Differential gene expression of ECM-related genes after in vivo CXL in rabbits

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Keratoconus

Extracellular matrix degeneration:

- ➔ **loss of collagen fibril orientation**
- ➔ **corneal thinning**
- ➔ **biomechanical weakening**

Aim of corneal cross-linking:

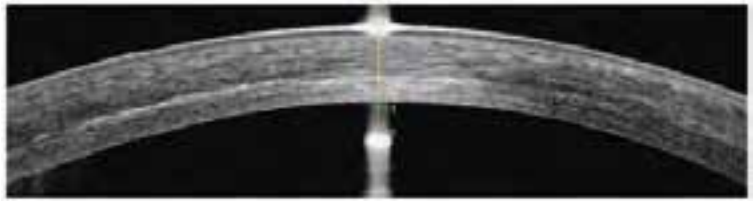
- **To increase the biomechanical resistance of the cornea.**
- **To stop ECM degeneration.**

Previous studies:

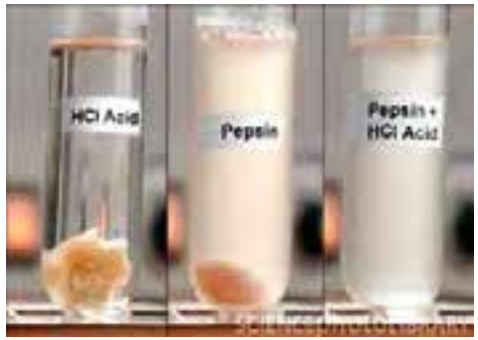
Mechanical, morphological and structural changes

