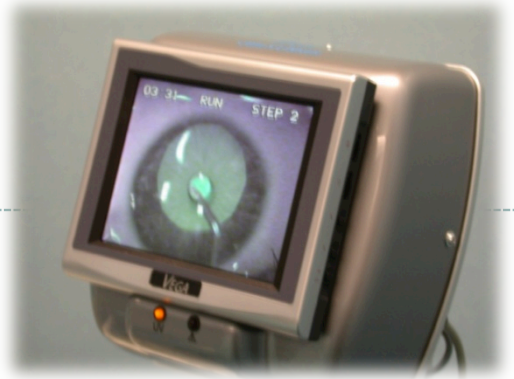
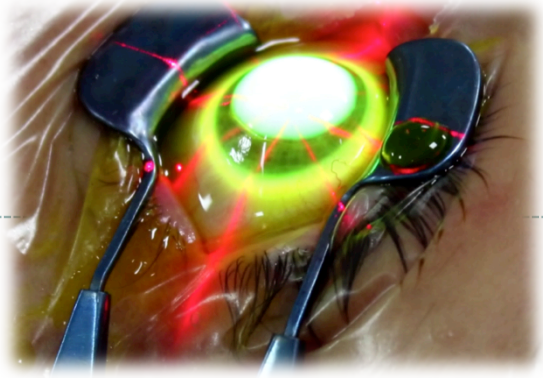
“ Magna Graecia ” University, Catanzaro, Italy
Ophthalmology Department

Corneal collagen crosslinking: Past, present and future of conservative treatment for keratoconus





Track 1: Cornea & External Eye Disease

Session Introduction

OMICS Group Conferences
Accelerating Scientific Discovery

4th International Conference on
Clinical & Experimental Ophthalmology
July 14-16, 2014 DoubleTree by Hilton Baltimore-BWI Airport, USA

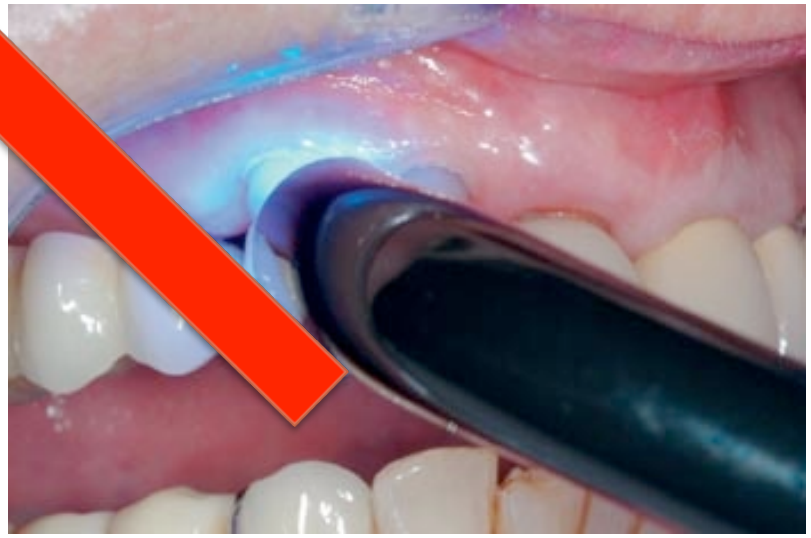
Miguel Rechichi MD, PhD

My financial interest…

The Past: Where Did Crosslinking history Begin???

The legend tells:
once upon a tir
in a SWISS
dentistry office…

Prof. Dr. Dr. Theo Seiler



The past: Crosslinking history

Early AMERICAN paper by CANNON, FOSTER e CHACE (IOWA, OKLAOMA) in 1970 showed relationship between age and collagen oxidation (crosslinking) that finally reduce its turnover.

Corneal collagen in keratoconic patients express more oxidated lisin radicals and hydroxilated protein

- **Exp Aging Res. 1977 Mar;3(2):87-105. Aging, and crosslinking in mammlian collagen. Cannon DJ, Davison PF.**
  The University of Iowa, Iowa City, USA.

- **Invest Ophthalmol Vis Sci. 1978 Jan;17(1):63-5.**
  Collagen crosslinking in keratoconus.
  Cannon DJ, Foster CS.
  The University of Iowa, Iowa City, USA

- **Free Radic Res Commun. 1991;12-13 Pt 2:591-4**
  Effect of oxygen free radicals on corneal collagen.
  Chace KV, Carubelli R, Nordquist RE, Rowsey JJ.
  Dean A. McGee Eye Institute, Oklahoma City, OK.

The past: Crosslinking history

- **In '90 Sady et al (OHIO University U.S.A) demonstrated higher protein glication (Maillard reaction) of corneal collagen in diabetic patients with an increace in biomechanic resistance.**
- **This and others aging studies was the base of actual crosslinking therapy that we actually use**

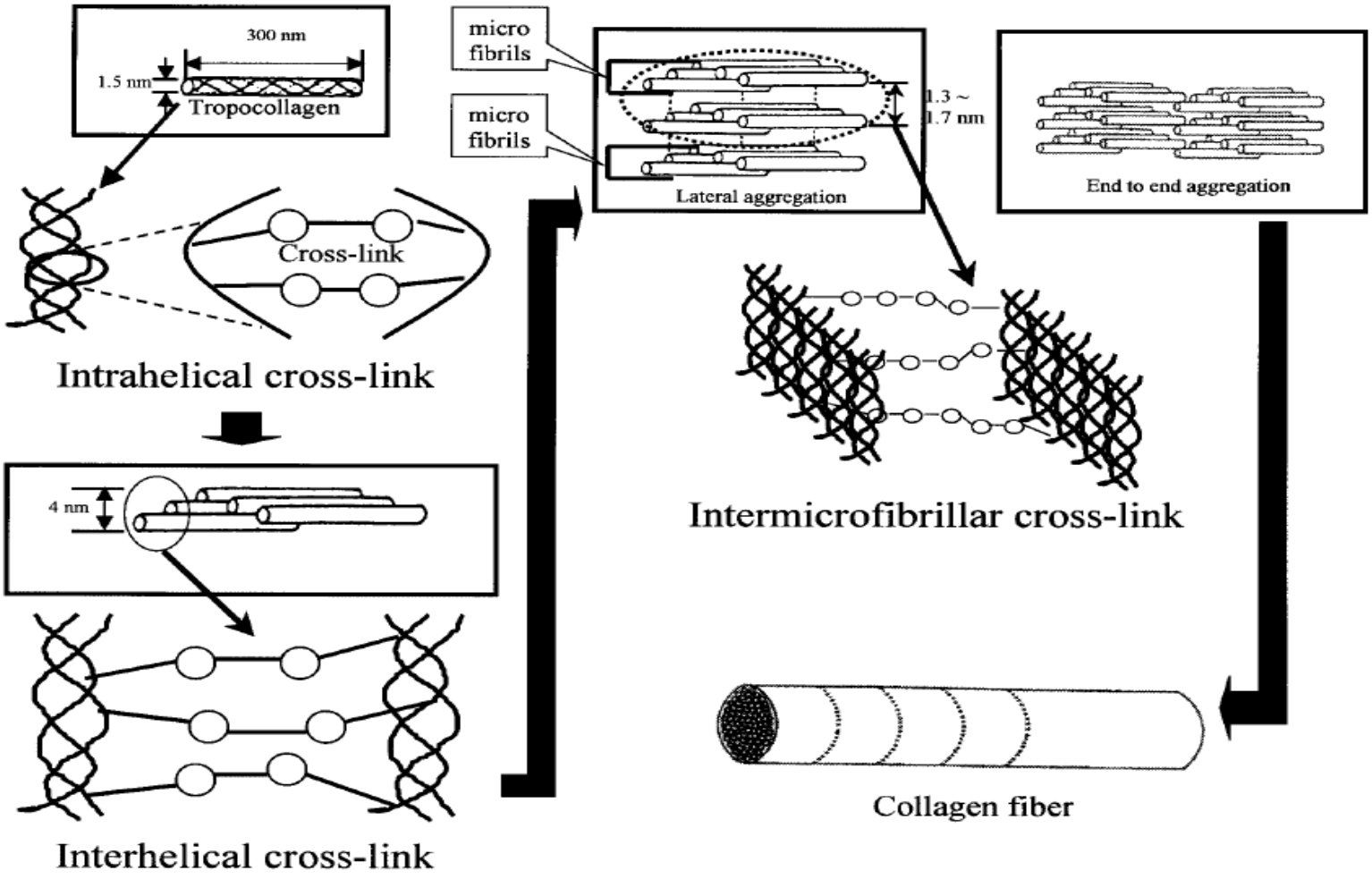
- **Biochem Biophys Res Commun. 1995 Sep 25;214:793-7**
  Advanced Maillard reaction and crosslinking of corneal collagen in diabetes.
  Sady C, Khosrof S, Nagaraj R. Department of Ophthalmology, Case Western Reserve University, Cleveland, OH 44106, USA.

Corneal Cross-linking

Chemical-physical process, natural or induced,that produces covalent intra-inter fibrillar links modifying collagen structure.

- **Natural Cross-linking : Age-related**
- **Pathologic Cross-linking : Diabetes**
- **Artificial Cross-linking : Chemical-Physic**

Cross-linking sites



Photochemical crosslinking improves the physicochemical properties of collagen scaffolds.

Chan BP, Sp KF: J Biomed Mater Res A. 75(2005)689-701

First studies on artificial Cross-Linking applied to the cornea began in 1995:

Spoerl e Seiler – Dresda University

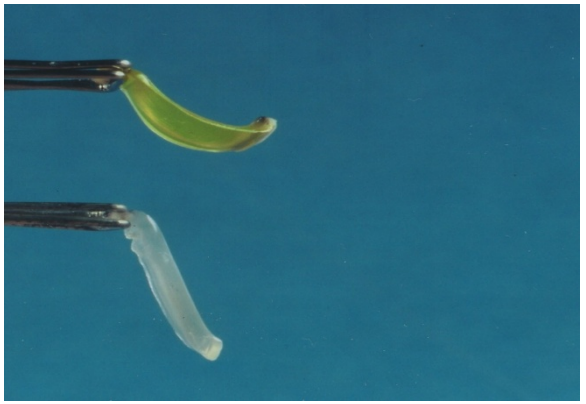
- **Exp Eye Res. 1998-1999 Jan;66(1):97-103.**
  Induction of cross-links in corneal tissue.
  Spoerl E, Huhle M, Seiler T.

  Department of Ophthalmology, University of Dresden, Dresden, Germany.

- **J Refract Surg. 1999 Nov-Dec;15(6):711-3**
  Techniques for stiffening the cornea.
  Spoerl E, Seiler T

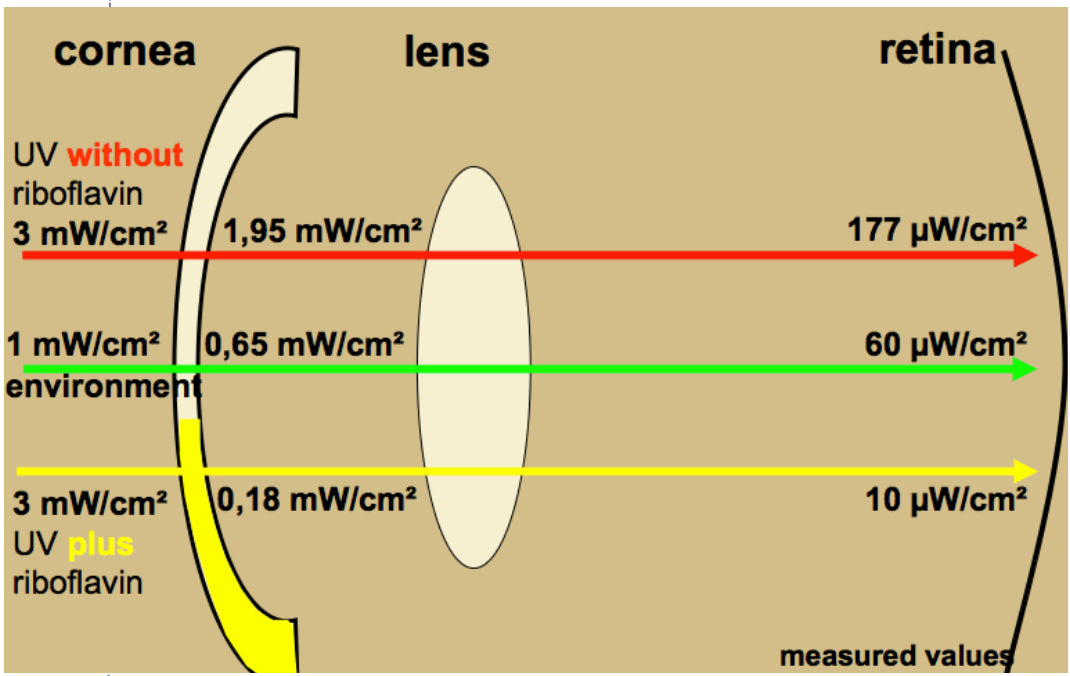
  University Eye Clinic, Dresden, Germany.

More than 10 years history!!!



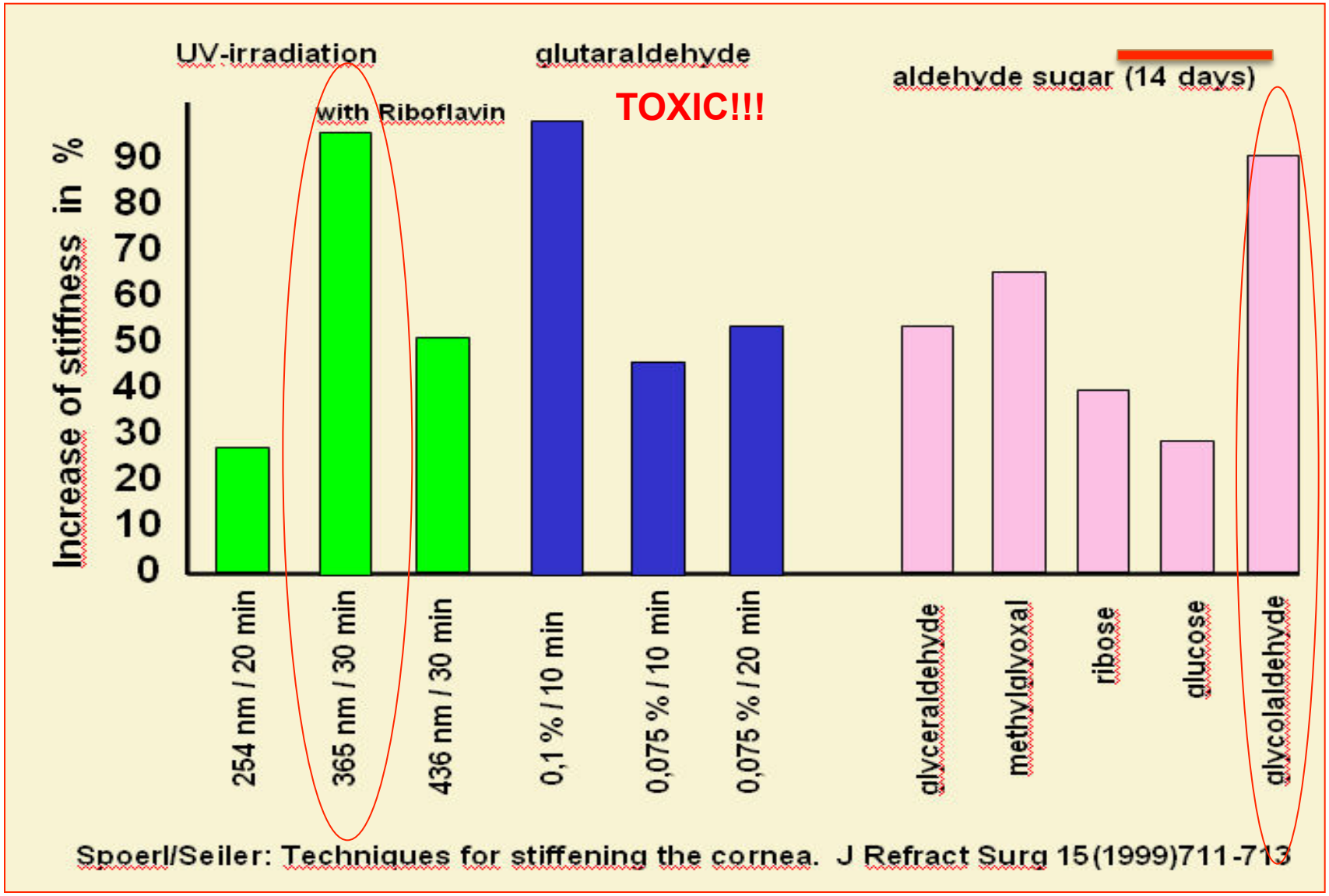
Why riboflavin + UV-A ?

- **Photosensitizer: interaction with carboxilic group for reactive Singulet Oxigen production**
- **Light filtering: <320 -400> μ light wavelenght, a well stained cornea absorbe more than 95% of radiation in a 400 μ corneal thickness**
- **Absence of direct cytotoxic effects on Keratocytes and/or endothelial cells**



Wollensak, Seiler, Spoerl; 2001

Why riboflavin and UV-A ?

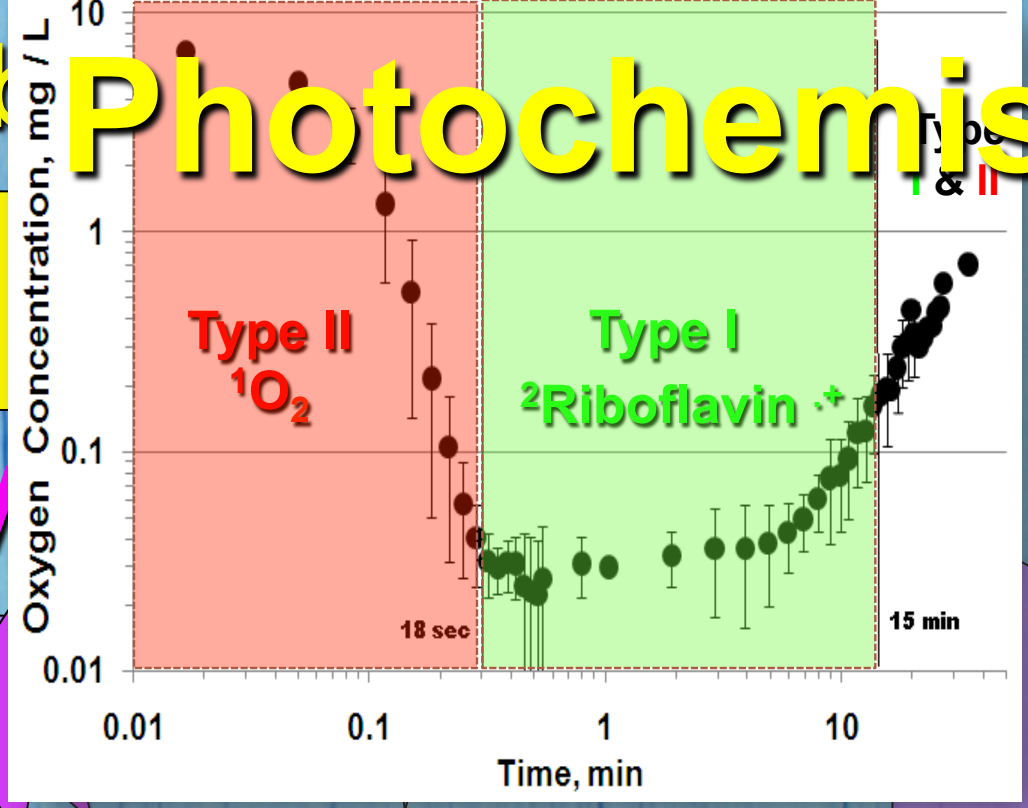


Spoerl/Seiler: Techniques for stiffening the cornea. J Refract Surg 15(1999)711-713

Photochemistry

Rib

UV



Combination

O* O* O*

10%

RF* RF* RF*

90%

Dresda: First Int Clinical Study

Am J Ophthalmol. 2003 May;135(5):620-7.

Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus.

Wollensak G, Spoerl E, Seiler T.
Department of Ophthalmology, Technical University of Dresden, Germany. gwollens@hotmail.com

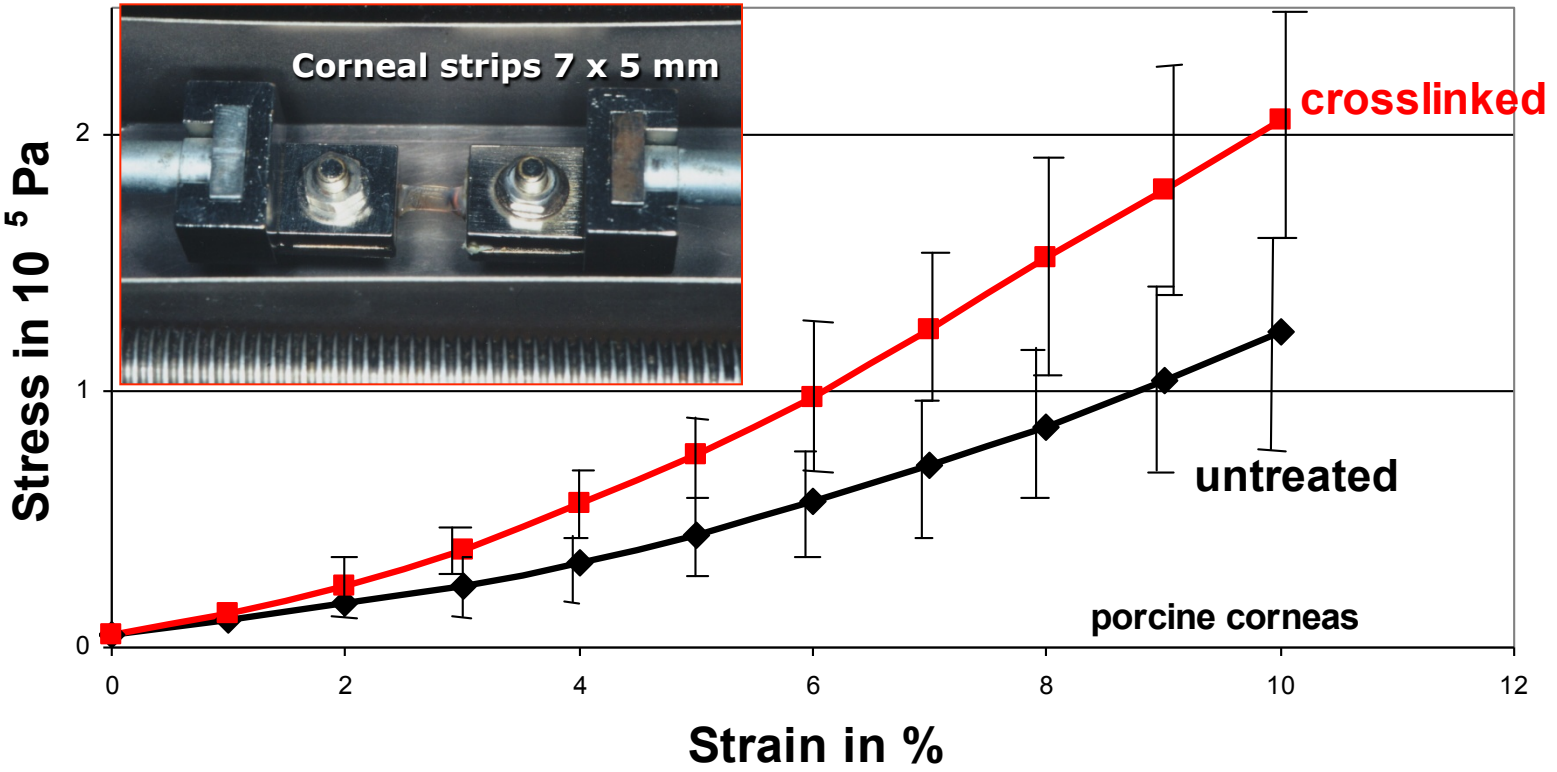
CONCLUSIONS: COLLAGEN CROSSLINKING MAY BE A NEW WAY FOR STOPPING THE PROGRESSION OF KERATECTASIA IN PATIENTS WITH KERATOCONUS. THE NEED FOR PENETRATING KERATOPLASTY MIGHT THEN BE SIGNIFICANTLY REDUCED IN KERATOCONUS. GIVEN THE SIMPLICITY AND MINIMAL COSTS OF THE TREATMENT, IT MIGHT ALSO BE WELL-SUITED FOR DEVELOPING COUNTRIES. LONG-TERM RESULTS ARE NECESSARY TO EVALUATE THE DURATION OF THE STIFFENING EFFECT AND TO EXCLUDE LONG TERM SIDE-EFFECTS.

The Dogma: Dresda Protocol:

Original Treatment Protocol

1. Removal of epithelium
2. Measurement of corneal thickness
   CCT>400µm isoosmolar riboflavin
   CCT<400µm hypoosmolar riboflavin (Hafezi 2009)
3. Riboflavin 0.1% with 20 % dextran T500
4. Riboflavin application:
   - 30 min with lid speculum (2007)
   - 30 min without lid speculum (2008)
5. Measurement of CCT immediately before irradiation (safety control 400µm) (2007)
6. Removal of riboflavin film before irradiation (2010)
7. UV 365-370 nm, 3mW/cm², 30 min, high intensity

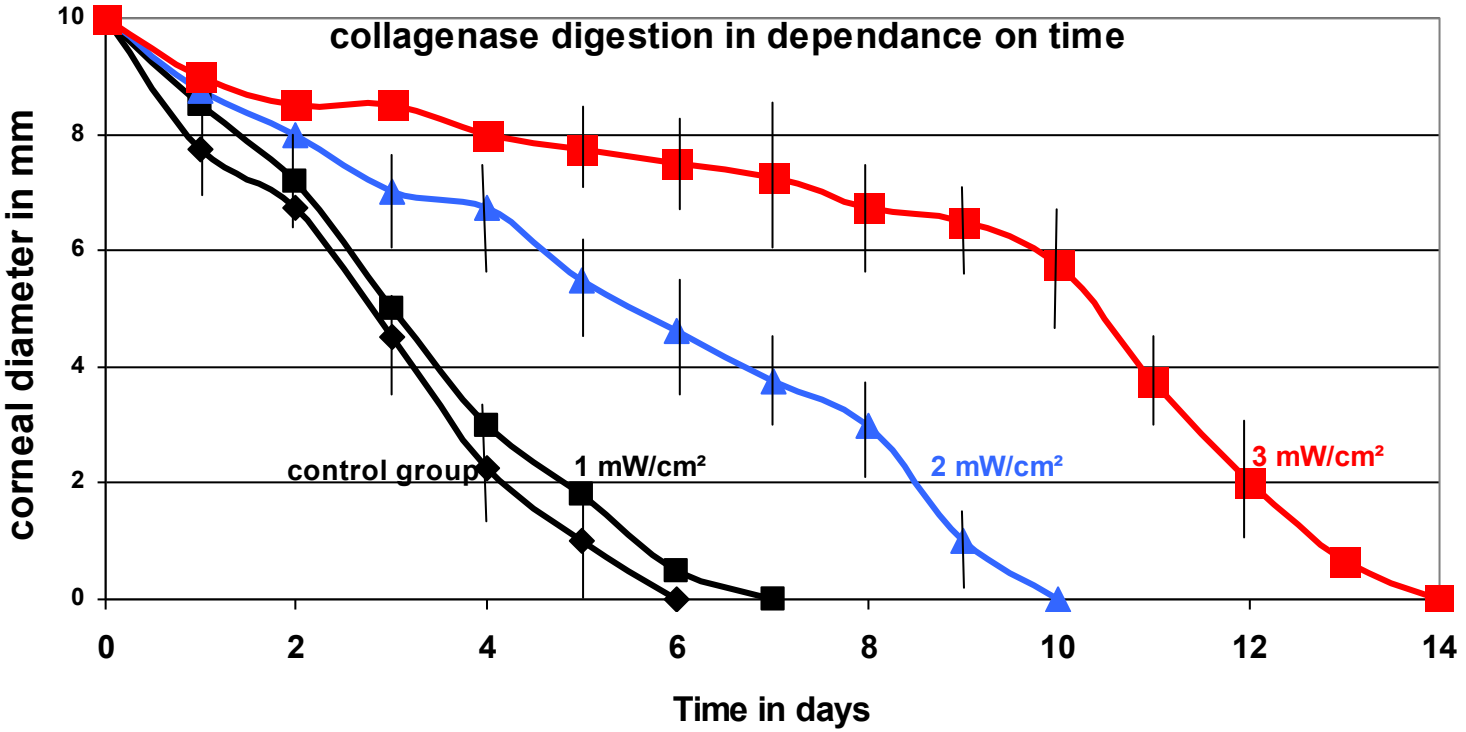
Stress-Strain test

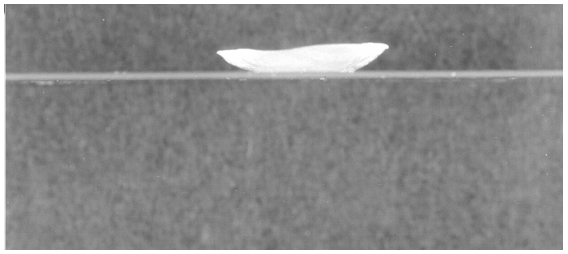


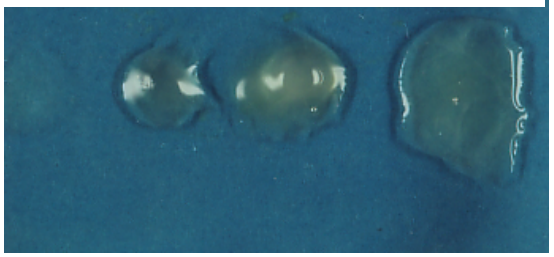
Increased rigidity by more than 300% Young's modulus increased by 4.5 x

Spoerl, Seiler: Exp Eye Res, 1998

CXL effects: increase resistance to enzymatic lysis

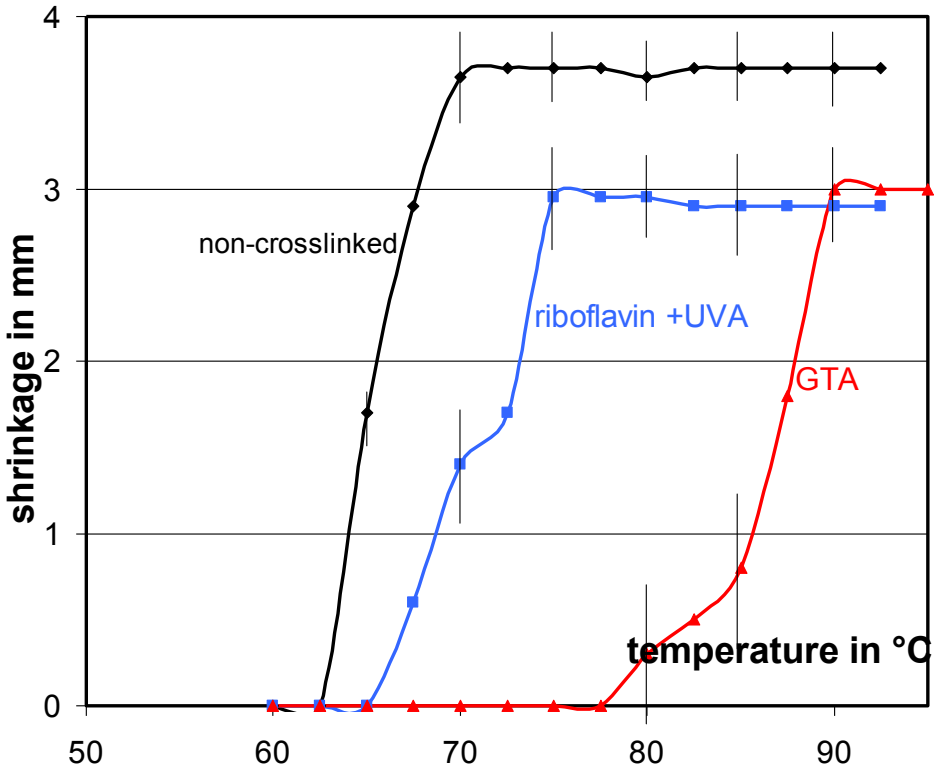


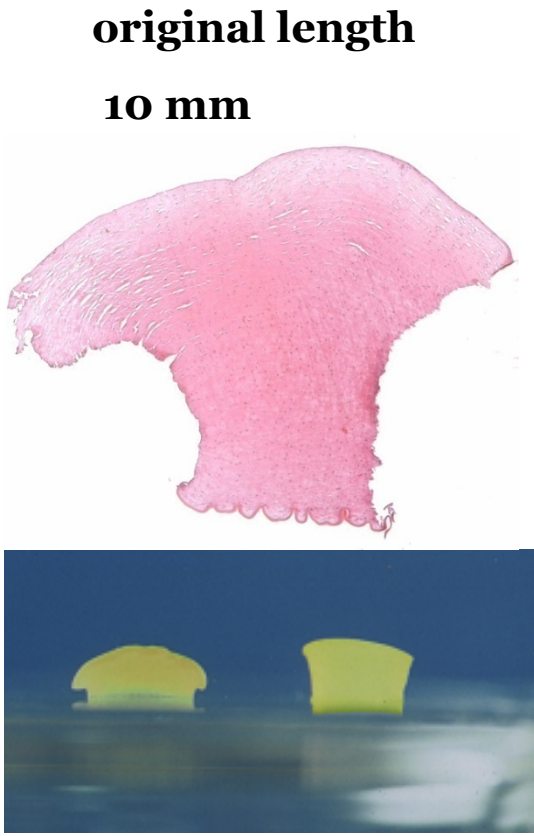




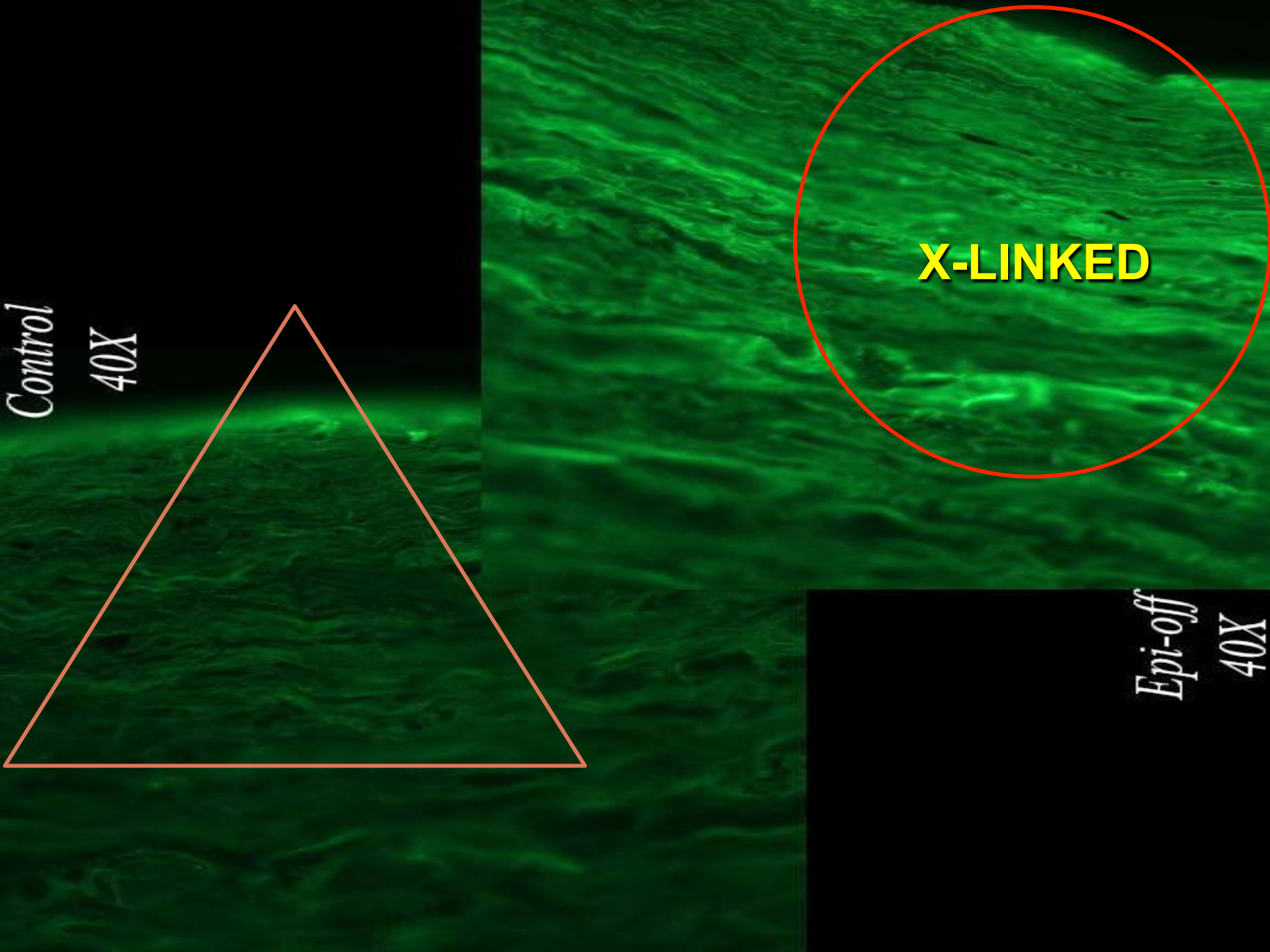
Spoerl, Wollensak, Seiler; Curr Eye Res, 2004

Cxl effects: increase of T (C°)





Spoerl, Wollensak, Seiler: Ophthalmologica 2004


X-LINKED
Control
40X
Epi-off
40X

Siena: first italian study
Secondo Studio Internazionale dopo Dresda

J CATARACT REFRACT SURG - **VOL 32, MAY 2006**

Parasurgical therapy for keratoconus by riboflavin–ultraviolet type A rays induced cross-linking of corneal collagen

Preliminary refractive results in an Italian study

Aldo Caporossi, MD, Stefano Baiocchi, MD, Cosimo Mazzotta, MD,
Claudio Traversi, MD, Tomaso Caporossi, MD

CONCLUSIONS: REFRACTIVE RESULTS SHOWED A REDUCTION OF ABOUT 2.5 D IN THE MEAN SPHERICAL EQUIVALENT, TOPOGRAPHICALLY CONFIRMED BY THE REDUCTION IN MEAN K. RESULTS OF SURFACE ABERROMETRIC ANALYSIS SHOWED IMPROVEMENT IN MORPHOLOGIC SYMMETRY WITH A SIGNIFICANT REDUCTION IN COMATIC ABERRATIONS.

