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

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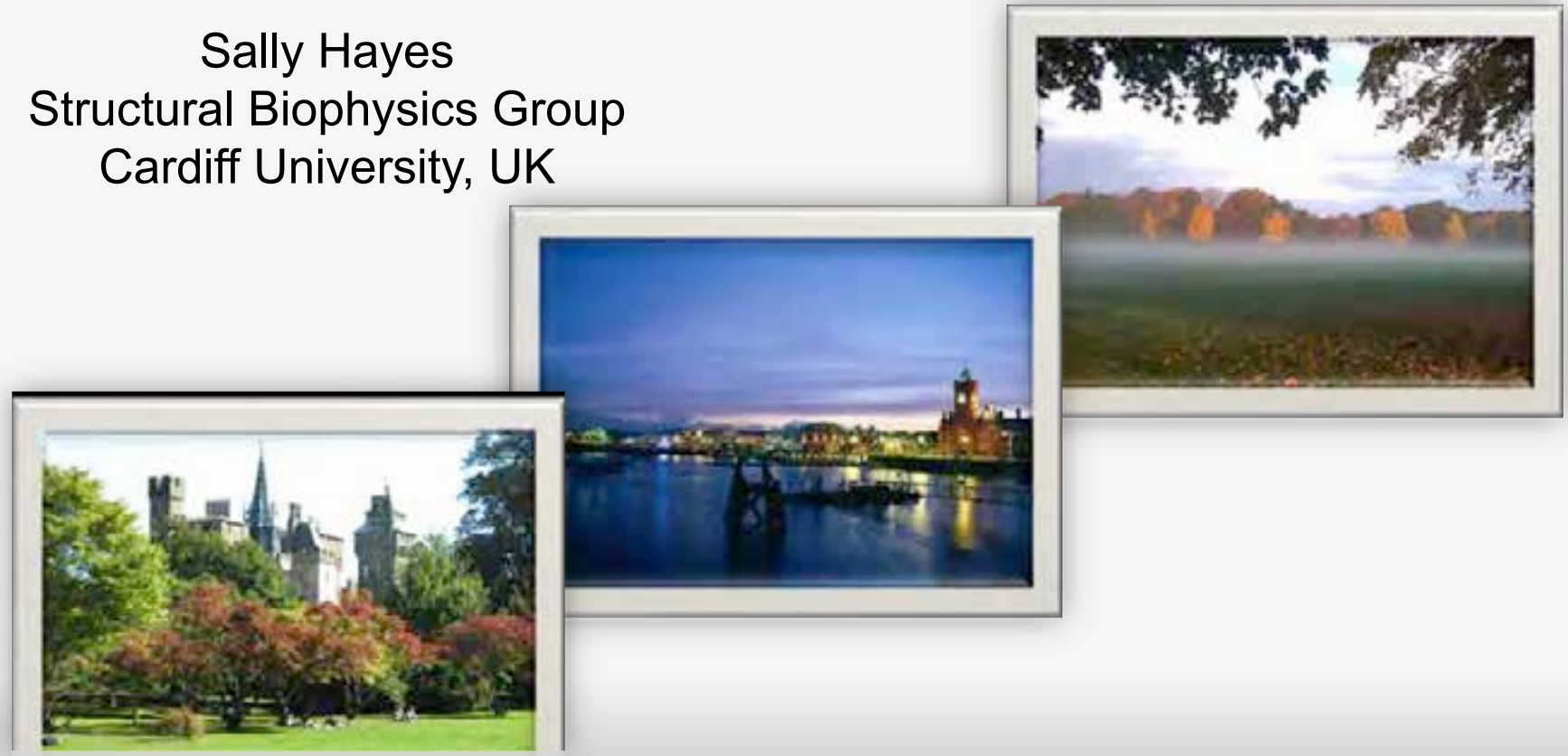
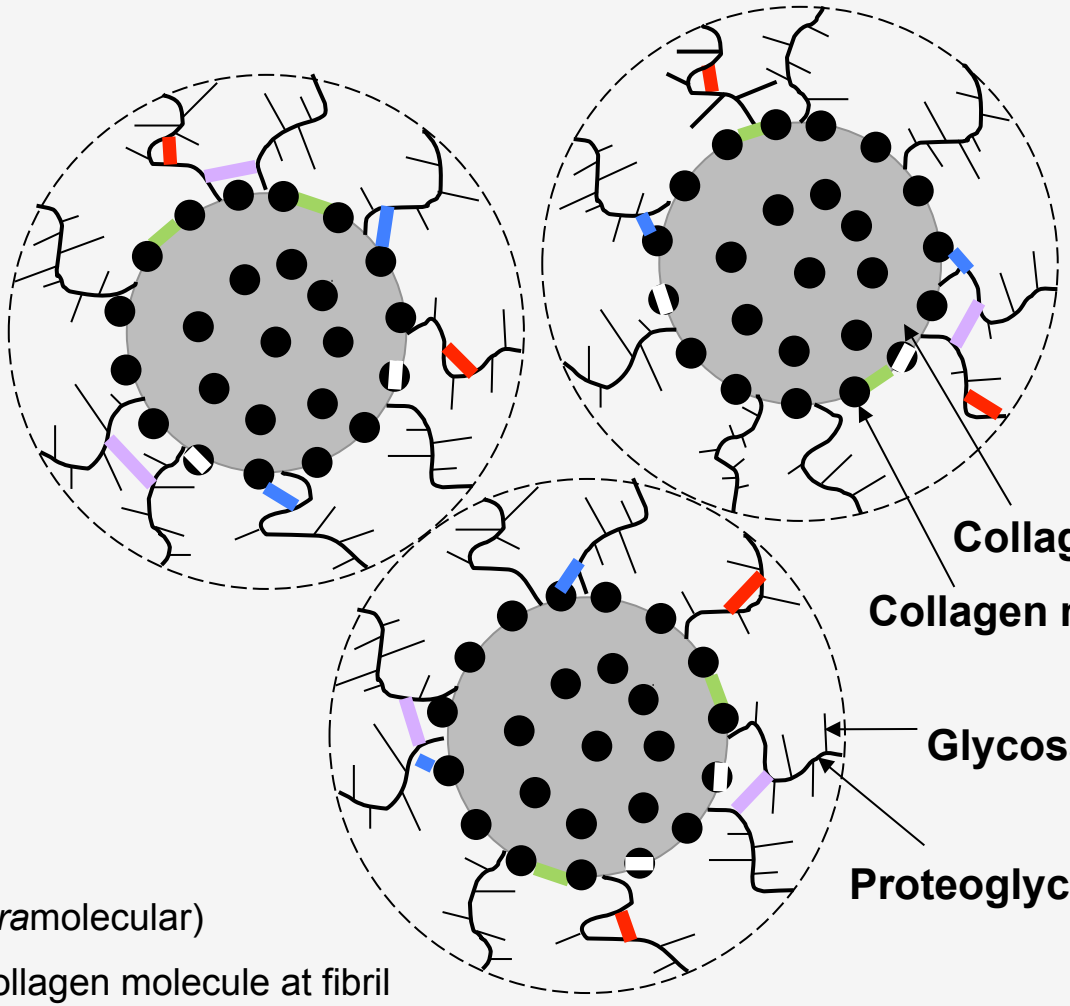


