</td>
</tr>
<tr>
<td>thin corneas</td>
<td>3 mW/cm² 30 min</td>
<td>7.2J/cm² - 10J/cm²</td>
</tr>
<tr>
<td></td>
<td>fluence 5.4 J/cm²</td>
<td>customized CXL</td>
</tr>
</tbody>
</table>

High intensity accelerated CXL
(same fluence of 5.4 J/cm²)

**Bunsen-Roscoe reciprocity law**
- 9 mW/cm² 10 min
- 18 mW/cm² 5 min
- 30 mW/cm² 3 min

Pulsed accelerated CXL
- 30 mW/cm² 6 min
- Fluence 5.4J/cm²

Pulsed accelerated CXL with high fluence
- 30 mW/cm² 8 min
- Fluence 7.2J/cm²
Safety aspects

Homogenous UVA light

- Diameter: 8-9 mm
- $I_o = 3 \text{ mW/cm}^2$

$I_e < 0.35 \text{ mW/cm}^2$
(toxic threshold of endothelial cells)

High absorption

- Removal of epithelium
- High concentration of riboflavin in stroma (0.1% and 20 min)
- Thickness of stroma >400 µm
Devices with higher irradiance
(second generation of cross-linking devices)

5 mW/cm²
Kera-X
Brasil

30 mW/cm²
KXL (Avedro)
USA

18 mW/cm²
CCL-365 (Peschke)
Switzerland

18 mW/cm²
Apollon
Turkey

9 mW/cm² 10 min
UV-X (IROC)
Avedro (USA)

10 mW/cm² 9 min
CBM Vega
Italien

CX-100 China
Beam profile

Higher energy in the periphery for deeper corneal stromal penetration - higher efficacy? same treatment: time 10 min

Different demarcation lines but same clinical effect  (Herber, 2015)

Mrochen et al. 2014
### keratoconus

<table>
<thead>
<tr>
<th>Local change of the cornea</th>
<th>Change of the whole cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>local epithelium changes</td>
<td>Thinning of peripherial lamellae</td>
</tr>
<tr>
<td>thinnest point</td>
<td>Corneal thickness is also thinner in periphery</td>
</tr>
<tr>
<td>Breaks in Bowman membrane</td>
<td>Genetic component</td>
</tr>
<tr>
<td>local biomechanical changes</td>
<td></td>
</tr>
</tbody>
</table>
Customized beam (fluence-time) profile

Profile consist of concentric superposition of 3 circular areas
Only the apex is irradiated.

Seiler TG, Fischinger I, Koller T, Zapp D, Frueh BE, Seiler T.
Customized fluence

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

• pulsed high intensity CXL
  (7.2J/cm²; 30mW/cm²; 8 min; 10s/10s)

• high intensity CXL
  (7.2J/cm²; 30mW/cm²; 4 min)

• standard CXL
  (5.4J/cm²; 3mW/cm²; 30 min)

• high intensity CXL
  (5.4J/cm²; 30mW/cm²; 3 min)

Aldahlawi NH. Enzymatic resistance of corneas crosslinked using riboflavin in conjunction with low energy, high energy, and pulsed UVA irradiation modes. IOVS 2016;57:1547-52
Demarcation line

Demarcation line represents the transition zone between acellular (treated) and cellular (untreated) corneal stroma. It is a tool for assessment of extent of CXL (not proven, only postulated)

Standard CXL 3 mW/cm² 30 min

Accelerated CXL 9 mW/cm² 10 min

# Depth of demarcation line

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth in µm</th>
<th>Irradiance</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard CXL</td>
<td>350.8±49.3</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td>Hyposomolar Riboflavin</td>
<td>262.9±45.6</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td>Accelerated CXL</td>
<td>288.5±42.4</td>
<td>9 mW/cm²</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>160 (150-180)</td>
<td>30 mW/cm²</td>
<td>4 min</td>
</tr>
<tr>
<td>Pulsed CXL</td>
<td>200 (190-215)</td>
<td>30 mW/cm²</td>
<td>8 min</td>
</tr>
<tr>
<td>Transepithelial CXL (BAC)</td>
<td>150</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td>Iontophoresis CXL (without compensation)</td>
<td>212±36</td>
<td>10 mW/cm²</td>
<td>9 min</td>
</tr>
</tbody>
</table>


Kymionis. Corneal stroma demarcation line after standard and high-intensity collagen cross-linking determined with anterior segment optical coherence tomography. *JCRS* 2014;40:736–40
Depth of demarcation line for several CXL techniques

Demarcation depth = 300 - 0.12 × (irradiance)^2

Standard deviation = 40 µm
Summary

• Determine the irradiation, the irradiation time, adjust the recommended distance
• Do not irradiate the limbus
• The depth of the demarcation line as a measure of the CXL effect depends on the irradiation time.
Many thanks for your attention