Biomechanical changes in human corneas after repeated collagen cross-linking treatment measured using scanning acoustic microscopy

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Aims

• To investigate the stiffness of eye-banked human corneas after applying cross-linking treatment.

• To compare the change in speed of sound after repeated treatment.

• To assess the change in speed of sound after cross-linking on different depths.
Materials & Methods

- 30 human corneas (10 male and 5 female donors)
- divided into three groups:
  - Group A: 5 corneas cross-linked once
    - Epithelium removed, 0.1% riboflavin applied for 30 minutes. UVA (365nm 3mW/cm²) applied for 30 minutes along with riboflavin
  - Group B: 5 corneas treated twice, 24 hours apart
  - Group C: 5 corneas treated three times, 24 hours apart
- Contralateral controls treated similarly, but without UVA
- The speed of sound, which is related to stiffness, was assessed using SAM

Figure 1 UVA/riboflavin irradiation on the human cornea mounted on a Baron anterior chamber.
Scanning Acoustic Microscope

- Main components:
  - transducer (sapphire rod with a zinc oxide piezoelectric film)
  - an acoustic lens
  - Coupling fluid
  - Z-stage

Figure 2 (A). Schematic diagram of scanning acoustic microscope (SAM). Sound wave generated in the machine propagates through the lens, medium and specimen generating reflections from the three interfaces. The contrast observed in SAM images contains phase information from the recombination of these signals. (B). An image of SAM 2000.
## Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Cornea</th>
<th>Speed of sound of anterior cornea mean ± SE (ms⁻¹)</th>
<th>Speed of sound of posterior cornea mean ± SE (ms⁻¹)</th>
<th>Increase factor</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>CXL</td>
<td>1677.38 ± 10.70</td>
<td>1603.90 ± 9.82</td>
<td>1.046</td>
<td>p=0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1595.23 ± 9.66</td>
<td>1577.13 ± 8.16</td>
<td>1.011</td>
<td>p=0.190</td>
</tr>
<tr>
<td>Group B</td>
<td>CXL</td>
<td>1746.33 ± 23.37</td>
<td>1631.60 ± 18.92</td>
<td>1.07</td>
<td>p=0.005</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1637.57 ± 22.15</td>
<td>1612.30 ± 22.23</td>
<td>1.015</td>
<td>p=0.564</td>
</tr>
<tr>
<td>Group C</td>
<td>CXL</td>
<td>1717.97 ± 18.92</td>
<td>1616.62 ± 17.58</td>
<td>1.063</td>
<td>p=0.008</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1628.69 ± 9.37</td>
<td>1597.68 ± 11.97</td>
<td>1.019</td>
<td>p=0.568</td>
</tr>
</tbody>
</table>

**Table** Speed of sound measures (mean ± SE) for the anterior and posterior stroma for the cross linked (CXL) and control corneas in each group. There was no significant difference found between the measures in the cross-linked corneas in the three groups.

**Figure 3** SAM images of (a) the anterior, and (b) the posterior stroma of a single cross linking treatment cornea, as well as images from the anterior (c) and posterior (d) stroma of the contralateral control cornea.
Results

Figure 4 Speed of sound across the stromal regions. The speed of sound shows a steady decline from the anterior to the posterior regions of the stroma, but with different patterns. Control corneas show a smoother pattern of decrease.
Results

• This decrease in the speed of sound, of the cross-linked corneas, was found to fit a cubic function for:

• The single treatment group
  \( y = 2.0947x^3 - 21.728x^2 + 47.47x + 1649R^2 = 0.9938 \)

• Two treatment group
  \( y = 1.6459x^3 - 15.962x^2 + 18.035x + 1742.6R^2 = 0.9999 \)

• Three treatment group
  \( y = 1.8108x^3 - 17.503x^2 + 24.417x + 1709.3R^2 = 0.9997 \).
Conclusions

• Using SAM, a greater speed of sound was demonstrated in the cross-linked corneas compared with the controls.

• A slightly greater stiffness increase (non-significant) was found in the twice-treated group compared with the single treatment group, suggesting that further cross-links may be induced when collagen cross-linking is repeated.
Future work

• Further research is required to explore the safety and efficacy of repeated CXL treatments

• Evidence based criteria relating to which patients to retreat and when is the optimum time to retreat need to be established.
References


Manchester Corneal Collagen Cross-Linking Group

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Development of the SAM was funded by the Wellcome Trust (WT085981AIA)
I Beshtawi’s PhD was funded by An-Najah National University/ Palestine

Thank you