Schematic of three collagen fibrils showing the likely location of riboflavin/ UVA-induced cross-links



- 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

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

Method

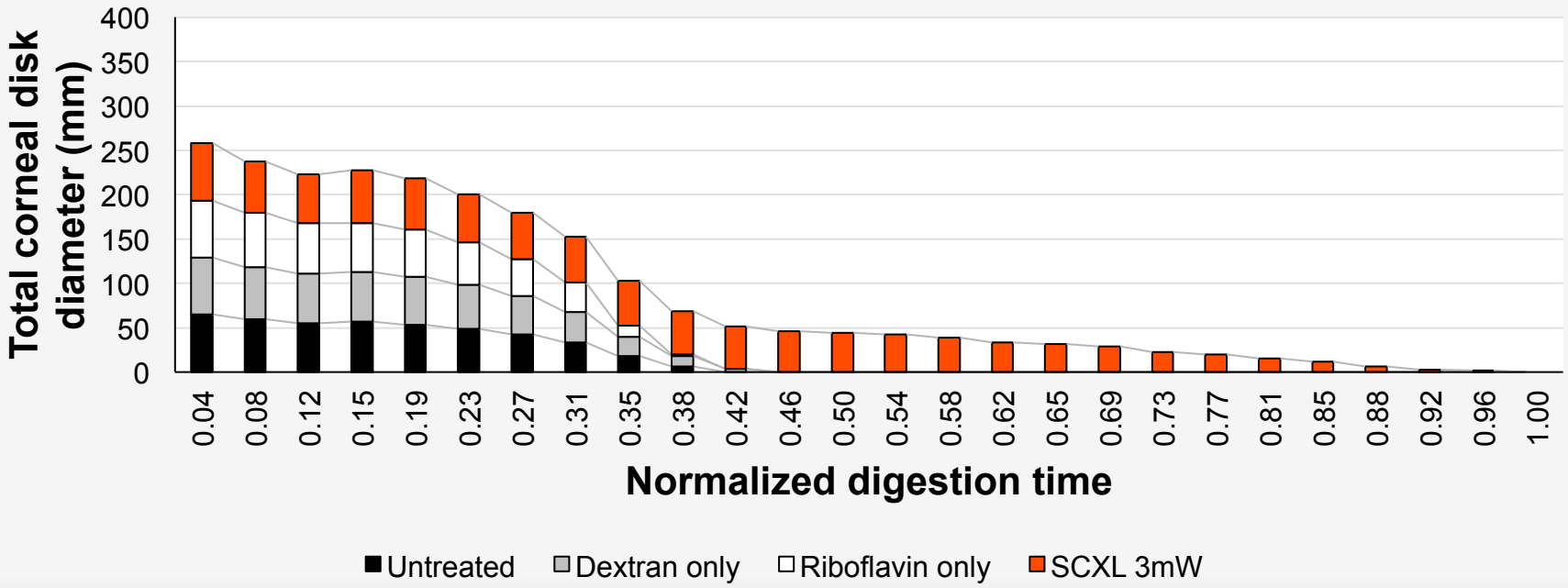
- Porcine eyes are obtained from the abattoir within ~ 4 hrs of death.
- Randomly divided into cross-linked and non-cross-linked treatment groups.
- After treatment, the cornea is removed and an 8mm disk trephined from the centre.




- 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.




6 porcine eyes/group	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**



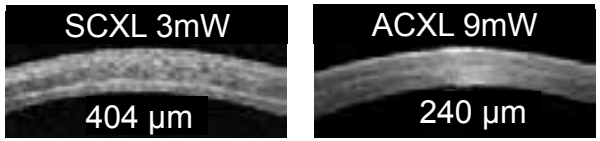
11 porcine eyes/ group	Average time for complete digestion (days)	Average dry weight (undigested tissue mass) at day 12 (g)
Untreated	11	0
Riboflavin+ 3 mW UVA 30 mins (SCXL 3 mW)	**25**	**0.0041**
Riboflavin + 9 mW UVA for 10 mins (ACXL 9 mW)	25	0.0020
Riboflavin+ 18 mW UVA for 5 mins (ACXL 18 mW)	25	0.0008

P< 0.0001

P< 0.0001

SCXL 3mW > ACXL 9mW > ACXL 18mW

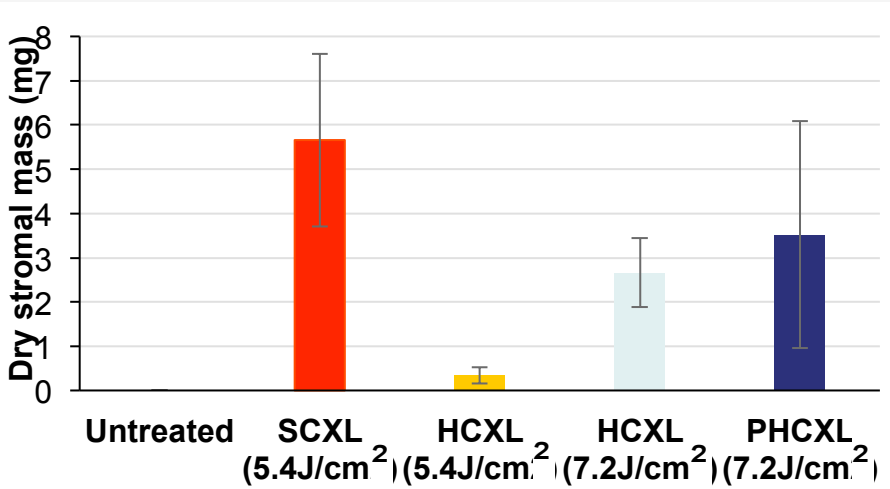
Same diameter but lower undigested tissue mass suggests shallower depth of cross-linking after ACXL.

