Corneal Collagen Cross linking (CCXL)
Myth, Realty and Future Refractive Applications

ESOIRS 2015
Rifaii Lecture

Osama Ibrahim M.D.
Professor of Ophthalmology
Alexandria University
Egypt

Avedro Ambassador
Origins of the 400um Limit

Spoerl et al calculated a minimum thickness of 400um (2x endothelial safety limit) through the application of the Lambert-Beer Law to the following protocol for CXL:

- Epithelial removal over central 9mm
- 30 minutes soaking with 0.1% riboflavin in 20% Dextran
- 3mW/cm² continuous irradiance for 30 minutes
- Reapplication of riboflavin at 5 minute intervals during irradiation

This calculation relies upon:

1. The applicability of the Lambert-Beer Law
2. The measured absorption coefficient of riboflavin
3. The measured damage threshold at the endothelium
4. The exact details of the cross-linking protocol
Riboflavin shielding

The CXL-effect is deepest in the center and reduced in the periphery (UV-X1000)

Corneal cross linking depth ~ 300 microns
Leave a safety margin from the endothelium um 50 microns
Consider an epithelium thickness of 50 microns

= 400 microns for a minimum corneal thickness
Basic Science:
Endothelial Cells Cytotoxicity Study – New Work

- Tests to be performed under GLP conditions
- Cell viability via MTT assay measured 48 hours post treatment.
- Cells used are both from an immortal line and primarily cultured cells:
  - HCEC-B4G12 Human corneal endothelial cell line (DSMZ, Germany)

COMSOL Model Assumptions:
- 20 min pre-soak with fast diffusion (6.5 x 10^-7 cm^2/s)
- 450 µm cornea, 400 µm stroma, 3 mm aqueous humor
- Rinse before UVA
- Boettner values for corneal UVA absorption
- 5% off at surface for Fresnel reflections
- 12 min UVA at 10 mW/cm^2 (7.2J/cm^2)

- Riboflavin Concentration to be used in study:
  - 0.01%
  - 0.02%
  - 0.04%

- O2 Concentration
  - 10%
  - 21%

- UVA Irradiance to be used:
  - 3 mW/cm^2
  - 30 mW/cm^2 (x 10)

Results from model at 400 um (stroma)

<table>
<thead>
<tr>
<th>CONC [% w/v]</th>
<th>UVA [mW/cm^2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125%</td>
<td>0.01</td>
</tr>
<tr>
<td>0.25%</td>
<td>0.02</td>
</tr>
<tr>
<td>0.50%</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Oxygen Availability During Cross-linking

- Role of oxygen in *photochemical kinetics* of corneal crosslinking, it is important to consider the maximum concentration of oxygen at the endothelium

- In previous CXL cytotoxicity studies, testing was performed in room air (20% oxygen), while the physiological concentration of oxygen at the endothelium (concentration in the aqueous) is 10%.

- Actual availability of oxygen during CXL may be even less, due to depletion of oxygen by the kinetics of the CXL reaction.

Depletion and gradual replenishment of dissolved oxygen below a 100 µm thick corneal flap, saturated with 0.1% RF, during 3 mW/cm^2 UVA irradiation at 25°C, demonstrating the distribution of predominant reaction types during light exposure.

Conclusions

• There is a 3X factor of endothelial cytotoxic dose between low and high irradiance (10X)

• Riboflavin concentration is not a factor

• Endothelial cell cytotoxicity is mediated by rate of available oxygen at endothelium to generate a sufficient concentration of reactive oxygen species (ROS).

Therefore in oxygen-limited endothelial environment, higher irradiances will require higher doses to achieve the same concentrations of ROS.

Conclusions

• Higher doses at higher irradiances and treatment of thinner corneas may be safely optimized by understanding the rate of oxygen consumption and replenishment as a function of depth.