- Elastic modulus

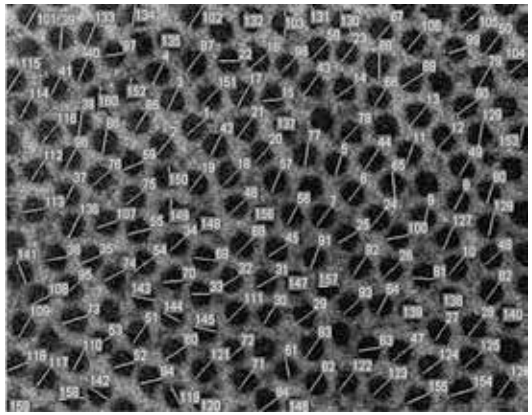
- Demarcation line



- Resistance to enzymatic digestion

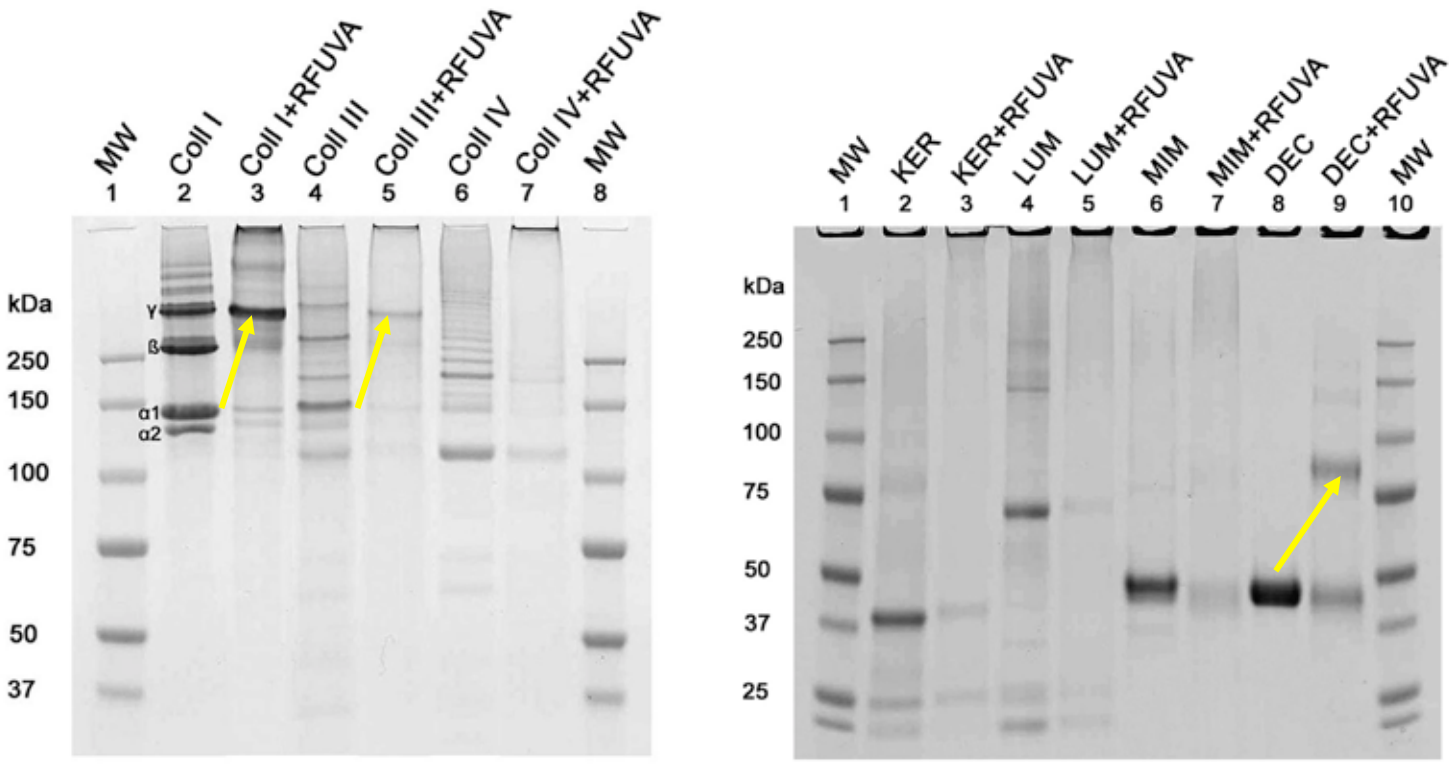


- Collagen fiber diameter



Proteomic level:

Cross-linking of collagens and / or proteoglycans?



REF. Zhang Y. J Biol Chem 2011

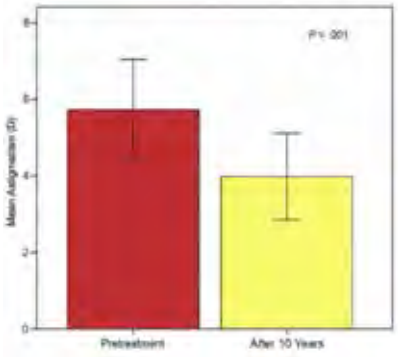
cross-links:

- **among collagens**
- **among proteoglycans**
- **between collagens and proteoglycans**

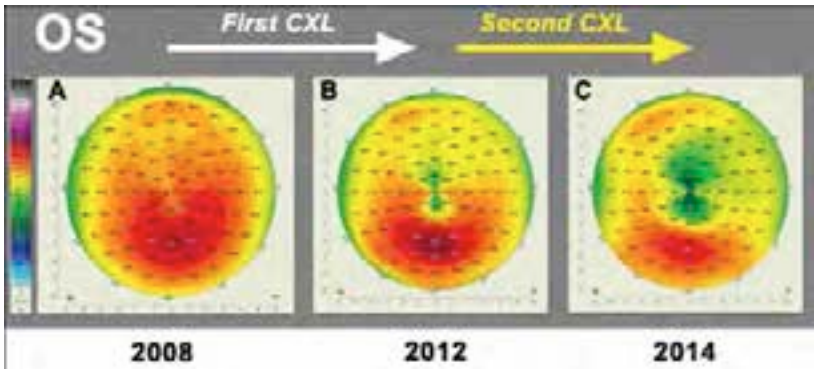
How long is cross-linking effective?

- **Stability for at least 10 years.**

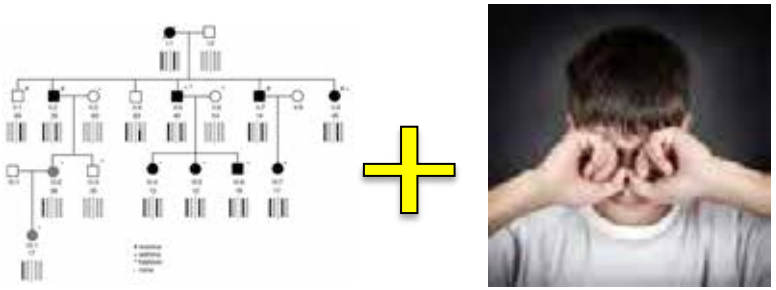
  Raiskup F. et al, 2015, JCRS



- **Progression after several years in some cases. However, re-stabilization after repeated CXL treatment.**



Hafezi F. et al, 2014, JRS



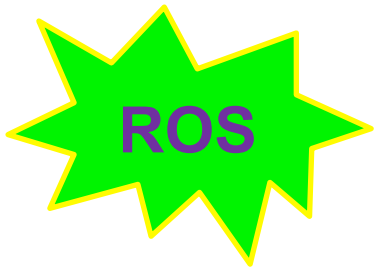
Keratoconus also has a genetic component.