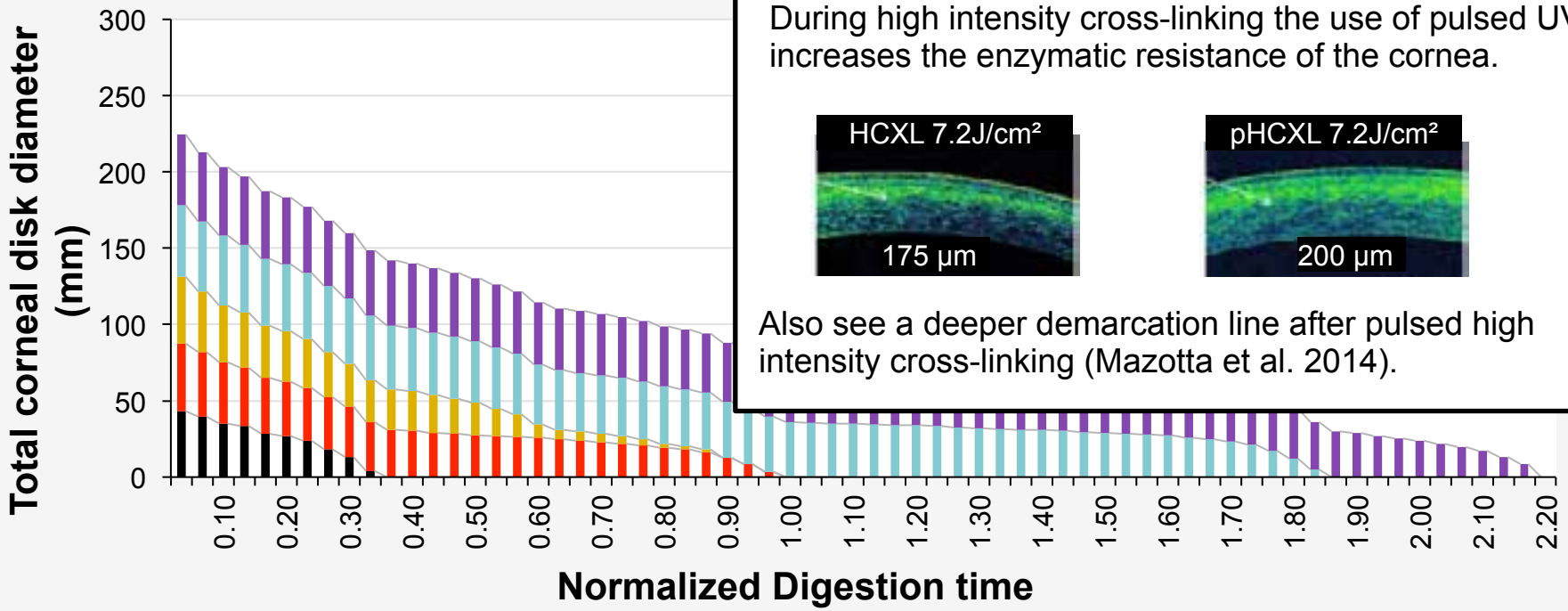


Also see a shallower demarcation line after ACXL (Kymionis et al. 2014).

Aldahlawi et al. 2015 JCRS

11 porcine eyes/group
Untreated
Riboflavin+3mW UVA 30 mins (SCXL 5.4J/cm²)
Riboflavin+30mW UVA 3 mins (HCXL 5.4J/cm²)
Riboflavin+30mW UVA 4 mins (HCXL 7.2J/cm²)
Riboflavin+30mW UVA 8 mins pulsed (p-HCXL 7.2J/cm²)





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).

Aldahlawi et al. 2016 IOVS

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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