SIENA 2006-2007 :
First industry-based riboflavin 0,1% (with dextran)

Siena EYE Cross Project: A. Caporossi, C. Mazzotta, S. Baiocchi

Siena 2004: First italian CXL Prototype

Caporossi-Mazzotta DUAL LED ARRAY CMS X LINKER, EXERION SAS-CNR, FIRENZE

Siena 2006: Second italian CXL Prototype

Five Led Array di Caporossi – Baiocchi – Mazzotta X Linker in collaboration with C.S.O. (Florence) FIRST CE mark!!!

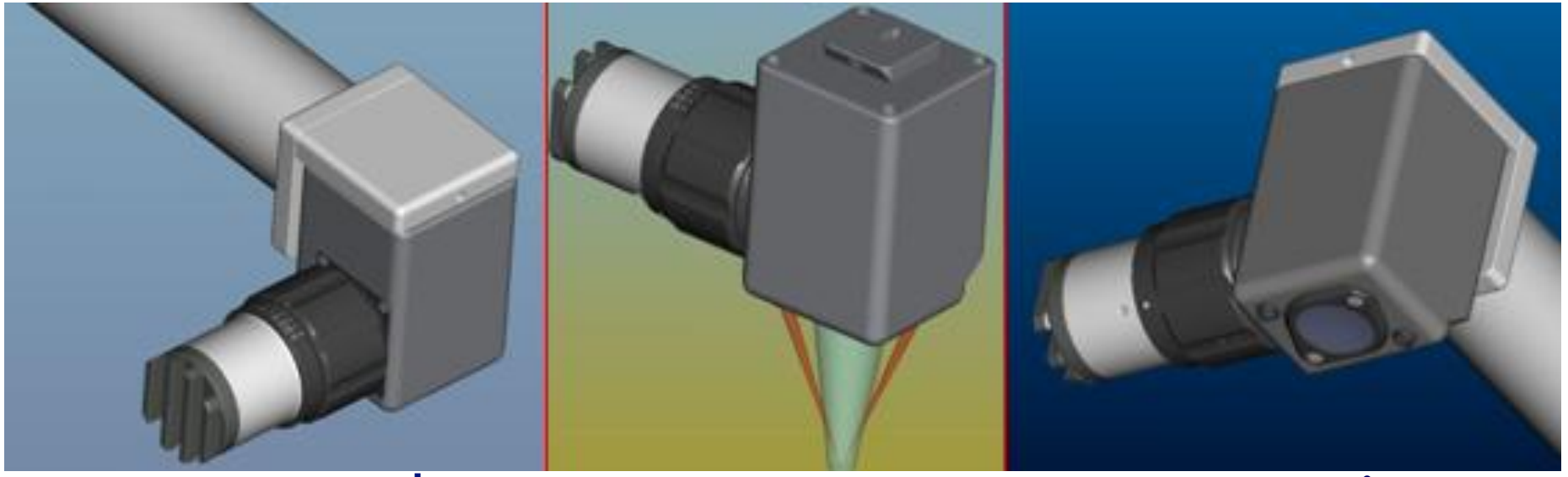
FIVE LED ARRAY CBM X LINKER, C.S.O.

SIENA 2007: The CBM Vega X Linker (mono-led)

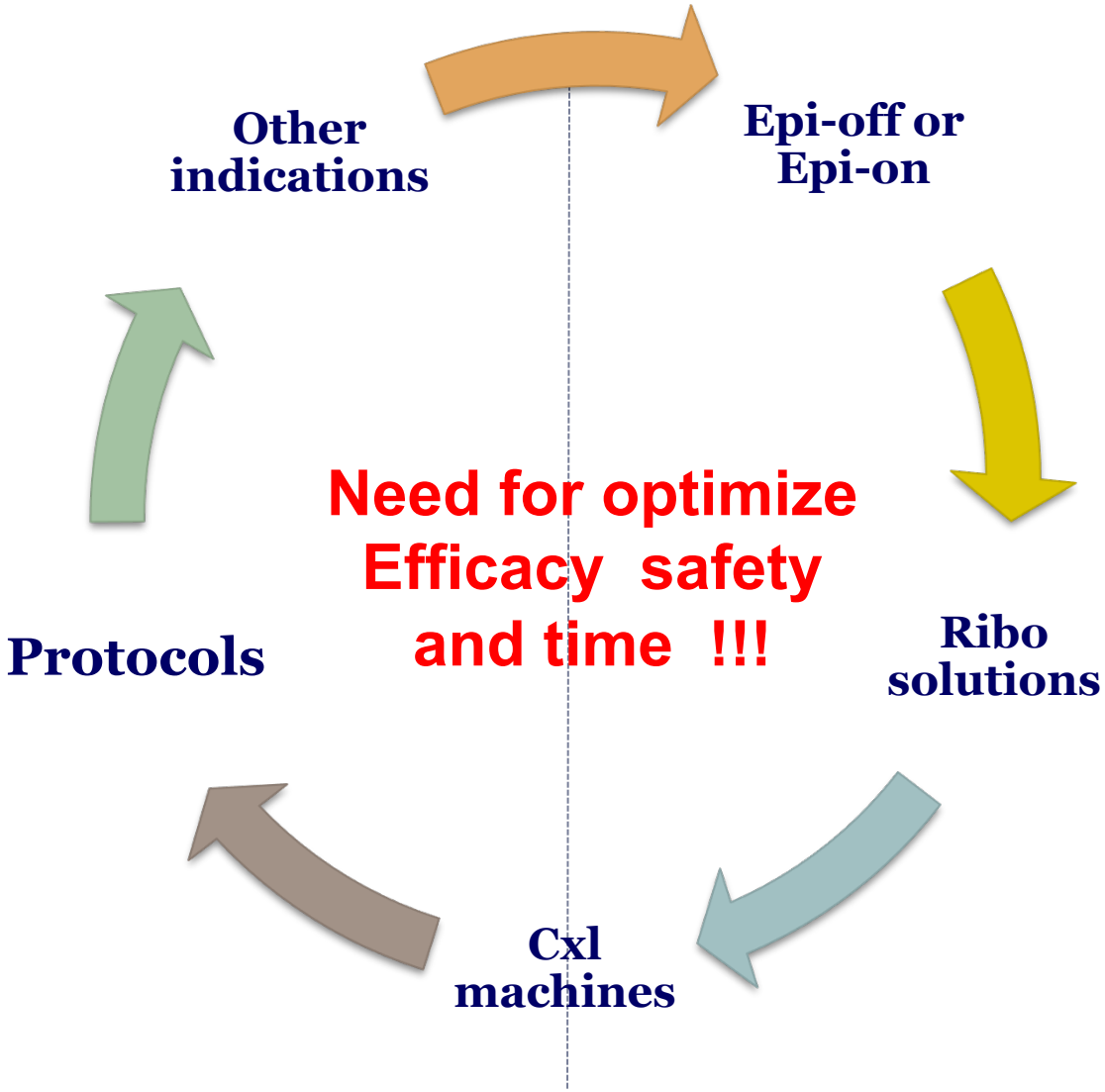
CBM VEGA X LINKER C.S.O.

FIRST WORLD MACHINE CE MARKED ONE-LED

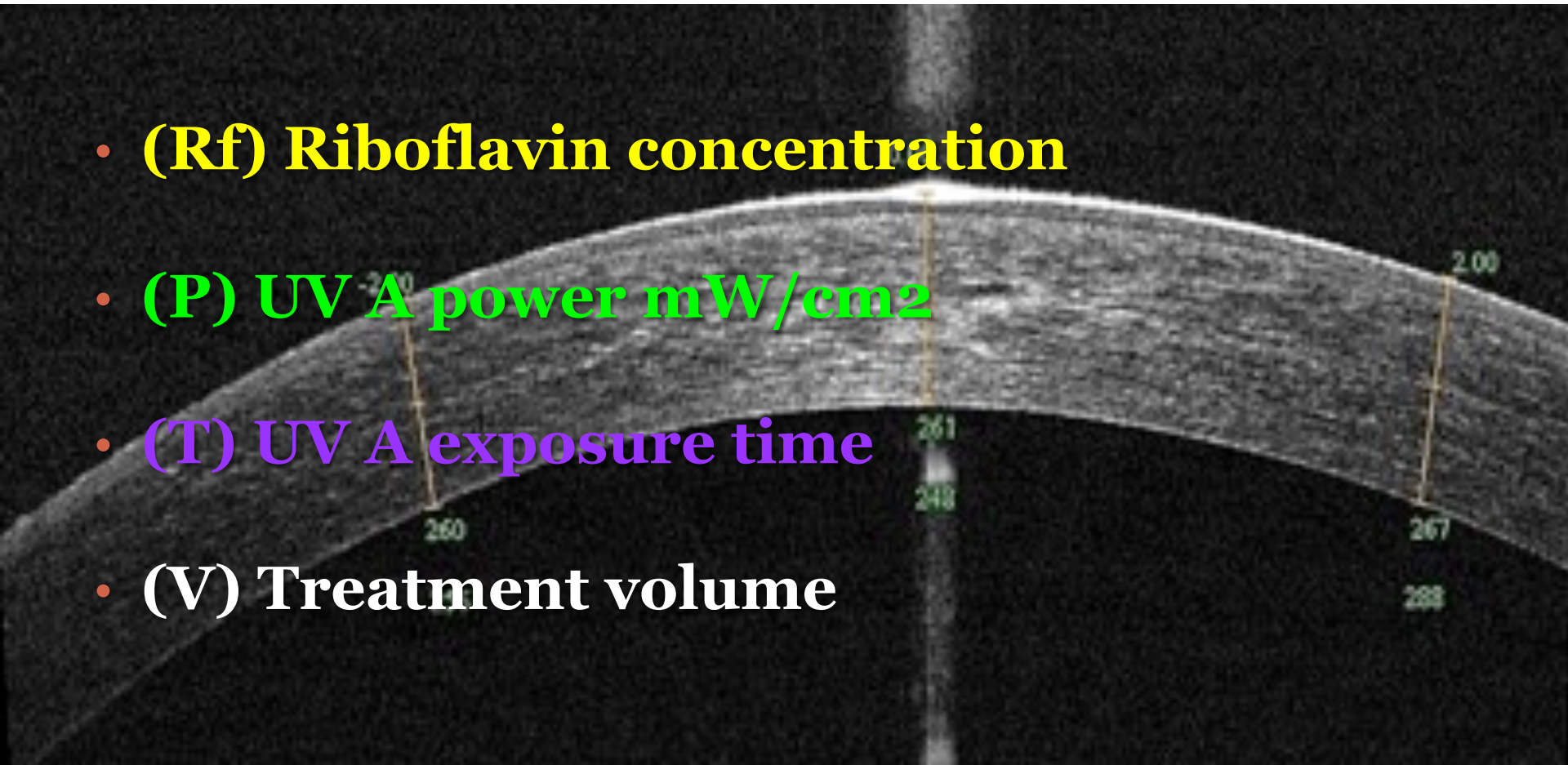
WITH REAL TIME COAXIAL MICRO-CAMERA



The present of CXL: hot topic!!!



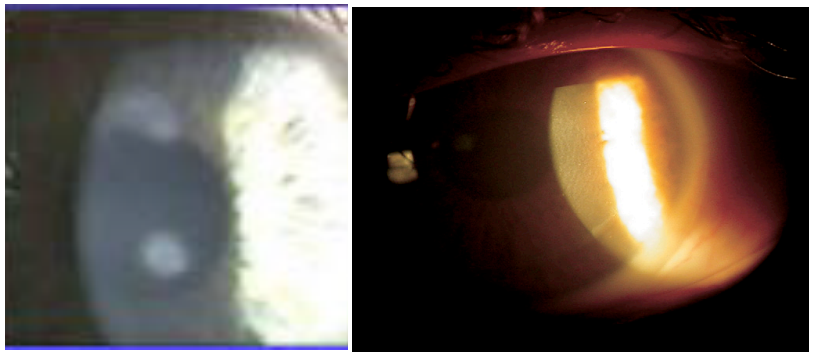
The present of CXL: more EB science but… Some alchemy !!!



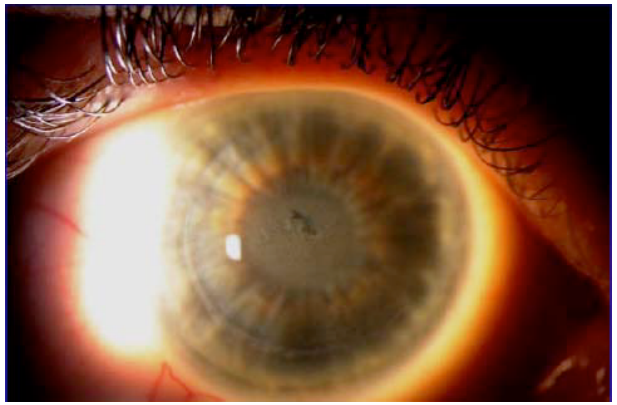
- (Rf) Riboflavin concentration
- (P) UV A power mW/cm2
- (T) UV A exposure time
- (V) Treatment volume

Epi-off CXL complication...

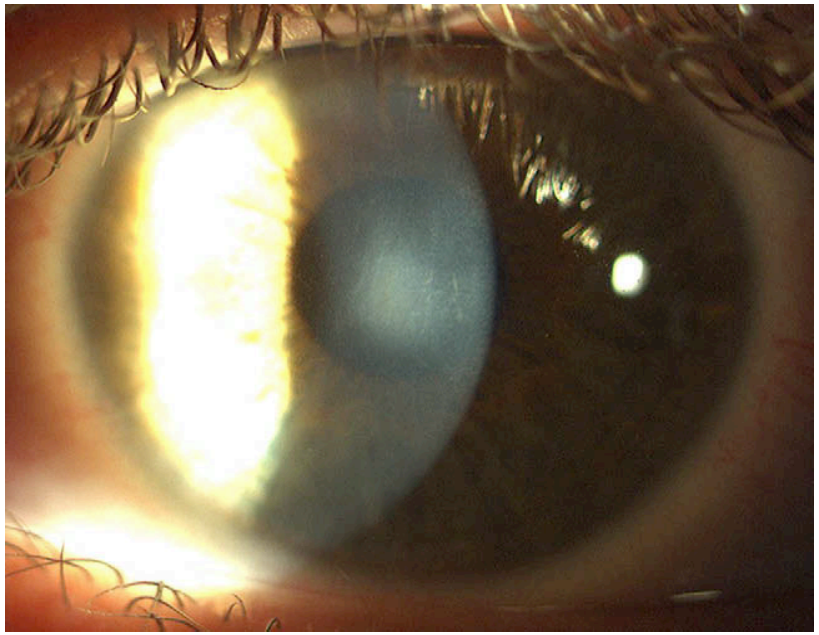
(Dextran 20% riboflavin)



Nummular or lamellar keratitis



Corneal edema



Stromal scar

Complication and failure rates after corneal crosslinking

Tobias Koller, MD, Michael Mrochen, PhD, Theo Seiler, MD, PhD

RESULTS: The study evaluated 117 eyes of 99 patients; approximately 90% completed the 12-month follow-up. The complication rate (percentage of eyes losing 2 or more Snellen lines) was 2.9% (95% confidence interval, 0.6%-8.5%). The failure rate of CXL (percentage of eyes with continued progression) was 7.6%. Age older than 35 years and a preoperative CDVA better than 20/25 were identified as significant risk factors for complications. A high preoperative maximum keratometry (K) reading was a significant risk factor for failure. Sterile infiltrates were seen in 7.6% of eyes and central stromal scars, in 2.8%.

CONCLUSIONS: Results indicate that changing the inclusion criteria may significantly reduce the complications and failures of CXL. A preoperative maximum K reading less than 58.00 diopters may reduce the failure rate to less than 3%, and restricting patient age to younger than 35 years may reduce the complication rate to 1%.

Limits of the Conventional CXL procedure

- Time consuming procedure: 1 hour
- Thin corneas with minimum corneal thickness under 400 (increased risk of endothelial cell loss)
- Corneal dehydration with Riboflavin 0.1% plus hmw 500-000 D - Dextran 20% solutions
- Intraoperative corneal thickness reduction with higher risk of haze development or endothelial damage (especially for thin corneas with borderline thinnest point values (≤ 450 µm with epithelium).
- High riboflavin concentration near endothelium in prolonged soaking time (oxidative damage)

Am J Ophthalmol. 2012 Jan;153(1):24-8. doi: 10.1016/j.ajo.2011.05.036. Epub 2011 Sep 8.

Corneal collagen cross-linking with riboflavin and ultraviolet-A irradiation in patients with thin corneas.

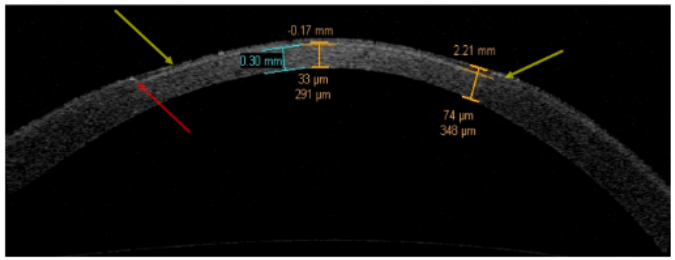
CXL in thin corneas with minimum corneal thickness less than 400 µm after epithelial removal seems to result in a significant endothelial cell density decrease postoperatively. This finding was not related to other

Ophthalmology. 2009 Dec;116(12):2336-9. doi: 10.1016/j.ophtha.2009.09.018. Epub 2009 Oct 22.

Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation.

Kymionis GD, Kounis GA, Portaliou DM, Grentzelos MA, Karavitaki AE, Coskunseven E, Jankov MR, Pallikaris IG.

AMERICAN JOURNAL OF OPHTHALMOLOGY



Am J Ophthalmol. 2014 Feb 26. pii: S0002-9394(14)00125-1. doi: 10.1016/j.ajo.2014.02.042. [Epub ahead of print]

Intraoperative Corneal Thickness Measurement by Optical Coherence Tomography in Keratoconic Patients Undergoing Corneal Collagen Cross-Linking.

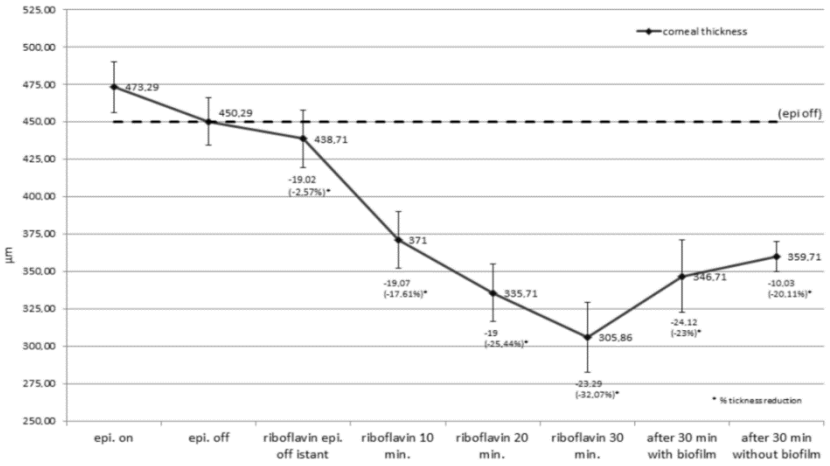
Mazzotta C[1], Caragiuli S[2].

RESULTS: The most significant decrease in thinnest point measurement was detected meanly in the first 10 minutes of corneal soaking. In this interval, the minimum recommended in various studies, a mean reduction of - 79.28 µm (- 17.61% of initial thinnest point value after removal of the epithelium) was recorded. No adverse events were recorded.