• Future studies to include different combinations of parameters: irradiance, oxygen concentration, riboflavin concentration, etc.
Uniformity is Key to Consistent Cross-linking

- Cool edges provide no cross-linking
- Center to edge beam uniformity insures consistent results

The CXL-effect is deepest in the center and reduced in the periphery (UV-X1000)

Only because of the light profile .... ?
The CXL-effect is deepest in the center and reduced in the periphery also observed after (KXL I treatments)

Optical Coherence Tomography and Confocal Microscopy Following Three Different Protocols of Corneal Collagen-Crosslinking in Keratoconus

optimized profile

UV-X2000
UV-X1000

I₀

8mm  4mm  0    4mm  8mm
Very similar demarcation lines due to eye motion

Mean eyes motions are compensated due to the donut beam profile

New requirements for CXL

• Allow device to compensate for the eye motion by eye-tracking
• Allow individulized light profiles to control the cross linking depth
• Allow higher energy dose for increased cross linking
• Integrate corneal tomography for treatment planning
Pig eyes: deswell the cornea with dextrane.

Pre-clinical Rabbit eye. Do not change formulation.

Isotonic Ribo

HPMC

LASIK Xtra

Trans-EPI

1996

2009

2015

Avedro’s Family of Riboflavin Formulations

<table>
<thead>
<tr>
<th>Name</th>
<th>Formulation</th>
<th>Procedure</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% Riboflavin 20% Dextran</td>
<td>Cross-Linking for Keratoconus &amp; Post-Lasik Ectasia</td>
<td>Epi-Off</td>
<td></td>
</tr>
<tr>
<td>0.1% Riboflavin HPMC</td>
<td>Cross-Linking for Keratoconus &amp; Post-Lasik Ectasia</td>
<td>Epi-Off</td>
<td></td>
</tr>
<tr>
<td>0.22% Riboflavin HPMC, BAC EDTA, TRIS</td>
<td>Cross-Linking for Keratoconus &amp; Post-Lasik Ectasia</td>
<td>Epi-On</td>
<td></td>
</tr>
<tr>
<td>0.22% Riboflavin Saline</td>
<td>Lasik Xtra for Corneal Strengthening During Lasik</td>
<td>Stromal Bed</td>
<td></td>
</tr>
</tbody>
</table>
Cross-linking in Refractive Correction

**Stabilize the Cornea**
- Restore baseline Strength
- Prevent Refractive Regression
- Protect against Ectasia

**Reshape the Cornea**
- Maintain or improve baseline strength
- Reduce refractive error
- Improve corneal shape

---

**Lasik Xtra**
- Evenly cross-link the central 9mm of the cornea to stabilize without inducing additional shape change

**PIXL**
- Differentially cross-link specific zones of the cornea to redistribute stress and alter corneal shape

---

**Application of Cross-Linking for Specific Goals: Lasik Xtra**

**Primary Goal:** Stabilize the cornea to reduce the risk of refractive drift and ectasia

**Requirements:**
1. Restore corneal strength to its pre-LASIK state
2. Maintain the accuracy and predictability of LASIK
3. Do not disrupt LASIK surgical flow
Lasik Xtra: Restoring corneal strength

Creation of the LASIK flap weakens the cornea by as much as 30%. Lasik Xtra treatment protocols are designed to apply sufficient stiffening to restore the cornea to its pre-LASIK level of stiffness, without excessive change.
**Lasik Xtra: Maintaining Predictability**

Pan-corneal treatment

- KXL system with homogenous beam profile and large depth of focus is used to cross-link the entire central 9mm of the cornea.
- Uniform treatment across the large treatment zone restores stability without redistributing corneal stresses (no shape change).
- This differs from treatment of keratoconus, where the cornea has a focal region of weakness.