- **How can CXL stop the progression of the disease on the long term?**

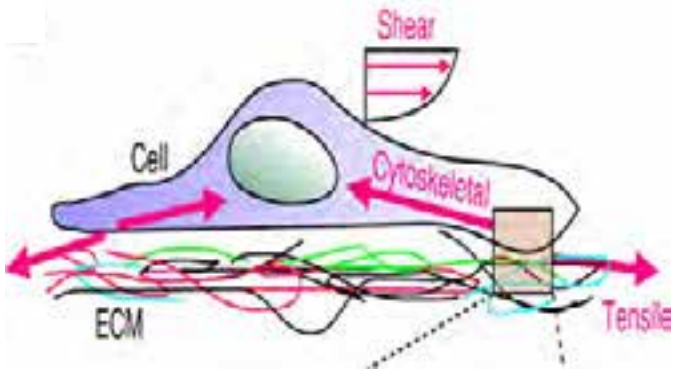
  - **Does CXL only have an immediate effect, or does it permanently affect gene expression?**

Possible mechanisms on the molecular level:

- **Triggering of mechanisms activated by oxidative stress**



- **Stimulation of mechanosensitive pathways**



Experimental set-up:

- **15 New-Zealand white rabbits**
- **Different CXL protocols to study different stiffening effects:**

3 mW/cm^2, 30 min
9 mW/cm^2, 10 min
18 mW/cm^2, 5 min

+

riboflavin-only control
virgin controls

Analysis:

- **1 week post treatment**
- **RNA extraction**
- **RNAseq (HighSeq 2500, Illumina)**
- **Statistical analysis considering**
  - **the overall effect of CXL**
  - **the graded stiffening effect**

Results:

- total of 9335 transcripts
- **297** significantly differentially expressed between CXL conditions and controls.
- **51%** of the differentially expressed genes were stiffening dependent.

Results:

Absolutely highest differentially expressed genes:

- **Down-regulation of enzyme activity**: Glycolysis (enolase 1 alpha, tranketolase), protease inhibition (cystatin, alpha-2-macroglobulin-like 1)
- **Up-regulation of enzymatic cross-linking**: (transgluatimnase 2)
- **Down-regulation of extracellular matrix (ECM) synthesis**: (collagen type-I alpha-1 / alpha-2, collagen type-VI alpha-2, keratocan)

Results:

Relatively highest differentially expressed genes:

- **Altered membrane transport** (cytohesin 1 interacting protein, solute carrier organic anion transporter family, solute carrier family 13 member 5) and receptor binding (EPH receptor B1, Kazal-type serine peptidase inhibitor domain 1, integrin, beta-like 1)
- **Down-regulation of ECM relevant components**: (cysteine-rich angiogenic inducer 61, keratocan, olfactomedin-like 1, thrombospondin 4, fibromodulin)
- **Up-regulation of cross-linking enzyme** transglutaminase 2

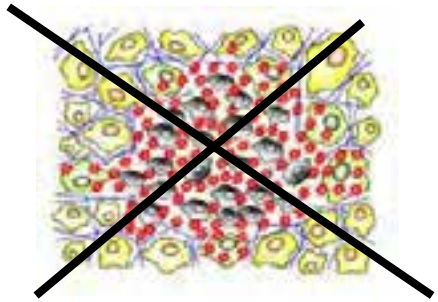
Reduced ECM degradation after CXL:

➢ **down-regulation of ENO1 after CXL**

- Enolase 1 (ENO1) overexpression has been reported in context with increased ECM degradation and cancer invasion.

➢ **TKT down-regulation after CXL**

- Transketolase (TKT) inhibition: to suppress tumor growth and lactate-based ECM degradation



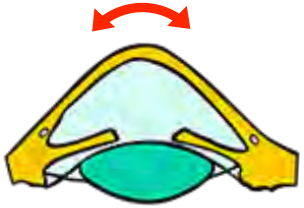
➢ **Down-regulation of collagen-I, collagen-VI and keratocan** confirms the reduced ECM degradation after CXL.

Gene expression is to 51% stiffening-dependent

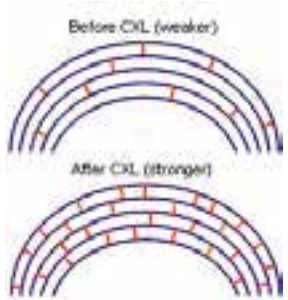
Potential mechanosensitive pathways:

- **Thrombospondin 4:** mechano-sensing molecule in the cardiac contractile response to mechanical stress
  - ➢ **up-regulation** in response to **hypertension**

- **Keratoconus**: constantly increasing corneal strain (progressive stromal thinning)
  - ➢ comparable to cardiac hypertension



- **after CXL**: thrombospondin 4 down-regulation
  - ➢ decreased corneal strain



Conclusions:

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CXL treatment affects gene expression.

Gene candidates have been identified suggesting that CXL treatment decreases the ECM degradation on the molecular level.

Differential gene expression is to 51% stiffening-dependent suggesting that mechano-sensitive pathways are involved.

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Thank you

for your attention

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