The most significant decrease in thickness was detected in the first 10 minutes of corneal soaking addressing the concept that prolonged soaking, over a minimum of 10 minutes, is not always recommended

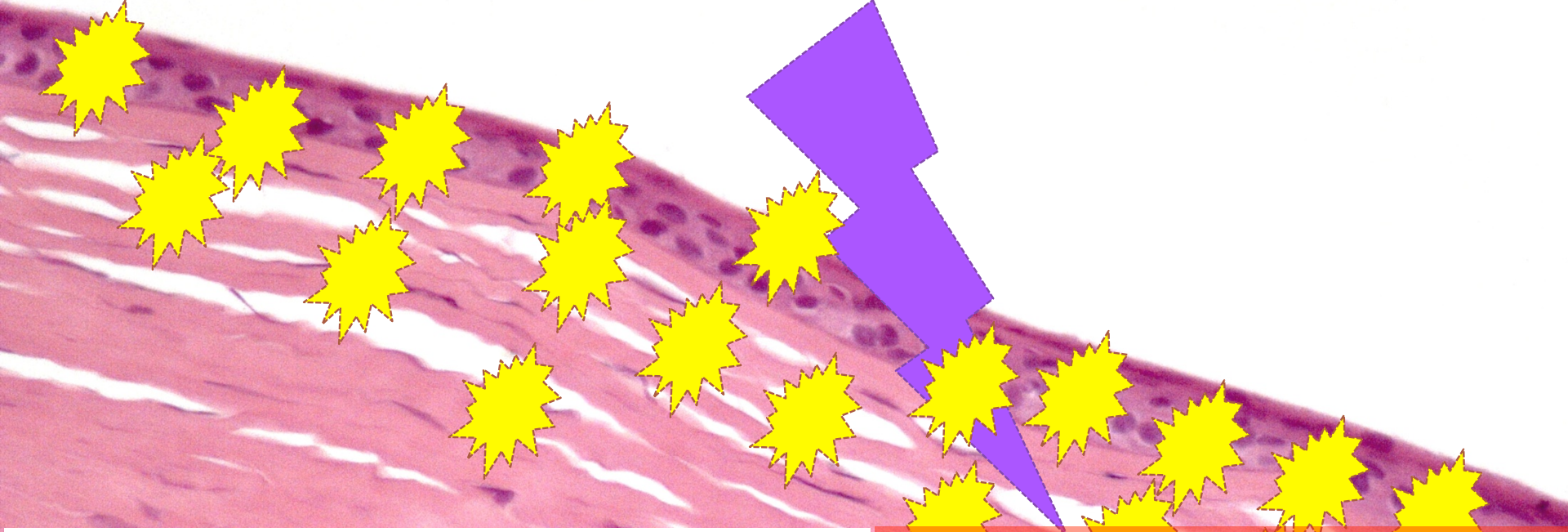
Corneal imbibition with classic Riboflavin 0.1%-Dextran 20% solutions should be prolonged to a maximum of 10 minutes (considering a 6% decrement corresponding about - 30 µm less on average at each 10 minutes interval, after the first 10 minutes).
10 minutes are sufficient to guarantee CXL safety and efficacy.

Stromal thickness should be measured before starting UV irradiation.

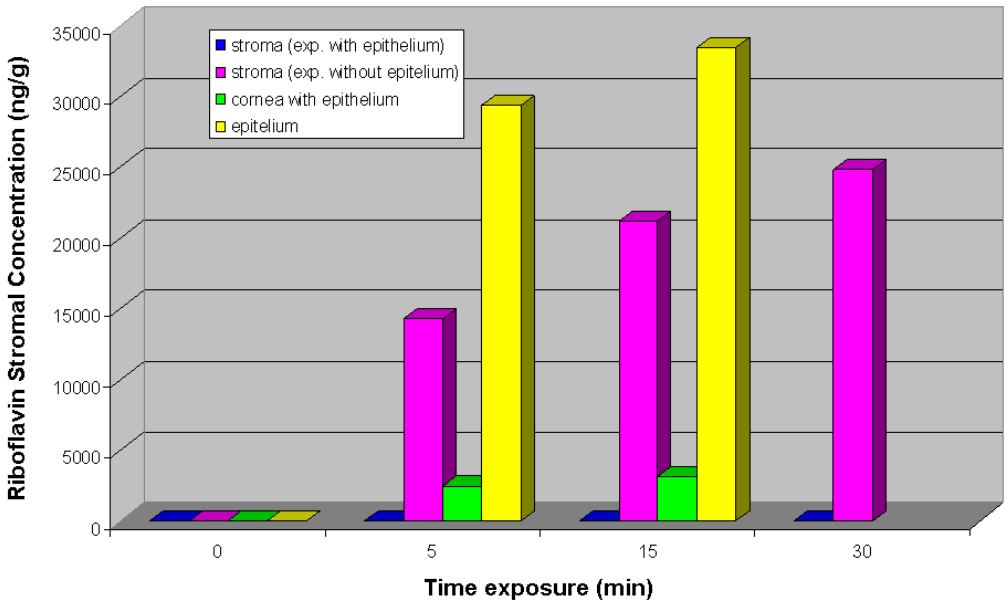


Epi-on or epi-off ???
This is the question !!!

	Epi-on	Epi-off
Pain	+	+++
Recovery time	+++	-
Infection risk	-	+
Pers epithelial defect	-	+
Endothelitis	?	+
HSV keratitis induction	?	+
Corneal Haze	?	+
Riboflavin penetration	80-100 micron	250-330 micron
Efficacy	+?	+++



With epithelium Vs Without epithelium stromal, epithelial and corneal concentration (numerical scale)



Epithelium in situ soaked by riboflavin induces a «shield» effect absorbing over 90% of UV, confining CXl penetration in a surface level under 100 µm even after ionthophoresis.

The epithelium should be always removed to ensure a deep interaction between UV A, Riboflavin and collagen molecules, maximizing the CXL effect !!!

TE CXL up to date

CXL TE	Caporossi et al 2012	Leccisotti et al 2010 JRS	Koppen , Tassignon et al. JCRS 2012	Filippello et al 2012 JCRS	Buzzonetti 2012
N° eyes	26	51	53	20	11
Age (range)	11-26	18-40	12-46	12-42	8-18
Follow-up m	24 m	12 m	18 m	18 m	18 m
Δ UCVA m	- 0.03 SL Epi-off + 0.16 SL	n.a.	n.a.	+ 0.13	n.a.
Δ BSCVA m	- 0.01 SL Epi-off + 0.22 SL	+ 0.1	- 0.02	+ 0.1	+ 0.24
Δ K MAX m	+ 0.51 D Epi-off - 1.0 D	+ 0.51	+ 0.30	- 3.07	+ 3.9
Δ Coma m	+ 0.05 µm Epi-off - 0.72 µm	n.a.	n.a	-0.1 µm	+ 0.53 µm

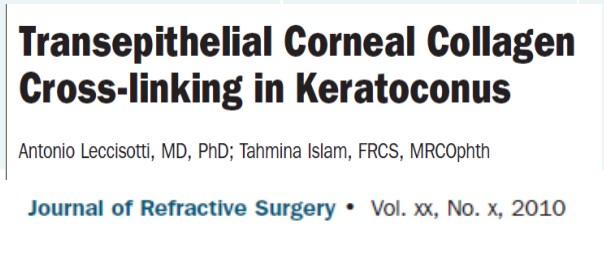

Eur J Ophthalmol 2012; 00 (00): 000-000 DOI: 10.5301/ejo.5000125

ORIGINAL ARTICLE

Transepithelial corneal collagen crosslinking for keratoconus: qualitative investigation by in vivo HRT II confocal analysis

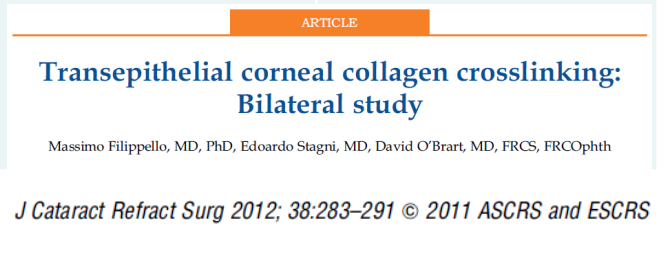
Aldo Caporossi[1], Cosimo Mazzotta[1], Stefano Baiocchi[1], Tomaso Caporossi[2], Anna Lucia Paradiso[1]

[1]Department of Ophthalmology, Siena University, Siena - Italy
[2]Department of Ophthalmology, Rome Catholic University, Rome - Italy


Transepithelial Corneal Collagen Cross-linking in Keratoconus

Antonio Leccisotti, MD, PhD; Tahmina Islam, FRCS, MRCOphth

Journal of Refractive Surgery • Vol. xx, No. x, 2010


ARTICLE

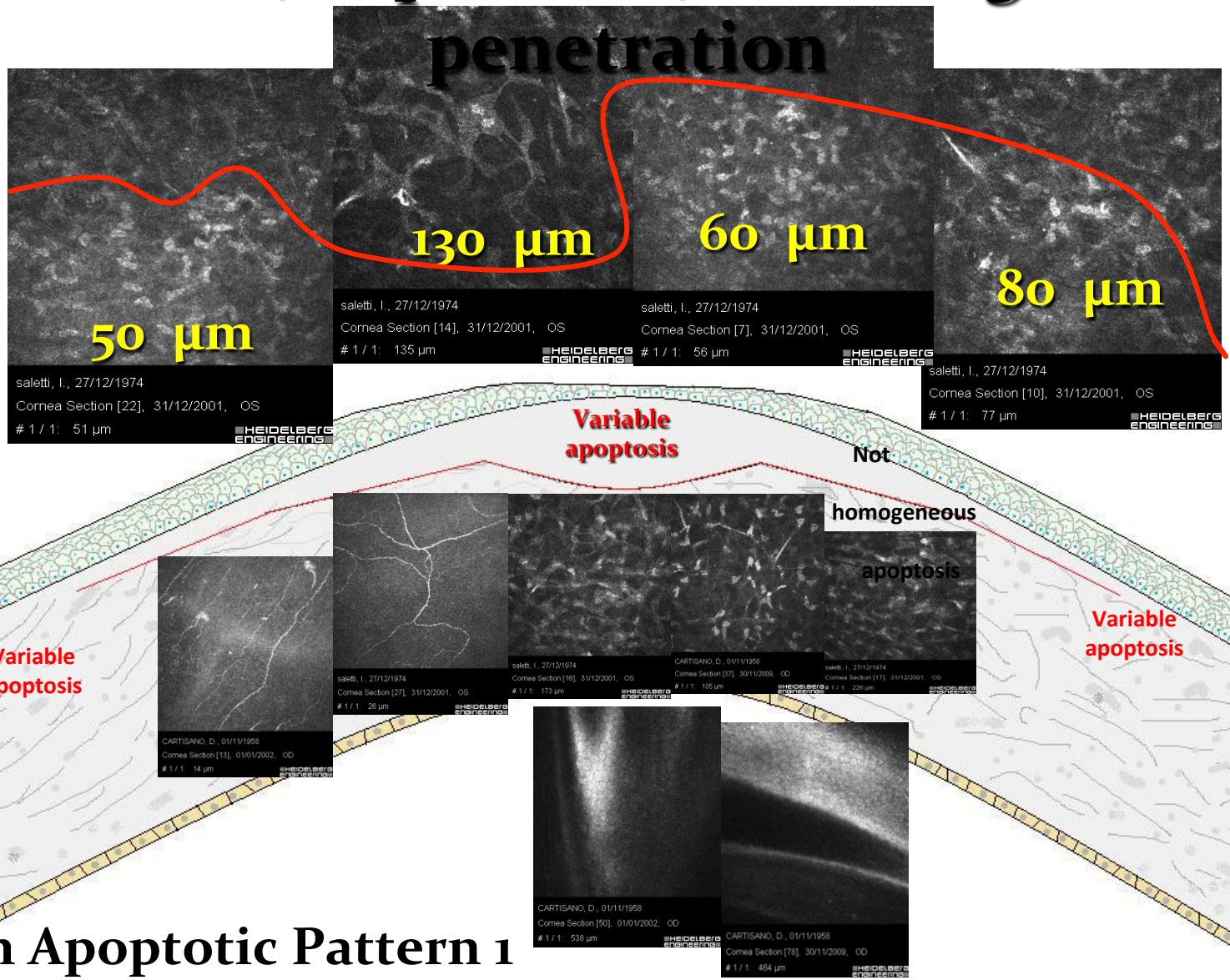
Transepithelial corneal collagen crosslinking: Bilateral study

Massimo Filippello, MD, PhD, Edoardo Stagni, MD, David O'Brart, MD, FRCS, FRCOphth

J Cataract Refract Surg 2012; 38:283–291 © 2011 ASCRS and ESCRS

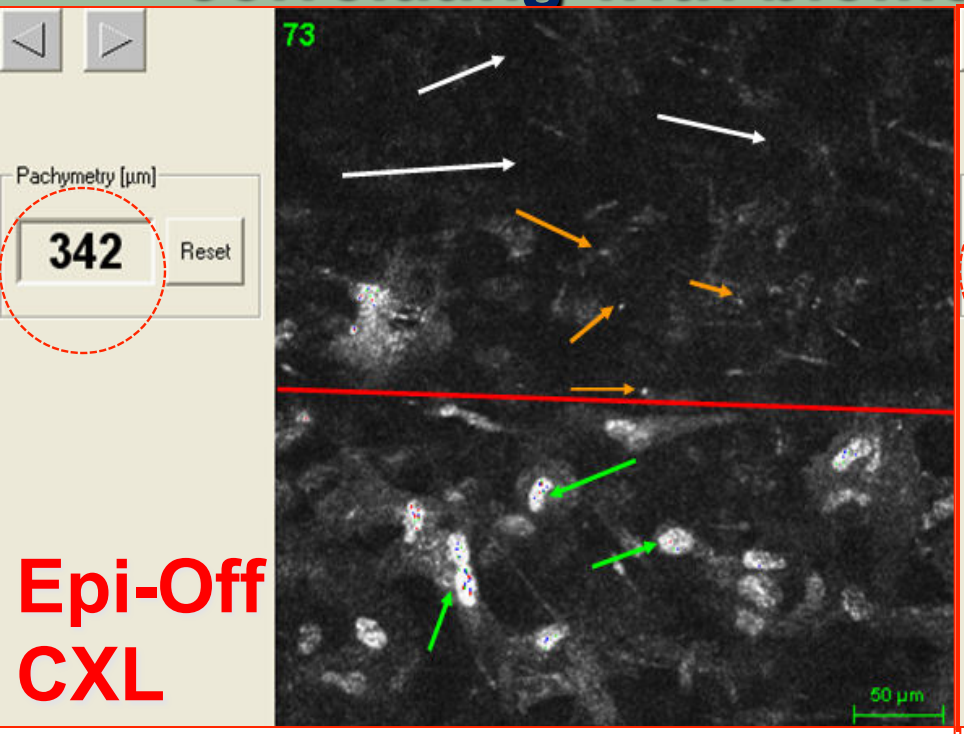
Epi-On CXL Apoptotic Pattern

Variable, Superficial, Inhomogeneous

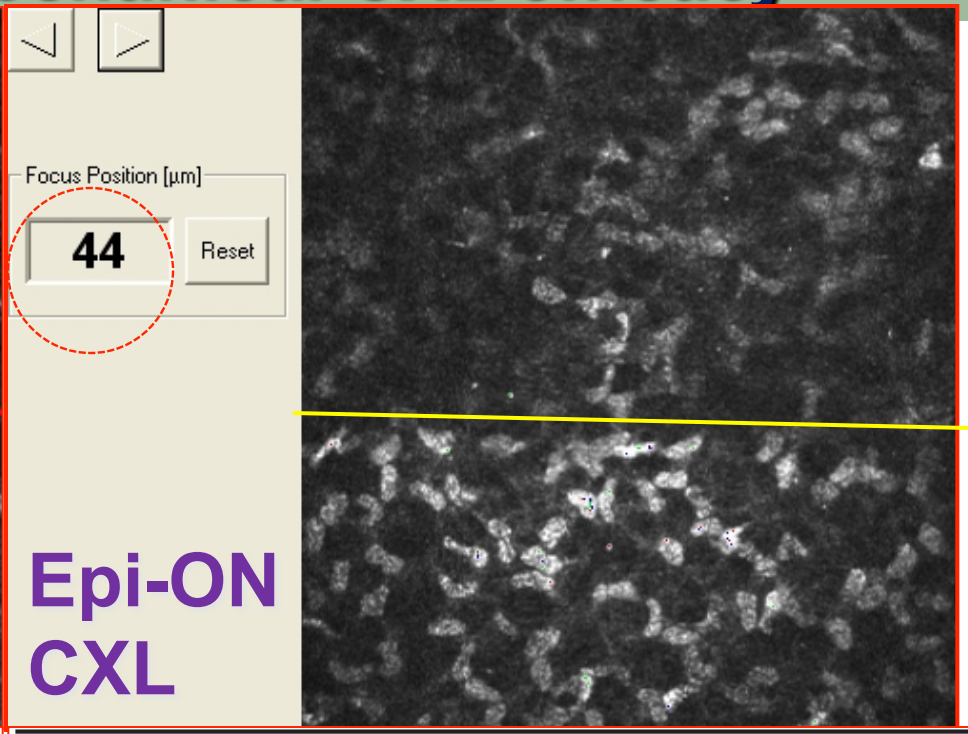


Epi-On Apoptotic Pattern 1

In Vivo Stromal Penetration: Epi-OFF and Epi-On correlating with biomechanical CXL efficacy



Epi-Off CXL



Epi-ON CXL

CLINICAL SCIENCE

Cornea. 2007 May;26(4):390-7

Treatment of Progressive Keratoconus by Riboflavin-UVA–Induced Cross-Linking of Corneal Collagen

Ultrastructural Analysis by Heidelberg Retinal Tomograph II In Vivo Confocal Microscopy in Humans

Cosimo Mazzotta, PhD, Angelo Balestrazzi, PhD,* Claudio Traversi, MD,* Stefano Baiocchi, PhD,* Tomaso Caporossi, MD,† Cristina Tommasi, MD,* and Aldo Caporossi, MD**

ORIGINAL ARTICLE

Eur J Ophthalmol. 2012 Jul;22 Suppl 7:81-8.

Transepithelial corneal collagen crosslinking for keratoconus: qualitative investigation by in vivo HRT II confocal analysis

Aldo Caporossi[1], Cosimo Mazzotta[1], Stefano Baiocchi[1], Tomaso Caporossi[2], Anna Lucia Paradiso[1]

[1]Department of Ophthalmology, Siena University, Siena - Italy
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Transepithelial corneal collagen crosslinking for progressive keratoconus: 24-month clinical results

Aldo Caporossi, MD, FRCS, Cosimo Mazzotta, MD, PhD, Anna Lucia Paradiso, MD, Stefano Baiocchi, MD, PhD, Davide Marigliani, MD, Tomaso Caporossi, MD

WHAT WAS KNOWN

- In vitro and in vivo studies show that the effect of transepithelial CXL is limited at about one third that of the epi-off procedure in terms of biomechanical and functional efficacy.

