The Effects of Low and High Intensity Collagen Cross-Linking on the Human Cornea

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Corneal collagen cross-linking treatment

• A combination of ultraviolet A and a photosensitizer (Riboflavin)
• The only treatment designed to halt the progression of keratoconus and to enhance the biomechanical properties of corneal collagen.
Corneal collagen cross-linking treatment

- Different cross-linking protocols are available, e.g. high intensity cross-linking
  - to improve the cross-linking delivery to patients
  - reduce the UV-A exposure time
  - avoid the epithelial removal

- Similar improvement between the low intensity and high intensity cross-linking in a long term (mean 48 months) randomized study (Kanellopoulos et al., 2012)

- Similar increase in stiffness with high and low intensity instruments experimentally on porcine corneas (Schumacher et al., 2011).
Aim

To compare the changes in corneal cell parameters and the biomechanical properties of the cornea following low and high intensity corneal collagen cross-linking protocols, on human corneas.
Materials and Methods

- 20 human corneas
  - (10 pairs, from 8 male and 2 female donors)
  - The age range of the donors was 61-86 years (73.70 ± 2.40) years

- The corneal pairs were divided into two groups:
  - Group A: 5 corneas cross-linked with low-intensity
    - Epithelium removed, 0.1% riboflavin applied for 30 minutes. UVA (365nm 3mW/cm²) applied for 30 minutes along with riboflavin
  - Group B: 5 corneas cross-linked with high-intensity
    - Epithelium removed, 0.1% riboflavin applied for 30 minutes. UVA (370 nm, 9mW/cm²) applied for 10 minutes along with riboflavin

- The contralateral control corneas in both groups had similar treatment but without UVA

Figure 1: UVA/riboflavin irradiation on the human cornea mounted on a Baron anterior chamber.
Materials and Methods

• Corneal thickness using Oculus Pentacam
• Histology assessment
  o Haematoxylin and Eosin staining
• TUNEL assay (Terminal deoxynucleotidyl Transferase (TdT) mediated dUTP-biotin Nick End Labeling)

Figure 2: The human cornea placed in anterior chamber and fixed on a holder during Scheimpflug Imaging.
Materials and Methods

- Scanning Acoustic Microscopy (SAM)
  - SAM provides an evaluation of the biomechanical properties of tissues by means of speed of sound.
  - It allows pixel-by-pixel analysis of tissue properties with a high spatial resolution
- SAM components:
  - an ultrasonic transducer (ultrasound waves (100 MHz-1 GHz))
  - an acoustic lens
  - a coupling fluid

Figure 3 (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.
No significant difference was found between the cross-linked corneas in both groups ($p=0.622$)
# SAM measurements

<table>
<thead>
<tr>
<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><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low-fluence</td>
<td></td>
<td></td>
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<tr>
<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>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><strong>Group B</strong></td>
<td></td>
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<tr>
<td>High fluence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXL</td>
<td>1665.06 ± 9.54</td>
<td>1589.89 ± 9.73</td>
<td>1.047</td>
<td>(p=0.001)</td>
</tr>
<tr>
<td>Control</td>
<td>1583.55 ± 8.22</td>
<td>1565.46 ± 8.13</td>
<td>1.012</td>
<td>(p=0.157)</td>
</tr>
</tbody>
</table>

**Table 1:** 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 both groups.

**Figure 5 (A).** SAM images of (a) the anterior, and (b) the posterior stroma of a low intensity cross linked cornea, as well as images from the anterior (c) and posterior (d) stroma of the contralateral control cornea.
SAM measurements

• The increase in the speed of sound between the cross-linked and control corneas was significant in both groups.

• The speed of sound in the CXL corneas (vs. controls) in group A & B increased by a factor of 1.051x (p=0.005)

• Furthermore, no significant differences were found in the speed of sound between the two protocols anteriorly (p=0.415) or posteriorly (p=0.340).
Results

• Additionally, the increase in the speed of sound was found to be depth-dependent, changing from the anterior part towards the posterior part of the corneal stroma and mainly occurred in the anterior one third of the stroma.

Figure 6. Speed of sound measurements of the cross-linked corneas in group A and B and their contralateral control from the anterior to the posterior regions of the stroma. Similar decrease in speed of sound was noted from both cross-linked groups.
Haematoxylin and Eosin (H&E) staining

- The epithelial layer is absent in all corneas
- A partial loss of the anterior stromal keratocytes was documented, down to 200μm in both treated groups, when compared with the controls.
- An intact and complete Descemet’s membrane and endothelium.

Figure 7: Haematoxylin and eosin staining 5x of (a) low intensity cross-linked cornea, (b) the contralateral control, (c) high intensity cross-linked cornea and (d) the contralateral control.
Assessment of cell apoptosis by TUNEL fluorescence

Figure 8: Assessment of cell apoptosis by TUNEL fluorescence staining 5x in (A) low intensity cross-linked and (B) contralateral control cornea, and (C) high intensity cross-linked and (D) contralateral control cornea.

Table 2: Number of apoptotic cells (mean ± SE) for the cross linked (CXL) and control corneas in each group.
Conclusions

• The safety of both techniques is confirmed with an advantage of reducing the treatment time using the higher-intensity technique.

• A higher speed of sound value was found in the treated corneas when compared with the controls.

• The comparison between the low and high intensity collagen cross-linking did not show any significant differences between the two groups (CCT, cell death and speed of sound).
References


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