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Within molecules (intra-molecular) 
Collagen molecule-collagen molecule at fibril surface (inter-molecular) 
Proteoglycan-collagen molecule (fibril surface) 
Within proteoglycan core proteins 
Proteoglycan core protein-proteoglycan core protein

Schematic of three collagen fibrils showing the likely location of riboflavin/UVA-induced cross-links

Hayes et al. 2013. PLoS One. 8 (1), e52860
Method

- Porcine eyes are obtained from the abattoir within ~ 4 hrs of death.
- Randomly divided into cross-linked and non-cross-linked treatment groups.
- After treatment, the cornea is removed and an 8mm disk trephined from the centre.

- Corneal disks are placed in 5ml pepsin digest solution (1g of 500 U/mg pepsin from porcine gastric mucosa in 10ml 0.1 M HCL at pH 1.4) and incubated at 23°C.
- Corneal disk diameter is measured daily until the point of complete digestion.
- Some corneal disks may be removed midway through the digestion process to obtain dry weight measurements.
<table>
<thead>
<tr>
<th>6 porcine eyes/group</th>
<th>Average time for complete digestion to occur (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>11</td>
</tr>
<tr>
<td>Dextran 20%</td>
<td>11</td>
</tr>
<tr>
<td>Riboflavin (with 20% dextran)</td>
<td>10</td>
</tr>
<tr>
<td><strong>Riboflavin (with 20% dextran) + 3 mW UVA for 30 minutes (SCXL 3 mW)</strong></td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>

**Diagram:**
- **Y-axis:** Total corneal disk diameter (mm)
- **X-axis:** Normalized digestion time
- **Legend:**
  - **Black:** Untreated
  - **Gray:** Dextran only
  - **Orange:** Riboflavin only
  - **Red:** SCXL 3mW
11 porcine eyes/ group

<table>
<thead>
<tr>
<th></th>
<th>Average time for complete digestion (days)</th>
<th>Average dry weight (undigested tissue mass) at day 12 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Riboflavin+ 3 mW UVA 30 mins (SCXL 3 mW)</td>
<td>25</td>
<td>0.0041</td>
</tr>
<tr>
<td>Riboflavin + 9 mW UVA for 10 mins (ACXL 9 mW)</td>
<td>25</td>
<td>0.0020</td>
</tr>
<tr>
<td>Riboflavin+ 18 mW UVA for 5 mins (ACXL 18 mW)</td>
<td>25</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

SCXL 3mW > ACXL 9mW > ACXL 18mW

Same diameter but lower undigested tissue mass suggests shallower depth of cross-linking after ACXL.

Also see a shallower demarcation line after ACXL (Kymionis et al. 2014).

Aldahlawi et al. 2015 JCRS
During high intensity cross-linking the use of pulsed UVA increases the enzymatic resistance of the cornea. Also see a deeper demarcation line after pulsed high intensity cross-linking (Mazotta et al. 2014).
Conclusions

• The intensity and depth of cross-linking varies with different protocols.

• High intensity/same energy protocols result in a shallower depth of cross-linking, possibly due to a more rapid oxygen consumption.

• High intensity/high energy protocols result in more cross-linking in the anterior-most stroma but the depth of cross-linking may be shallower.

• Pulsing UVA during high intensity/high energy procedures can increase the enzymatic resistance of the cornea by increasing oxygen availability.

• The amount of cross-linking needed to stop keratoconus progression is not yet known.
Acknowledgements

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