WHAT THIS PAPER ADDS

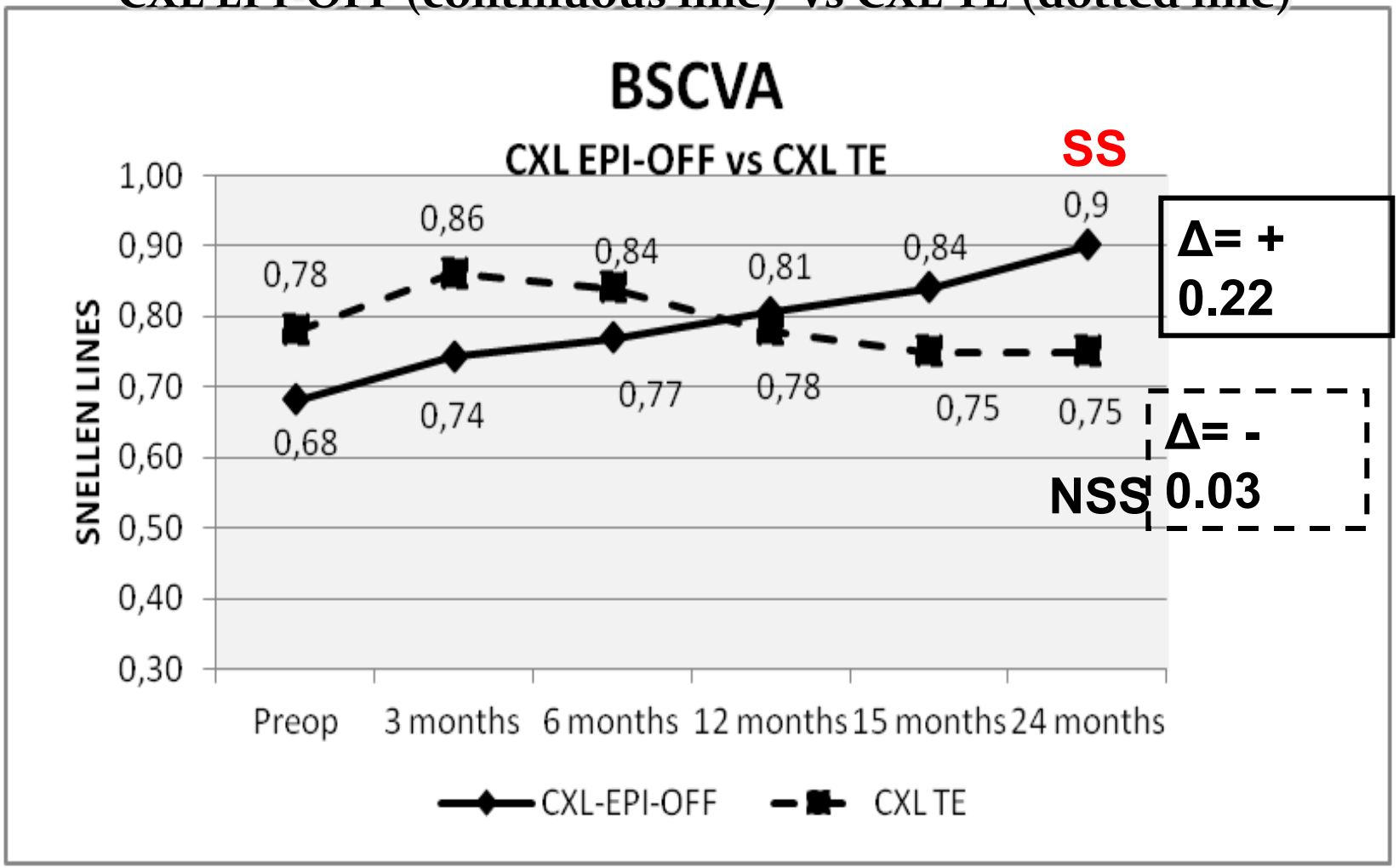
- Results indicate that transepithelial CXL is not indicated for pediatric patients 18 years and younger or for patients younger than 26 years with progressive keratoconus and a thinnest corneal point over 400 μm due to its limited efficacy in terms of keratoconus stabilization and functional improvement after 24 months of follow-up.

J Cataract Refract Surg 2013; 39:1157–1163 © 2013 ASCRS and ESCRS

Cross-Linking EPI-OFF vs TE

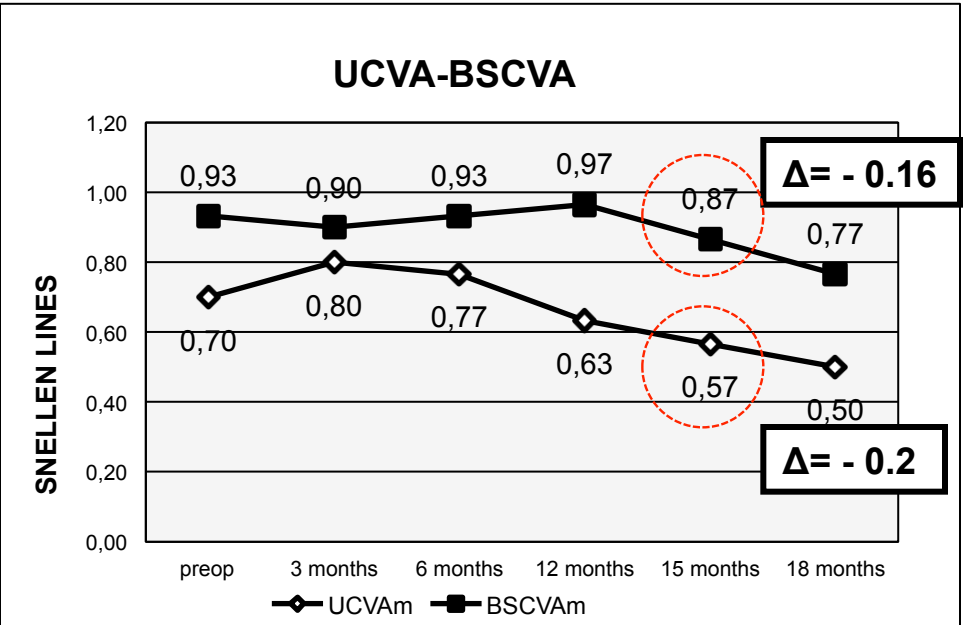
follow-up 24 m

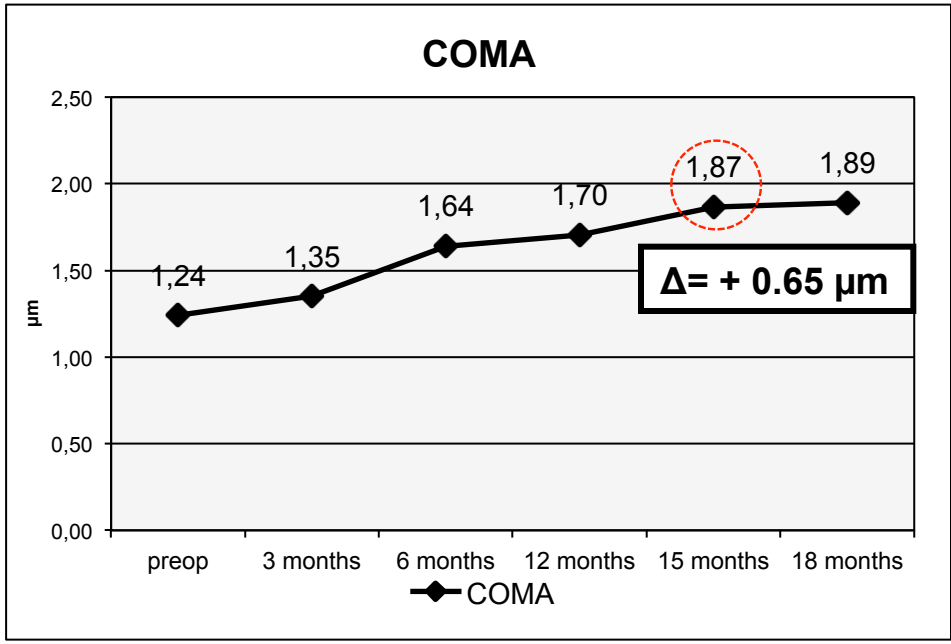
CXL EPI-OFF (continuous line) vs CXL TE (dotted line)

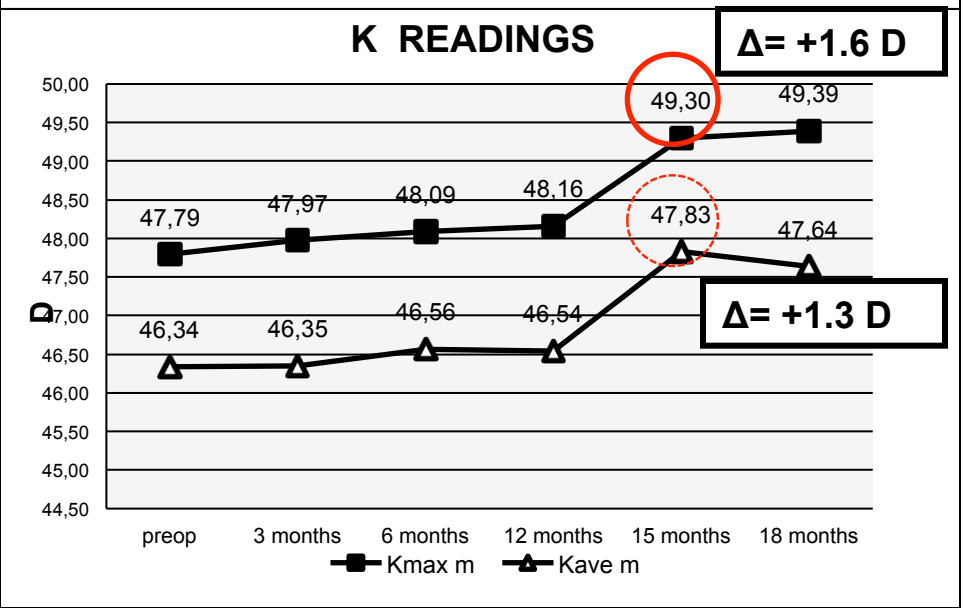


SS: Statistically Significant **NSS: not statistically significant**

Re-treatments at 24 months f-up

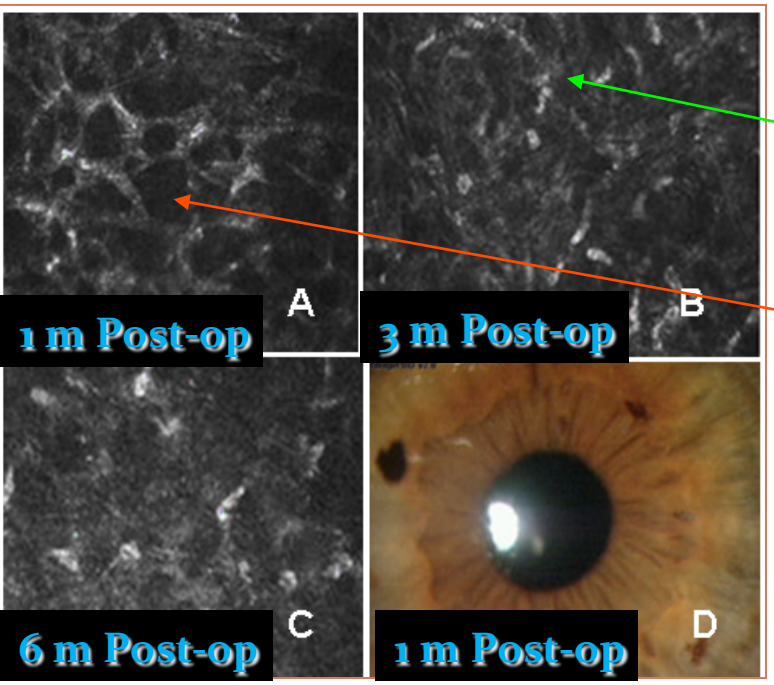


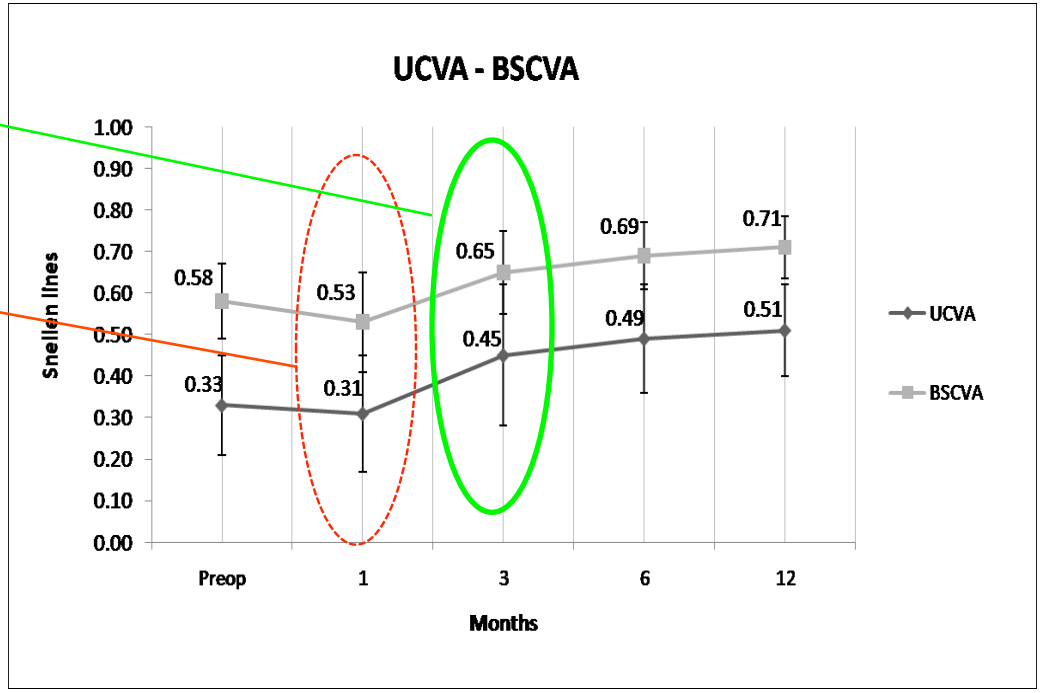




Over 90 % retreated after Epi-On
At 24 months follow-up under 18 y
Over 40 % retreated after epi-on
At 24 months follow-up over 18 y
None retreated after Epi-OFF !!!

MORPHO-FUNCTIONAL CORRELATIONS
STROMAL HEALING AFTER CXL





In the 4-6 weeks post CXL there was a tendency (with statistically not significant values) towards slightly reduced visual acuity (VA) and more glare one month postoperatively.

Visual improvement generally started 4-6 weeks after treatment becoming statistically significant between the third and sixth month.

Morphological and functional correlations in riboflavin UV A corneal collagen cross-linking for keratoconus

Cosimo Mazzotta,[1] Tomaso Caporossi,[2] Rosario Denaro,[1] Cristina Bovone,[1] Caterina Sparano,[1] Anna Paradiso,[1] Stefano Baiocchi[1] and Aldo Caporossi[1]

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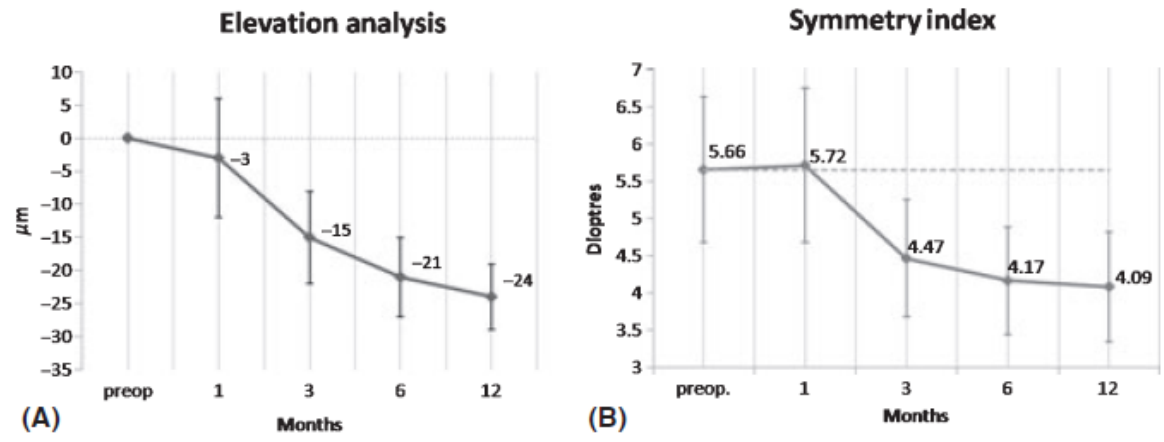
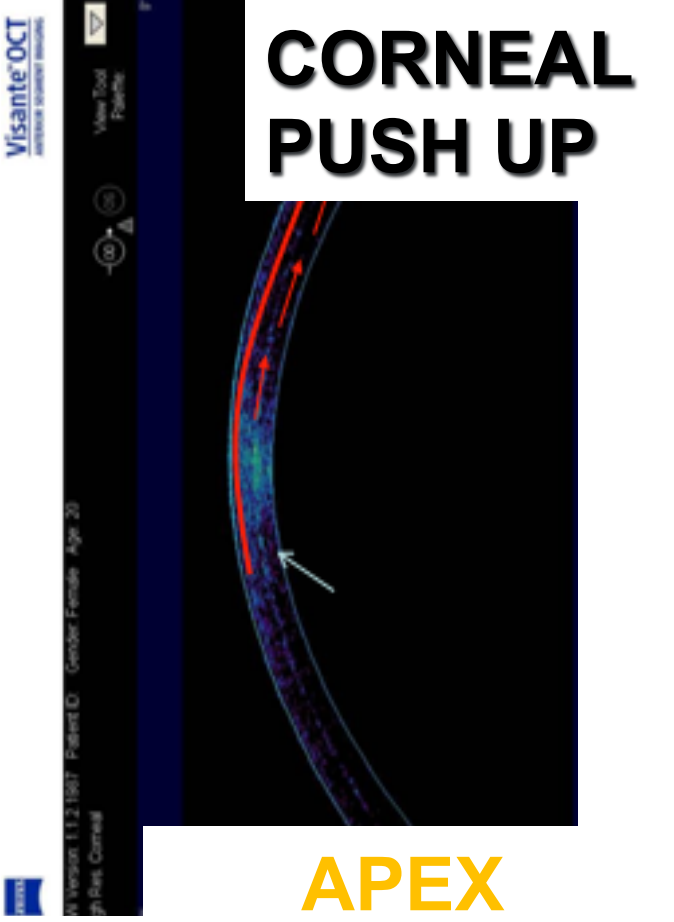
· Acta Ophthalmologica 2010 ·





How to enhance riboflavin penetration preserving epithelium ???

- Epithelium surgical disruption plus dextran riboflavin (S. Daya)
- Enhancers riboflavin solution: BAK, EDTA, trometamol
- Epithelium grid marking (P. Vinciguerra)
- Iontoforesis
- Epithelial island CXL (Mazzotta)
- Epithelium surgical disruption (pockmarking) plus enhancers??? (Rechichi)

Epithelial-disruption collagen crosslinking for keratoconus: One-year results

Miguel Rechichi, MD, Sheraz Daya, MD, Vincenzo Scorcia, MD, Alessandro Meduri, MD, Giovanni Scorcia, MD

PURPOSE: To evaluate the efficacy of epithelial-disruption collagen crosslinking (CXL) for progressive keratoconus using a corneal disruptor device and a riboflavin solution designed for a transepithelial technique.

SETTING: Magna Graecia University Eye Clinic, Catanzaro, Italy.

DESIGN: Prospective comparative case series.

