Enzymatic Resistance of Corneas Crosslinked Using Riboflavin in Conjunction With Low Energy, High Energy, and Pulsed UVA Irradiation Modes

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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.
| Total corneal disk diameter (mm) | Normalized digestion time |
|---------------------------------|--------------------------|
| Untreated                       | 0.04 – 1.00              |
| Dextran 20%                     | 0.04 – 1.00              |
| Riboflavin (with 20% dextran)   | 0.04 – 1.00              |
| Riboflavin (with 20% dextran) + 3 mW UVA for 30 minutes (SCXL 3 mW) | 0.04 – 1.00 |

### 6 porcine eyes/group

| Treatment                           | Average time for complete digestion to occur (days) |
|-------------------------------------|-----------------------------------------------------|
| Untreated                           | 11                                                  |
| Dextran 20%                         | 11                                                  |
| Riboflavin (with 20% dextran)       | 10                                                  |
| Riboflavin (with 20% dextran) + 3 mW UVA for 30 minutes (SCXL 3 mW) | 25                                                  |
| 11 porcine eyes/ group | Average time for complete digestion (days) | Average dry weight (undigested tissue mass) at day 12 (g) |
|-------------------------|-------------------------------------------|----------------------------------------------------------|
| Untreated               | 11                                        | 0                                                        |
| Riboflavin+ 3 mW UVA 30 mins (SCXL 3 mW) | 25                                        | 0.0041                                                   |
| Riboflavin + 9 mW UVA for 10 mins (ACXL 9 mW) | 25                                        | 0.0020                                                   |
| Riboflavin+ 18 mW UVA for 5 mins (ACXL 18 mW) | 25                                        | 0.0008                                                   |

Riboflavin+ 3 mW UVA 30 mins (SCXL 3 mW) > Riboflavin + 9 mW UVA for 10 mins (ACXL 9 mW) > Riboflavin+ 18 mW UVA for 5 mins (ACXL 18 mW)

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.
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