**Lasik Xtra: Maintaining Clinical Flow**

Accelerated technique

- Direct application of dextran-free riboflavin to stromal bed to reduce soak time.
- Riboflavin applied immediately after ablation to avoid extra flap handling.
- Avoiding excessive riboflavin in the flap reduces shielding to maintain lower total UVA dose requirement.
- High irradiance accelerated protocol to reduce UVA treatment time.
Modifying Cross-Linking for Specific Goals:

**PiXL**

**Primary Goal:** Correct refractive error using cross-linking alone to reduce or eliminate risks associated with conventional refractive surgery

**Requirements:**
1. Induce predictable refractive change of clinically significant magnitude
2. Have the ability to be customized for specific patient characteristics or treatment goals
3. Achieve outcomes that are stable for a sufficient duration

---

**Requirements for true refractive correction with PiXL**

- Sharply defined edges
  - Eye Tracking

- Complex patterning
  - Digital Micromirror Device (DMD)

- More stiffening than standard technology
  - Pulsed Illumination
  - Higher Energy Doses
Mosaic/KXL II System

A. Main Console with USB flash drive and treatment activation slot
B. System Keyboard
C. System Body with lift system
D. Articulating Arm
E. Heads Up Display
F. Optical Head
G. Wheels

Customized UVA Patterning
Mosaic – Software Controlled UV Projection using DMD

- Customizable UVA pattern
- Individually controlled mirrors (8 bit modulation possible)
- System maintains 60 Hz update frequency to DMD
- Three-axis motion control +/- 10 mm in each axis
- Treatment range: 15.5 mm + 20 mm of motor motion
- Beam Uniformity: < 2%.

© 2015 Avedro.
Mosaic – Eye Tracking

Positioning
- Frame rate pupil tracking
- Average eye tracking response time: 7.4 ms
- Average time between DMD updates: 16.6 ms

Focus assessment
- Automated assessment of focus
- Intensity gradients at pupil boundary
- Used during fully automated Z alignment
- Standard deviation ~200 um.

Mosaic – Precise application of UVA

Best focus following automated Z alignment
- 20%-80% transition width: 100 um

Step edge projection+ following Z alignment
3D profile fit across edge

Simulated eye motion blur*+ UV projection measured with beam gauge

After simulated eye motion*
- 20%-80% transition width: 210 um

3D profile fit across edge

* Assumes standard deviation of 100 um eye motion in a worst case instruction lag of 100 ms.
Mosaic – Iris Registration

- Cyclotorsional image alignment using Iris Registration technique
- Average difference (Algorithm – Mean of observers): 0.23 degrees (95% CI 0.9 to 1.2 degrees)

© 2015 Avedro.

Iris Rotational Alignment

- Iris image is “unwrapped” for polar coordinate representation
- Binary texture representation of the polar coordinate image

Pupil and Limbus segmentation on Mosaic display, represented by the red and green circles.
Corneal Tomography

Biomechanical Properties

Customized Treatment Plan

Riboflavin is applied to the corneal surface

The patient is positioned under the Mosaic KXL II device, and an iris tracker precisely aligns the device with the patient’s eye

A customized UVA treatment pattern is applied
Customization of treatment

Oculus Pentacam Tomography

Customized UVA Pattern

Avedro Mosaic Treatment Design

PiXL for Keratoconus

Professor Anders Behndig
Redistribution of Corneal Stress

- UVA applied
- Center (myopia), the Midperiphery (hyperopia), or Bowtie (astigmatism) Riboflavin-soaked cornea
- Focal stiffening cross-linked regions
- “Bulging” untreated regions (response to normal intraocular pressure)

Images Adapted From: Professor John Marshall, MBE, PhD

Clinical Measurement Of Corneal Biomechanics

Goal: Develop a technique to directly map corneal stiffness properties in a clinical setting

Potential Applications:
- Measure Effect of Cross Linking
- Early Diagnosis of Ectasia
- Pre-Op Screening of Refractive Patients
- Assessment of other tissues
- PiXL treatment planning using individual biomechanical properties

What is the elastic modulus of this area?
Current clinical techniques for measuring biomechanics

- Air puff tonometry
- Optical coherence elastography
- Dynamic ultra high speed Scheimpflug imaging
- Dynamic OCT imaging
- Quantitative ultrasound spectroscopy

Disadvantage: All require applying a mechanical force to achieve measurement

Brillouin Scattering Spectroscopy

Brillouin scattering is created by the interaction between photons and acoustic phonons in a material.