METHODS: The most severely affected eye of patients with bilateral progressive keratoconus was treated. The fellow eye served as a control. Follow-up was 12 months. A corneal disruptor device was used to create pockmarks in the epithelium. Riboflavin solution was applied for 30 minutes and irradiation for 30 minutes. Three days postoperatively, patients were asked to assess the level of pain.

RESULTS: The study comprised 28 patients (mean age 28 years). The mean postoperative pain score was 4.3, 2.6, and 2.1 at 1 day, 2 days, and 3 days. The mean preoperative uncorrected (UDVA) and corrected (CDVA) distance visual acuities improved from 0.73 logMAR ± 0.21 (SD) and 0.30 ± 0.11 logMAR to 0.48 ± 0.15 logMAR and 0.25 ± 0.1 logMAR, respectively, at 12 months (P=.02). The mean spherical equivalent refraction decreased 0.96 diopter (D). The mean baseline apical keratometry, apical gradient curvature, average pupillary power, inferior–superior index, and cone area were 59.21 D, 8.91 D, 47.9 D, 11.49 mm^2, and 10.32 mm^2, respectively. At 12 months, these values were 56.18 D, 7.32 D, 41.34 D, 9.65 mm^2, and 7.75 mm^2, respectively. No adverse effects were observed.

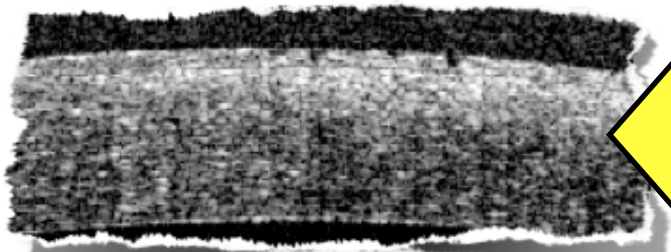
CONCLUSIONS: Corneal epithelial-disruption CXL was safe and effective in medium-term stabilization of keratoconus with an improvement in topographic and refractive parameters and less patient discomfort.

Financial Disclosure: No author has a financial or proprietary interest in any material or method mentioned.

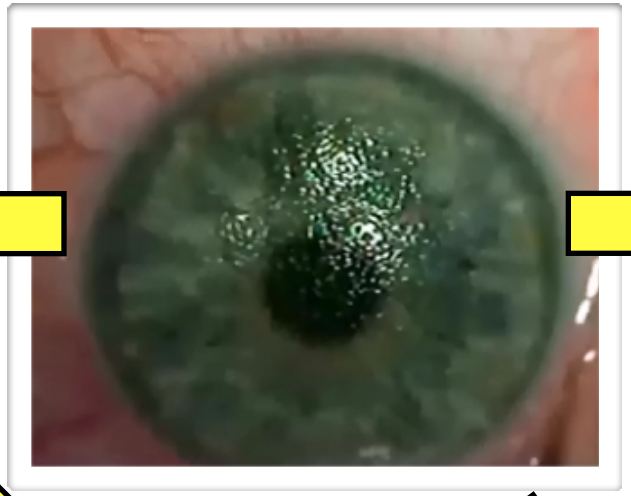
J Cataract Refract Surg 2013; ■:■–■

Online Video

Why epi-disruption?

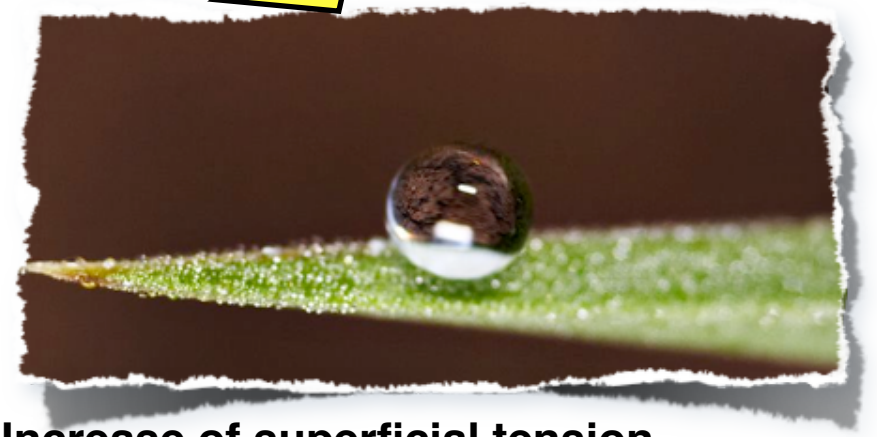


Enhance penetration through direct access to superficial stroma



Microvilli-like action

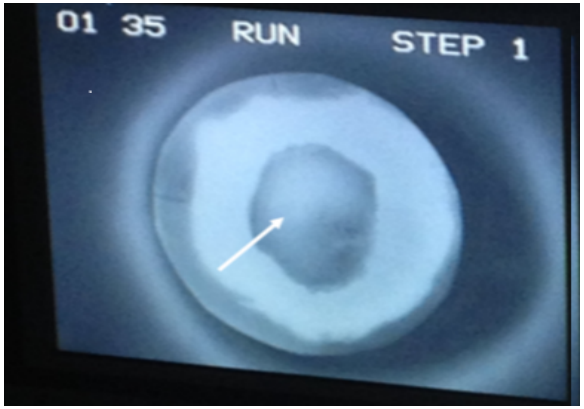
Preserve an epithelial “net” that promote a fast reepithelization

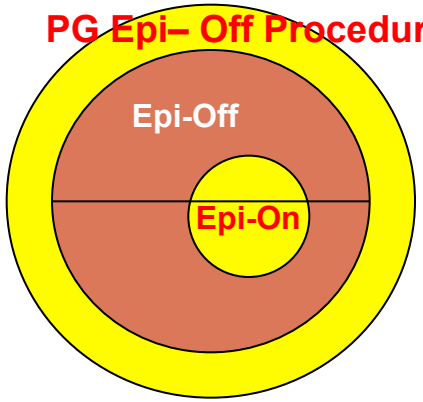


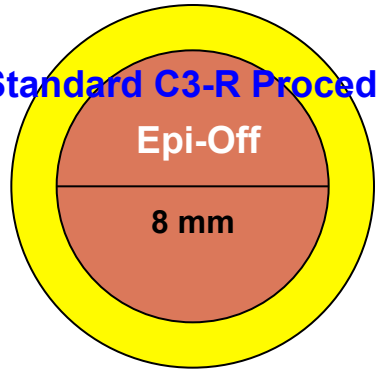
Increase of superficial tension

Pachymetry guided Mazzotta's Epithelial Island CXL EI-CXL) for thin corneas under 400

- Diameter 9 mm ring abrasion (peri-central Epi-Off) – apex Epi-on







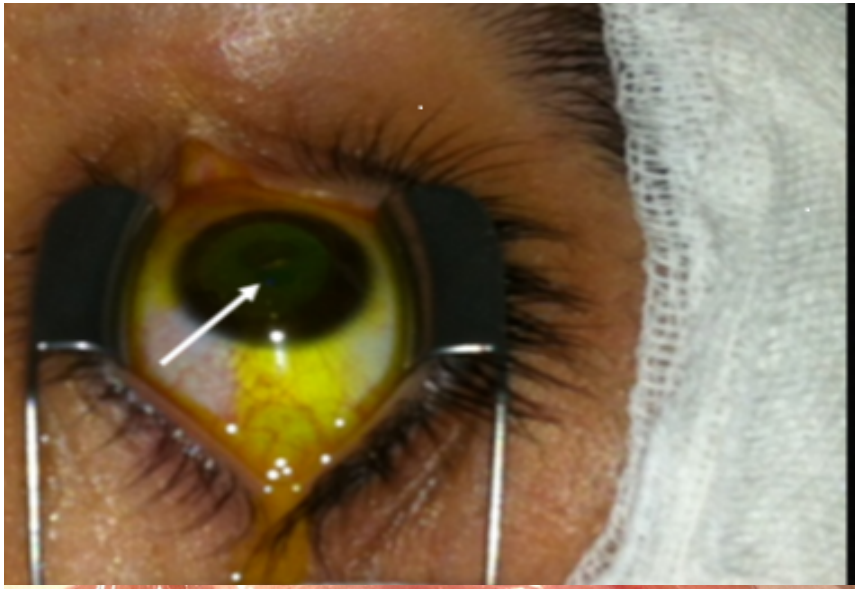
- Epithelial island 3 mm on the kone apex
- Preoperative Riboflavin 0,1% dextran freee corneal soaking for 10 minutes
- Standard UV A irradiation 3mW/cm² (6 steps of 5 minutes)
- Soft therapeutic contact lens bandage 4 days

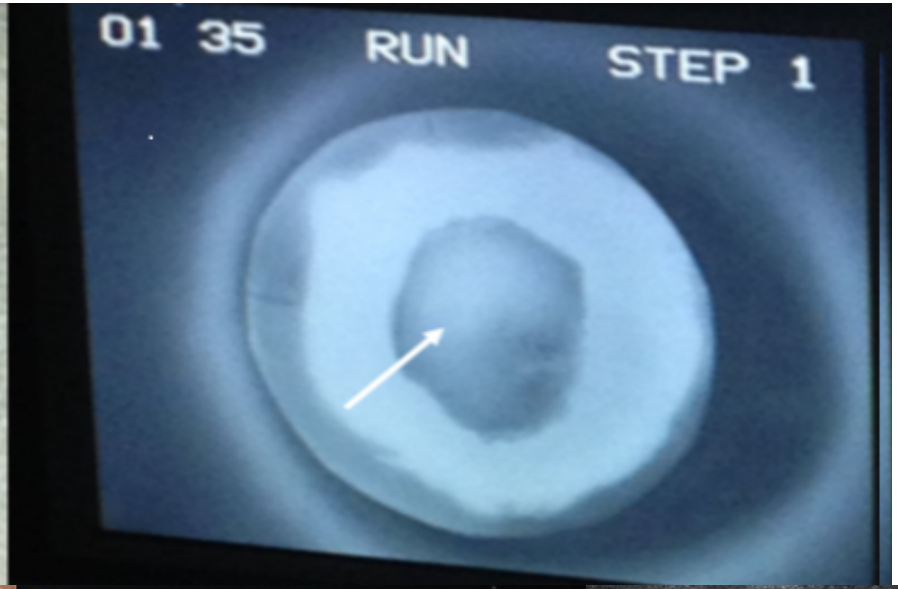
Journal: Clinical Ophthalmology

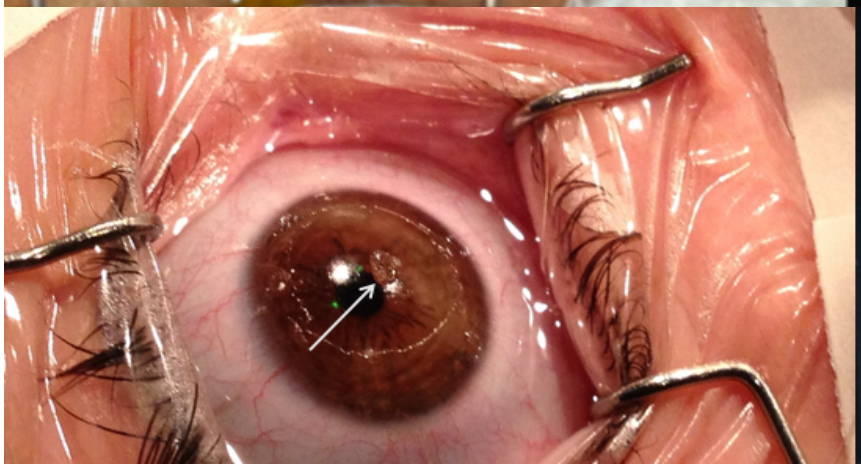
Paper Title: Customized Epithelial Debridement for Thin Ectatic Corneas Undergoing Corneal Cross-Linking: Epithelial Island Cross-Linking Technique (EI-CXL)

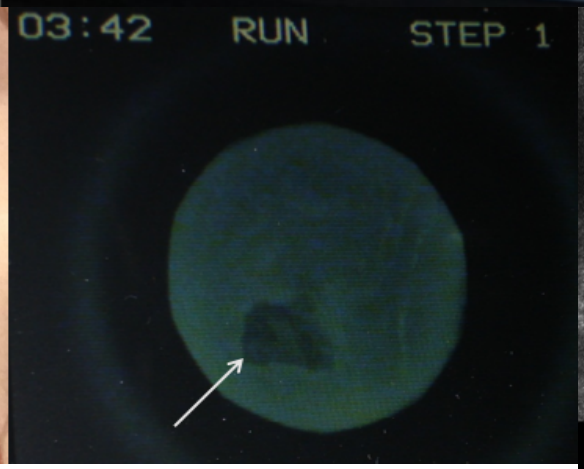
Authors: Cosimo Mazzotta MD, PhD and Vincenzo Ramovecchi MD

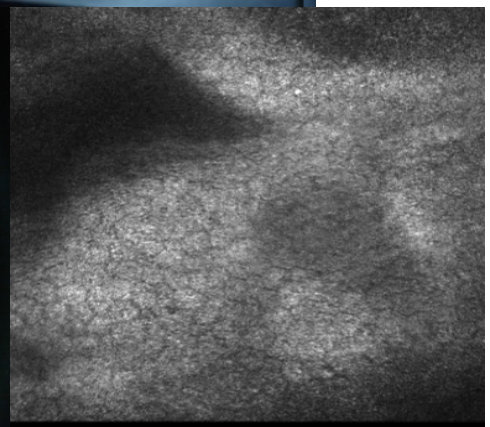
Accepted May 2014 in press



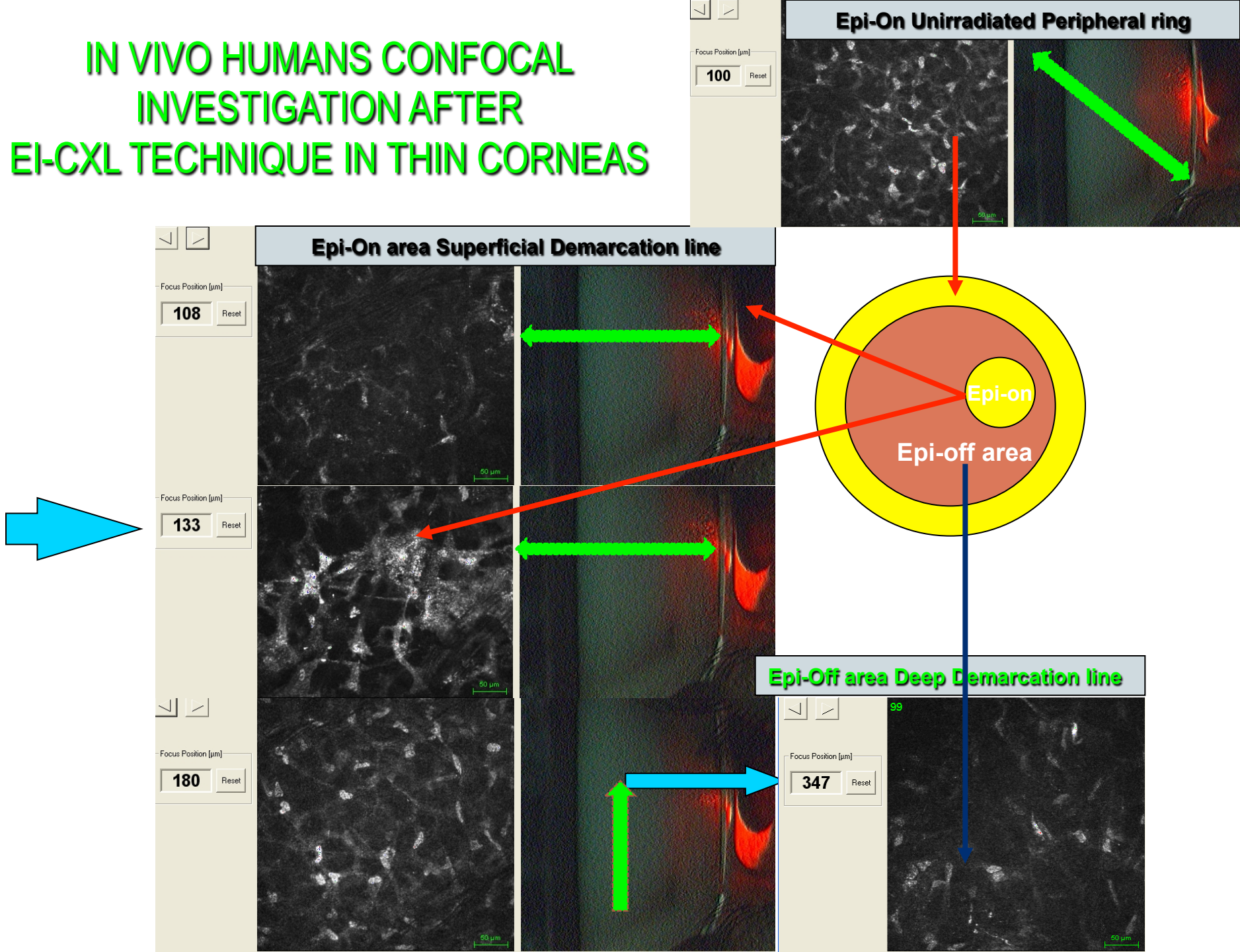




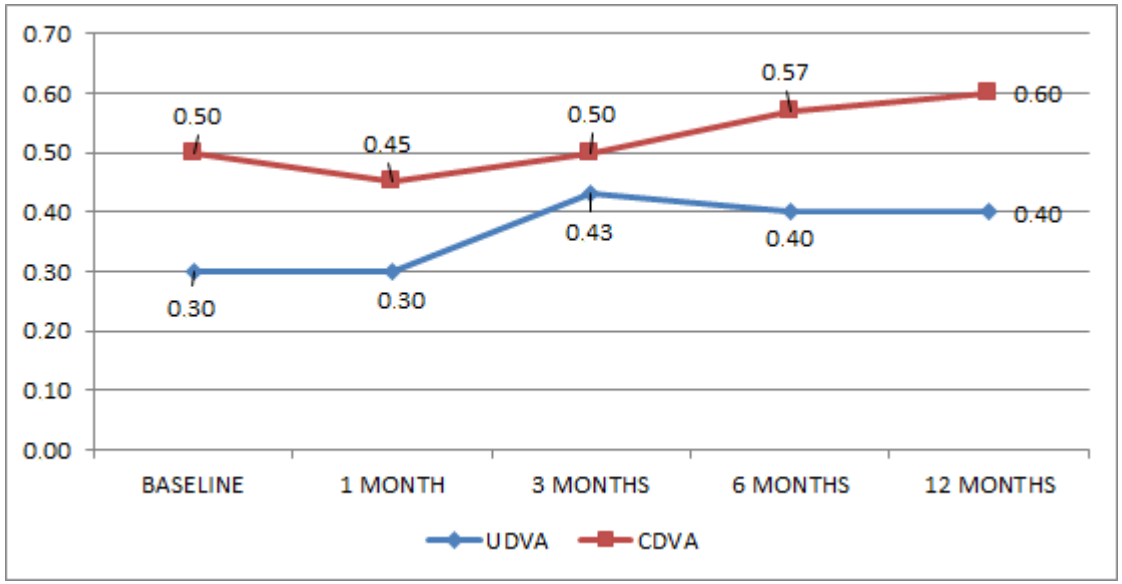




IN VIVO HUMANS CONFOCAL INVESTIGATION AFTER EI-CXL TECHNIQUE IN THIN CORNEAS



MORPHO-FUNCTIONAL CORRELATIONS IN EPITHELIAL ISLAND CXL FOR THIN CORNEAS



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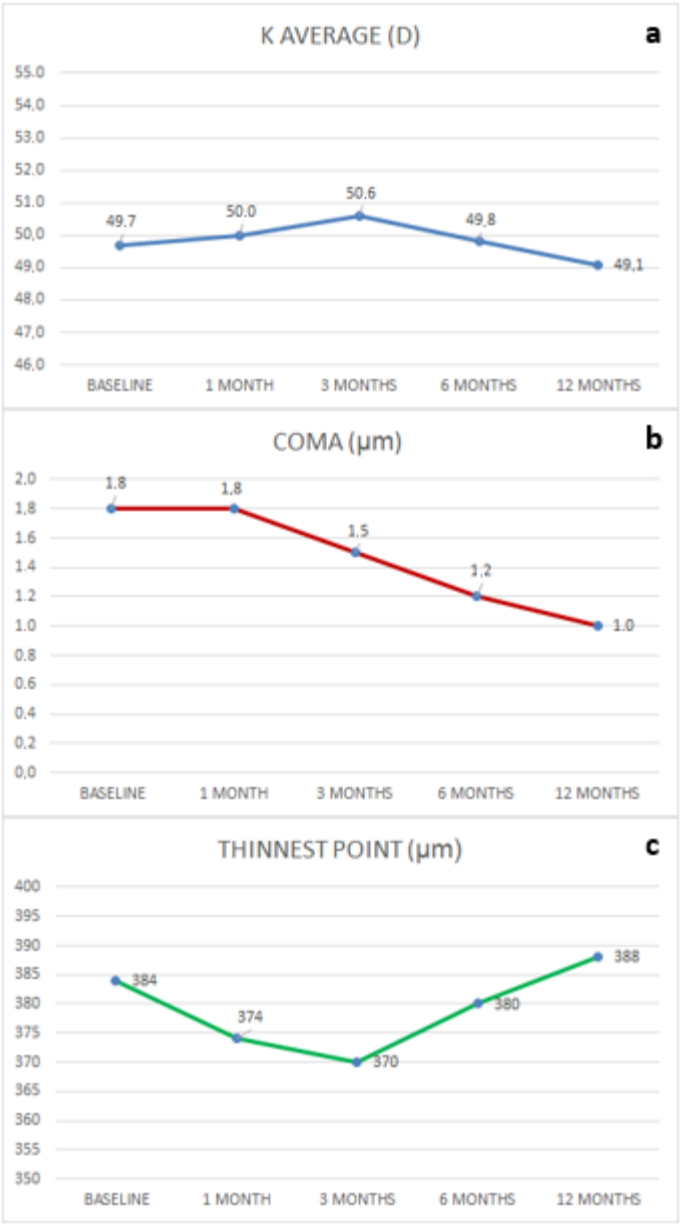
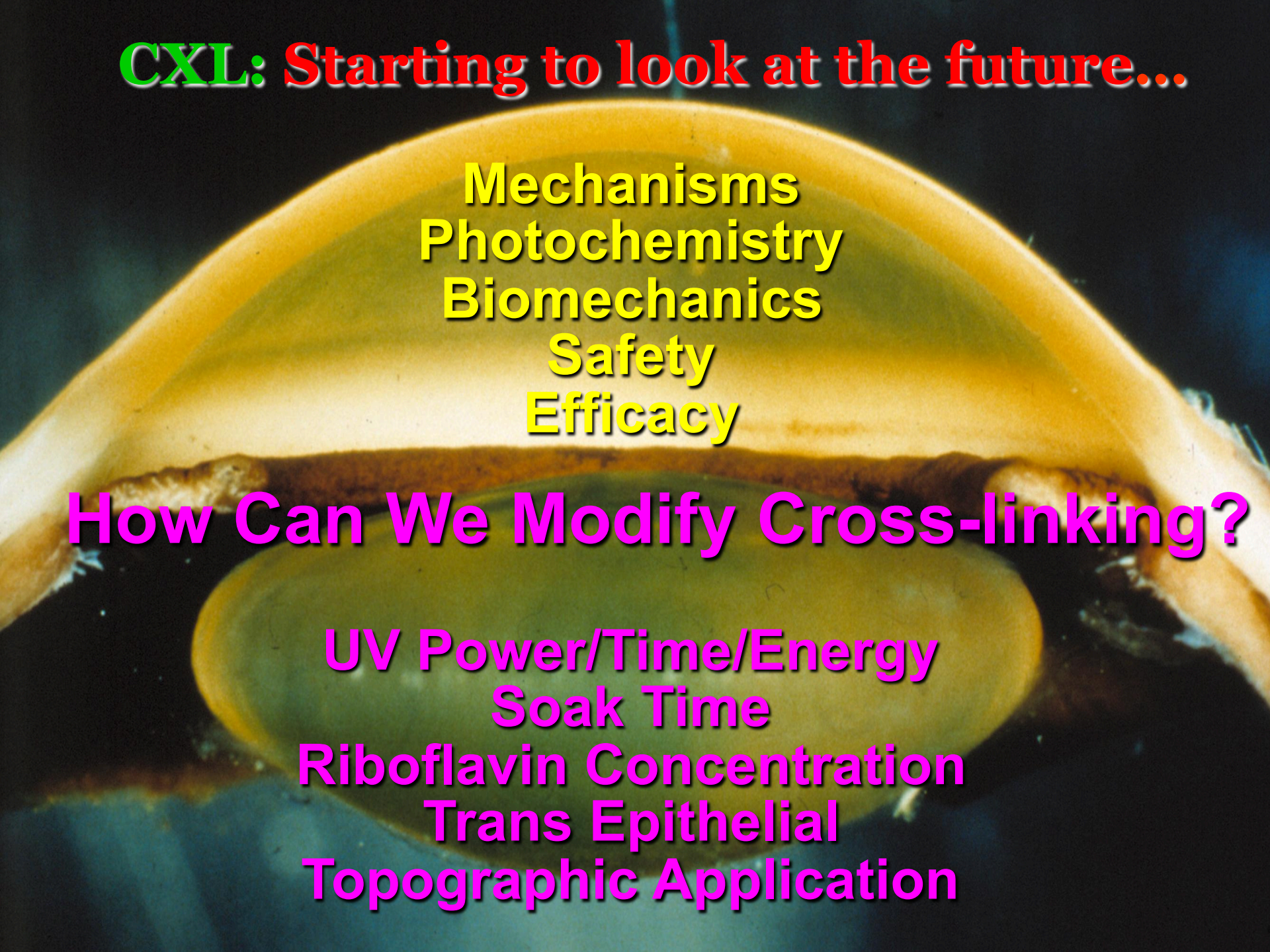


Mazzotta C, Ramovecchi V. EI-CXL for thin Corneas, Accepted May 2014, in press, Journal of Clinical Opthalmology


CXL: Starting to look at the future...
Mechanisms
Photochemistry
Biomechanics
Safety
Efficacy
How Can We Modify Cross-linking?
UV Power/Time/Energy
Soak Time
Riboflavin Concentration
Trans Epithelial
Topographic Application

How to solve corneal shrinking??

- **REMOVE DEXTRAN!!!!**
- **Use dextran-free iso-osmolar solution!!!**

Expert Opinion on Orphan Drugs (2013) 1(3):235-240

Drug Evaluation

EXPERT OPINION

1. Introduction
2. Keratoconus

Riboflavin 0.1% (VibeX) for the treatment of keratoconus

Cosimo Mazzotta†, Stefano Baiocchi, Tomaso Caporossi, Stefano Caragiuli, Anna Lucia Paradiso & Aldo Caporossi

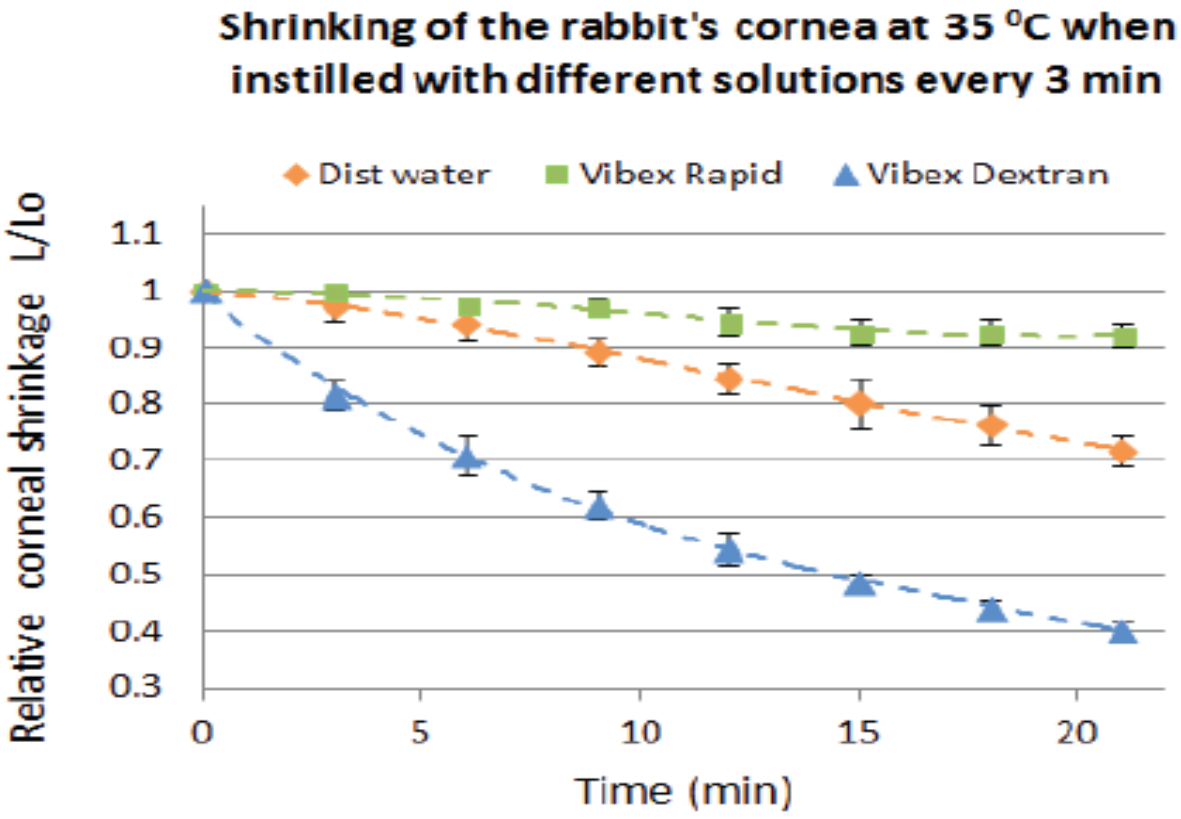
†*Siena University, Policlinico Santa Maria alle Scotte, Department of Ophthalmology, Siena, Italy*

Box 2. Drug summary.

Drug name	VibeX Rapid™
Pharmaceutical company	Avedro, Inc. (Waltham, Massachusetts, MA, USA)
Indication	Keratoconus and secondary corneal ectasia
Composition	100 mL of solution contains: riboflavin 0.1 g, HPMC, disodium hydrogen phosphate, sodium phosphate monobasic dihydrate, sodium chloride, water for injectable solution
Mechanism of action	Ophthalmic medical device used for corneal crosslinking treatment
Route of administration	Topically

Compared with VibeX riboflavin 0.1% – dextran 20% formula, the VibeX Rapid has been proposed for a faster and homogeneous corneal soaking, avoiding the intraoperative corneal thinning often occurring with the standard riboflavin solutions containing high molecular weight dextran as excipient.

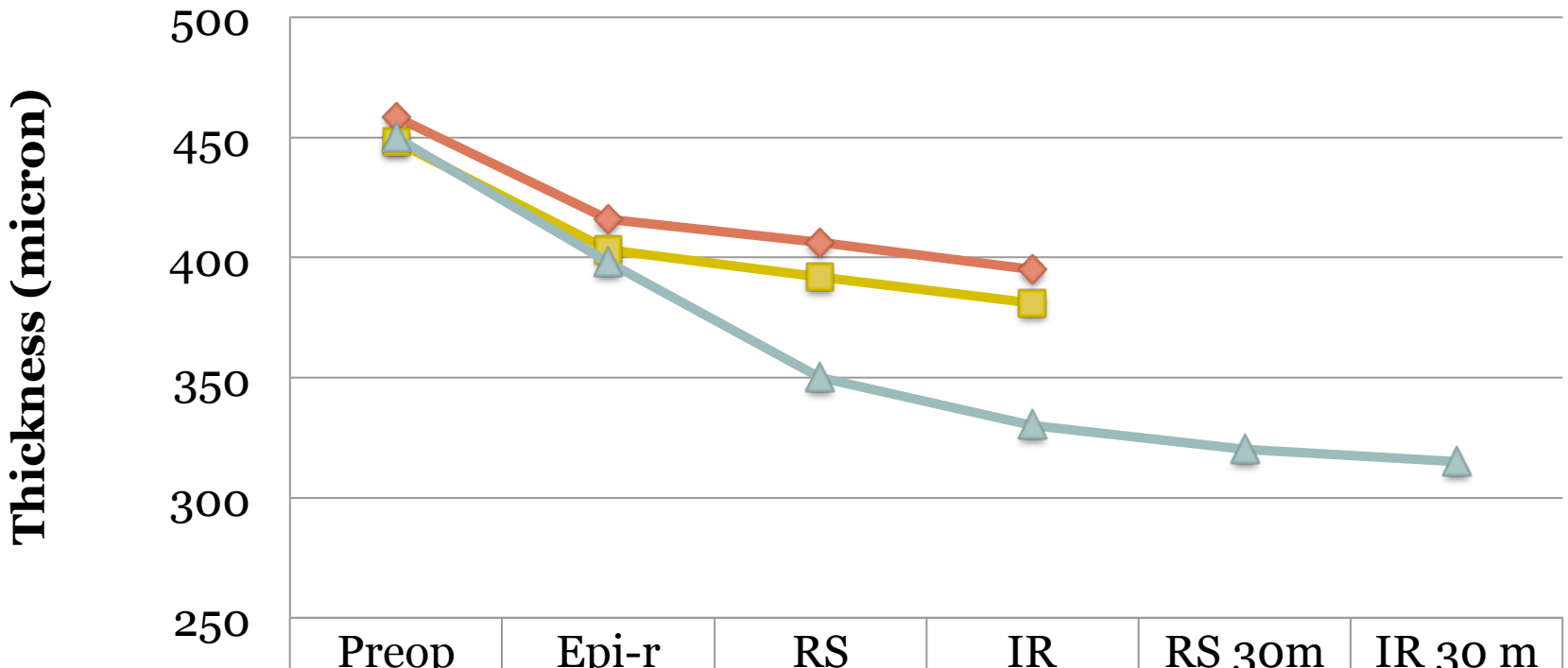
VibeX Rapid™ dextran-free Riboflavin 0.1% plus HPMC solution



Suggestions from lab…

Intraoperative Corneal OCT after Accelerated crosslinking with dextran free Riboflavin solution

Comparison between old and new cxl protocol



	Preop	Epi-r	RS	IR	RS 30m	IR 30 m
CCT	458,5	415,7	406,4	395		
IPCT	448	403	392	381		
Conv CXL	450	398	350	330	320	315

No intraoperative corneal thickness reduction during entire procedure!!!

M. Rechichi, C. Mazzotta, M. Lanza et al. in press

Newer cxl machines…

KXL

KXL 2

- 10 mW/ 9 min
- 30 mW/ 3 min
- 18 mW / 5 min
- 45 mW / 2 min
- UVA intensities
- Riboflavin solutions
- Optimized beams profiles
- Adjunctive substances

Penske CCL vario

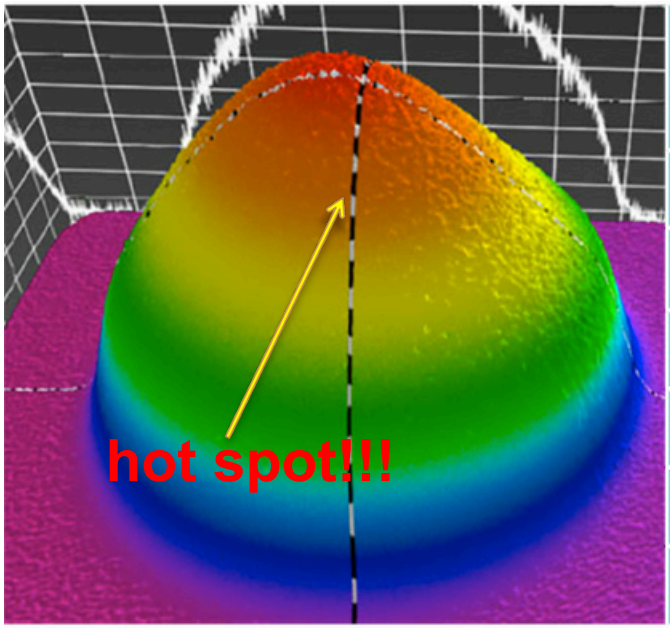
Iroc uvx-2000

Why use newer cxl machine?