Phonons: quantum of the vibration of the crystalline lattice of the material.

The photon may lose energy (Stokes process) or gain energy (anti-Stokes process) from this interaction.
Brillouin Scattering Spectroscopy

The change in energy of the photon from the interaction with the crystalline lattice of the material corresponds to a shift in frequency in the Brillouin spectrum.

\[ M' = \frac{\rho \lambda^2 \Omega^2}{4n^2} \]

This shift is related to the elastic modulus (M’) of the material where, \( \rho = \) mass density, \( \lambda = \) wavelength, \( n = \) the refractive index.

\[ M' = \frac{E'(1-\sigma)}{(1+\sigma)(1-2\sigma)} \]

Elastic modulus (M’) is related to Young’s modulus.

Avedro Benchtop Brillouin-Scattering System

EMCCD camera

Fourier lens

M-VIPA

Achromatic lens

SpF Second OB

RB absorption cell

Future fiber moving-platform connector

Alignment mirror 1 – Future Dichroic

Future Telecope

Alignment via QWP reflection

780-nm laser @ ~35 mW

780-nm ASE cleanup filter

FiberDock 1

PM SMF

Dichroic Mirror

Reference laser fixation marker

580 nm lens

Future LED

780 nm band filter
Fig. 5. Spectral orders 1, 2, m... of Brillouin and Rayleigh scattering lines at 1-second exposure: a) water; b) methanol; c) PMMA. A1/S1 – anti-Stokes/Stokes Brillouin scattering components of 1st order; R1 – Rayleigh scattering, 1st order.

10 μs exposure: A1 R1 S1 A2 R2 S2 ...

Fig. 6. Spectra of Brillouin and Rayleigh scattering lines of Fig. 5 recorded at 10 microsecond single-pixel exposure.

1 sec exposure with new optical system

10 microsecond exposure with new optical system

Brillouin Spectroscopy in Cross-Linking

Scarcelli et al measured differences in treated and untreated porcine corneas that are in agreement with Avedro laboratory findings for the same dose

They also demonstrated that Brillouin Spectroscopy could show effect as a function of depth, and was sensitive enough to detect differences between cross-linking protocols

Brillouin Spectra of Live Human Eye

Without Contact Lens

- Distance does not change (Brillouin Frequency Shift)
- Frequency is relatively constant in stroma as subject moves in and out

With Contact Lens

- Distance changes (Brillouin Frequency Shift)
- Frequency changes between Contact lens and stroma as subject moves in and out

Brillouin Spectroscopy: Clinical System in Development

Potential Applications:
- Measure Effect of Cross Linking
- Early Diagnosis of Ectasia
- Pre-Op Screening of Refractive Patients
- Assessment of other tissues
- **PIXL treatment planning using individual biomechanical properties**

Future Applications:
- More Accurate IOP Measurement
- Assessment of Other Tissues
  - Lens, Lamina, Retina...?
Biomechanical Customization of PiXL Treatment Design

Corneal Tomography

Biomechanical Properties

Customized PiXL Treatment Plan

PiXL for Post-Cataract Myopia Case Example

52 year old Female
Pseudophakia OD
Refraction OD: -1.25 -0.25 x 180
BCVA 20/20, UCVA 20/200
PiXL For Post-Cataract Myopia Case Example: 9 Month Post-op

-0.50 sphere
UCVA: 20/25
BCVA: 20/20

9 Months    Pre-OP

Case from: Dr. Pavel Stodulka

PiXL

The Future of Refractive Correction

- Post- IOL tune-ups
- Post- refractive surgery enhancements (SMILE)
- Low myopia
- All patients with keratoconus

.. and this is just the beginning.
Thank you

Osama Ibrahim