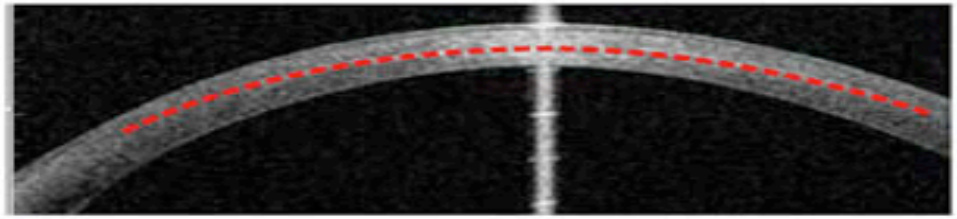
Flat Non-optimized

Beam profile

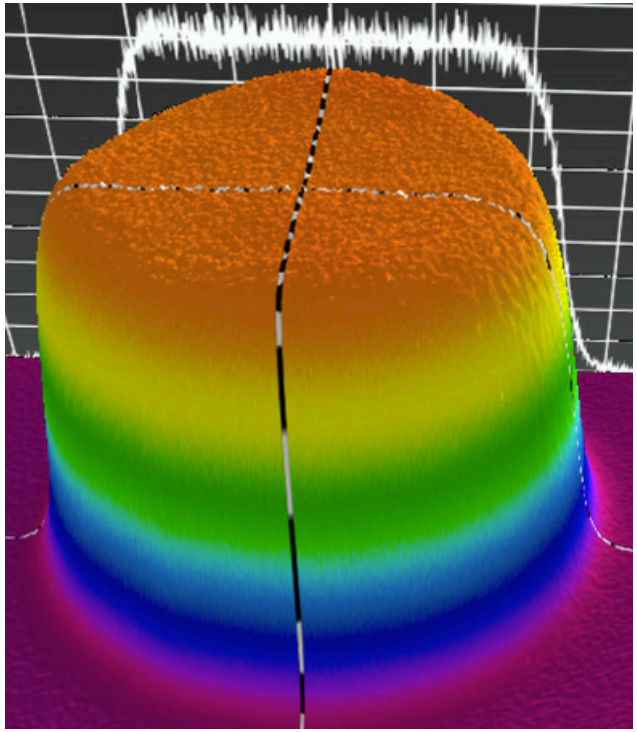
TopHat Optimized

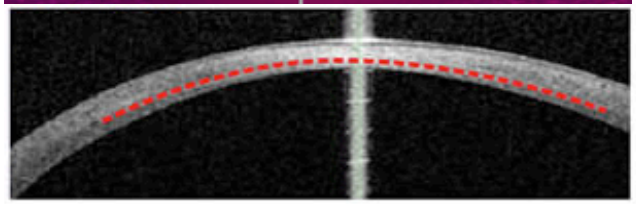


Annulus (mm)	Total Surface Exposure Energy (J)
Central 2	8.4
2 to 4	6.5
4 to 6	5.9
6 to 7	5.9
7 to 9	5.0
9 to 11	3.7



Less cross-linking in periphery





Improved cross-linking in periphery

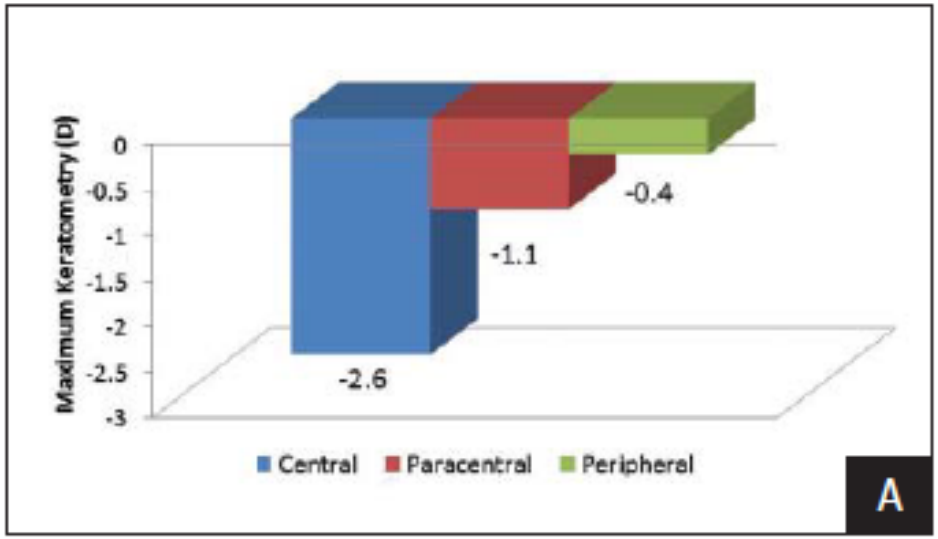
uneven energy distribution
Energy delivery greatly affected by defocus (>10% over 1mm)

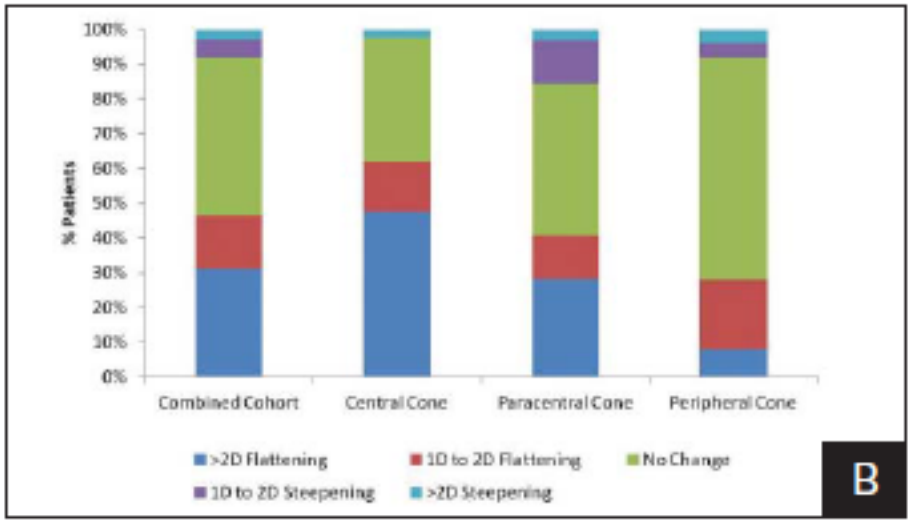
More homogeneous energy delivery

Why use newer cxl machine?

Effect of Topographic Cone Location on Outcomes of Corneal Collagen Cross-linking for Keratoconus and Corneal Ectasia

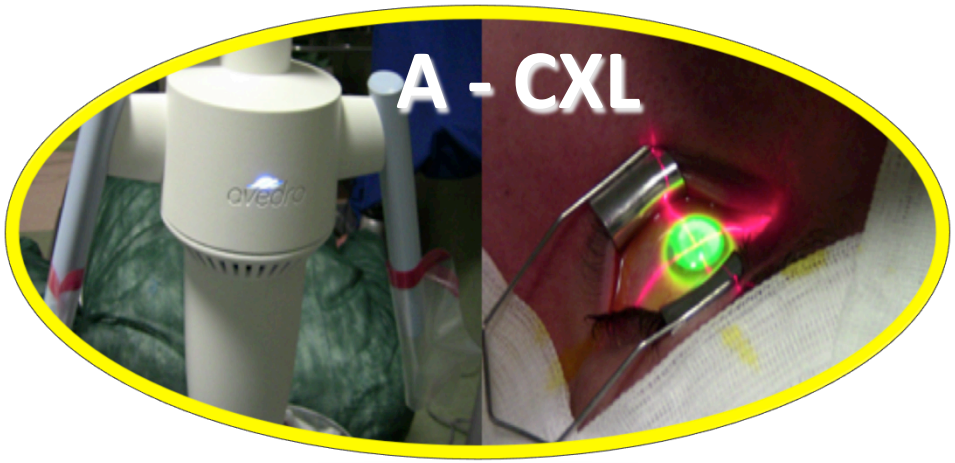
Steven A. Greenstein, MD; Kristen L. Fry, OD, MS; Peter S. Hersh, MD





CONCLUSIONS: After CXL, more topographic flattening occurs in eyes with centrally located cones and the least flattening effect occurs when the cone is located peripherally. This cone-location effect is found in eyes with both keratoconus and ectasia. [*J Refract Surg.*

Shortening the CXL Procedure is possible by



1. **Remodulating the Epi-Off Riboflavin Diffusion into the Stroma by using other excipients (HPMC) as Riboflavin vehicles (dextran free) instead of Dextran 20%,**

2. **Ionthophoresis in Epi-on Riboflavin delivery (5 min plus preparation)**

3. **Increasing UV-A Fluence / cm ^{2} up to 3 mW (9 - 45 mW / cm ^{2})**

4. **Reducing UV-A Exposure Time (10 min or less)**

The Photo-chemical Basis of Accelerated Crosslinking

J. Physiol. (1952) 118, 135–139

THE BUNSEN-ROSCOE LAW FOR THE HUMAN EYE
AT VERY SHORT DURATIONS

By G. S. BRINDLEY*
From the Physiological Laboratory, University of Cambridge

Bunsen-Roscoe Law of Reciprocity

The Law Stated that Photochemical process in a tissue depends on the absorbed energy dose

$$Effect = I_X \; t$$

Theoretically biological effect is proportional to the total energy dose delivered in the tissue

Exposure time and OXIDATIVE DAMAGE

Journal of Photochemistry and Photobiology B: Biology 79 (2005) 197–207

UVA-induced oxidative damage and cytotoxicity depend on the mode of exposure

Helga Merwald, Gabriele Klosner, Claudia Kokesch, Manon Der-Petrossian, Herbert Hönigsmann, Franz Trautinger *

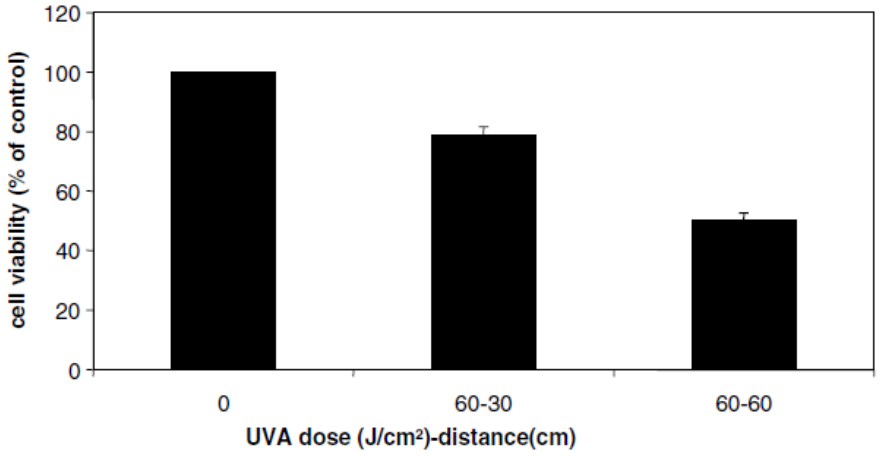
Division of Special and Environmental Dermatology, Department of Dermatology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

that at a constant dose low intensity exposure with a long exposure time enhances cytotoxicity (Fig. 4).

A main result of our experiments is that not only the cumulative dose but also the timing of exposures is crucially important for the amount of oxidative damage to the cell. Preirradiation within a short interval prior to a

damage. Similar effects were observed without fractionation after prolonged exposure at decreased intensity

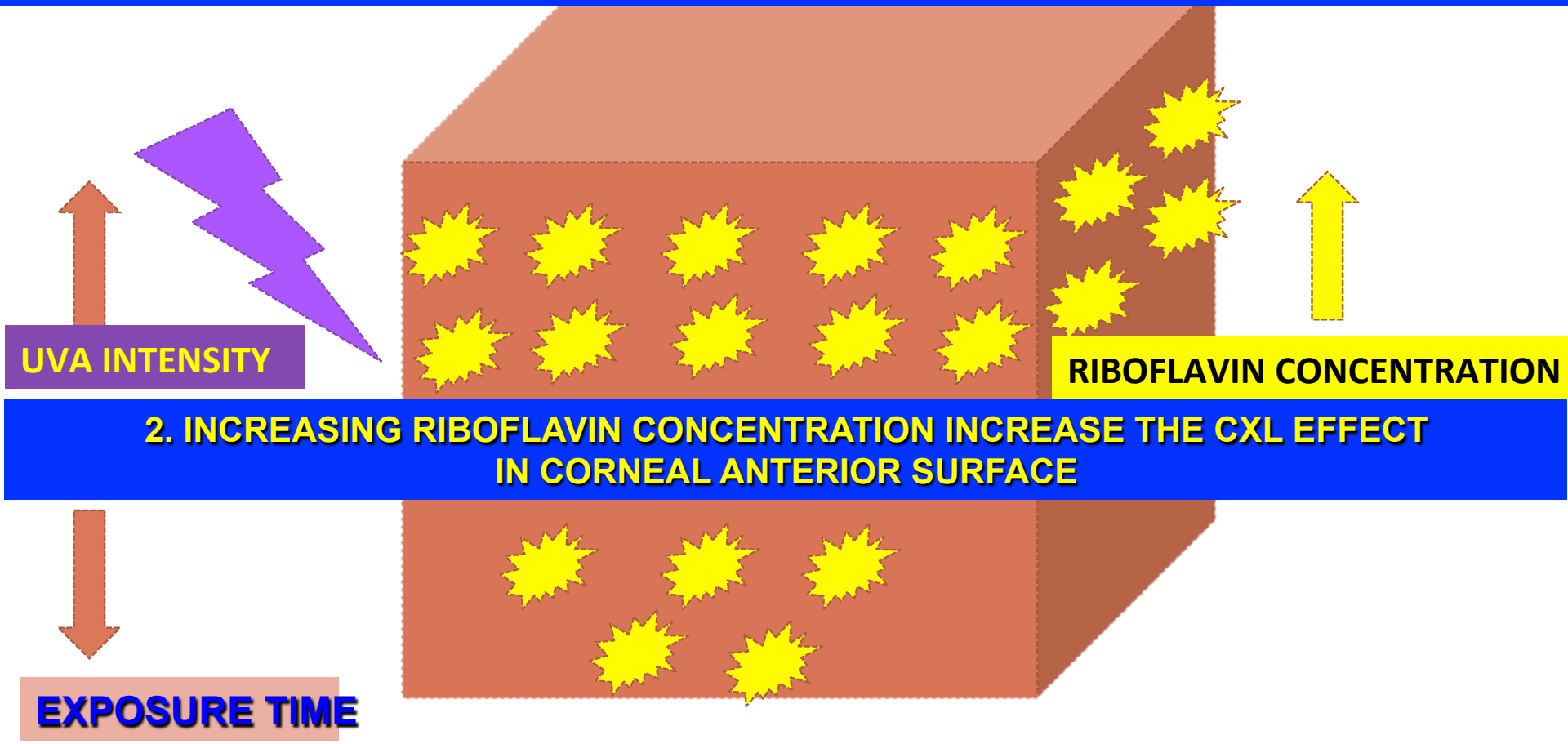
In summary, we provide evidence that the modification of UVA radiation intensity at a constant dose has different effects on cell survival. Thus, we conclude that the rule of Bunsen and Roscoe, that was originally established for simple photochemical reactions, cannot be safely transferred to complex biological systems. Spe-



INCREASING EXPOSURE TIME INCREASES THE TREATMENT PENETRATION AND PHOTO-OXIDATIVE EFFECTS

RIBOFLAVIN -UV A-TIME INTERACTIONS

1. INCREASING UV A INTENSITY INCREASE PHOTONS IMPACT IN CORNEAL SURFACE WITH GRATER COMPACTION IN THE ANTERIOR STROMA



2. INCREASING RIBOFLAVIN CONCENTRATION INCREASE THE CXL EFFECT IN CORNEAL ANTERIOR SURFACE

3. INCREASING THE EXPOSURE TIME INCREASE THE INTERACTION BETWEEN UV A PHOTNS, RIBOFLAVIN AND COLLAGEN MOLECULES INCREASING THE DEPTH OF CROSSLINKING

UV A power

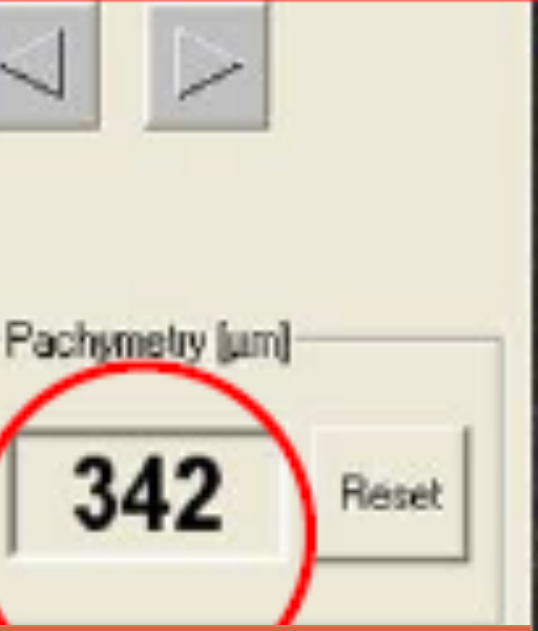
Bunsen-Roscoe: law of reciprocity

The stromal compaction reduces the treatment penetration !!!

- Increasing UV A power from 3 mW to 45 mW (mantaining the enrgy dose delivered in the tisssue at 5.4 J or 7.2 J/cm^2)
- Increase the photons impact on stromal surface maximizing the CXL effect in the anterior superficial stroma

Epi-Off CXL

Riboflavin concentration



Increased riboflavin concentration in the anterior stroma reduces the penetration of UV A that are bloked in a superficial level

Epi-Off CXL

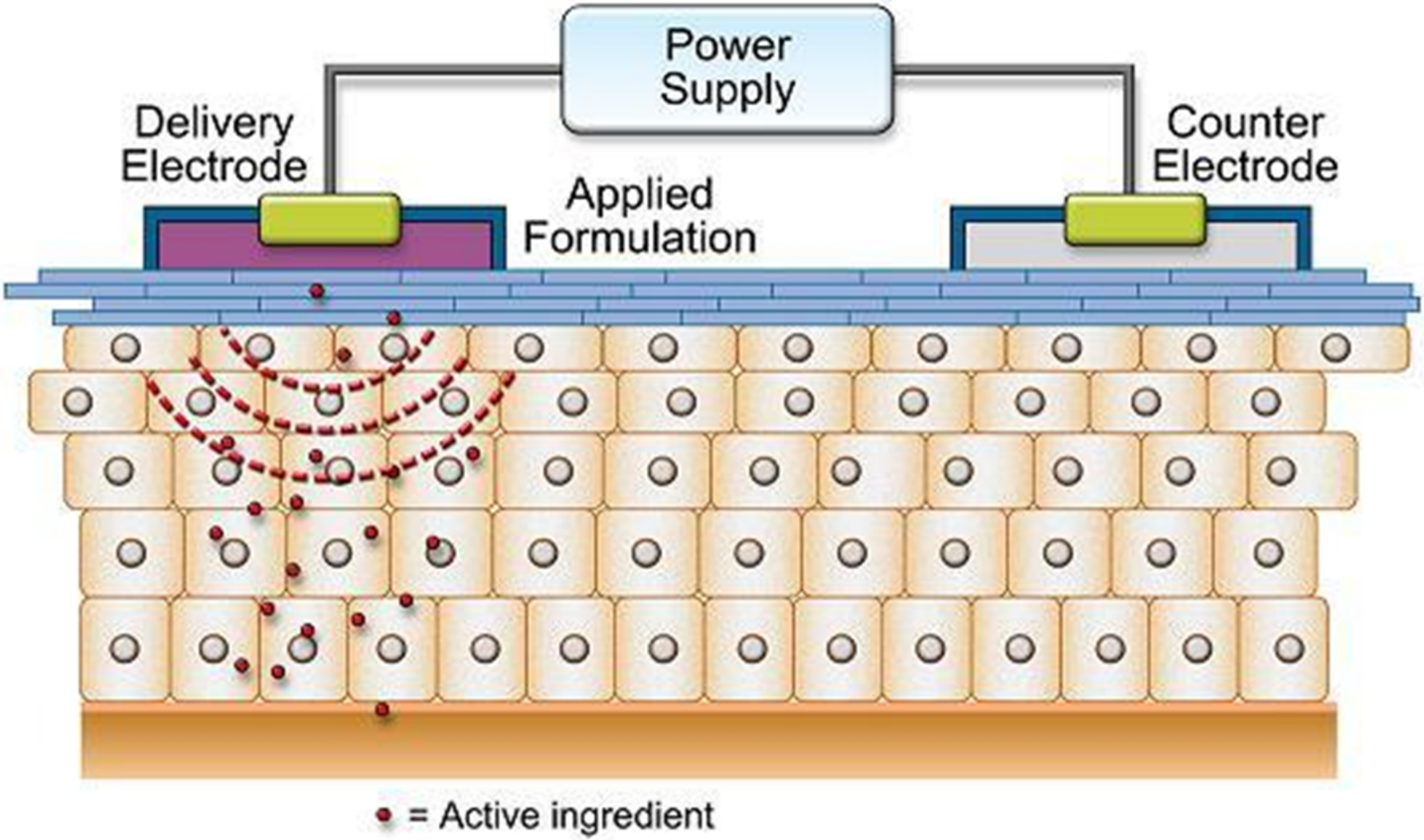
- Can be increased by:

1. Increasing the concentration of the solution (0.1% to 0.25%)
2. Increasing soaking time: 10, 20, 30 min)

3. Iontophoresis

Increasing riboflavin concentration into the stroma increase the absorbtion of UV A radiation in strromal surface. Major is the concentration of riboflavin greater is the absorbtion of UV A photons and free radical release in the anterior stromal surface

Iontophoresis principles…



High Fluence Iontophoretic Corneal Collagen Cross-linking: In Vivo OCT Imaging of Riboflavin Penetration

Paolo Vinciguerra, MD
Miguel Rechichi, MD
Pietro Rosetta, MD
Mario R. Romano, MD, PhD

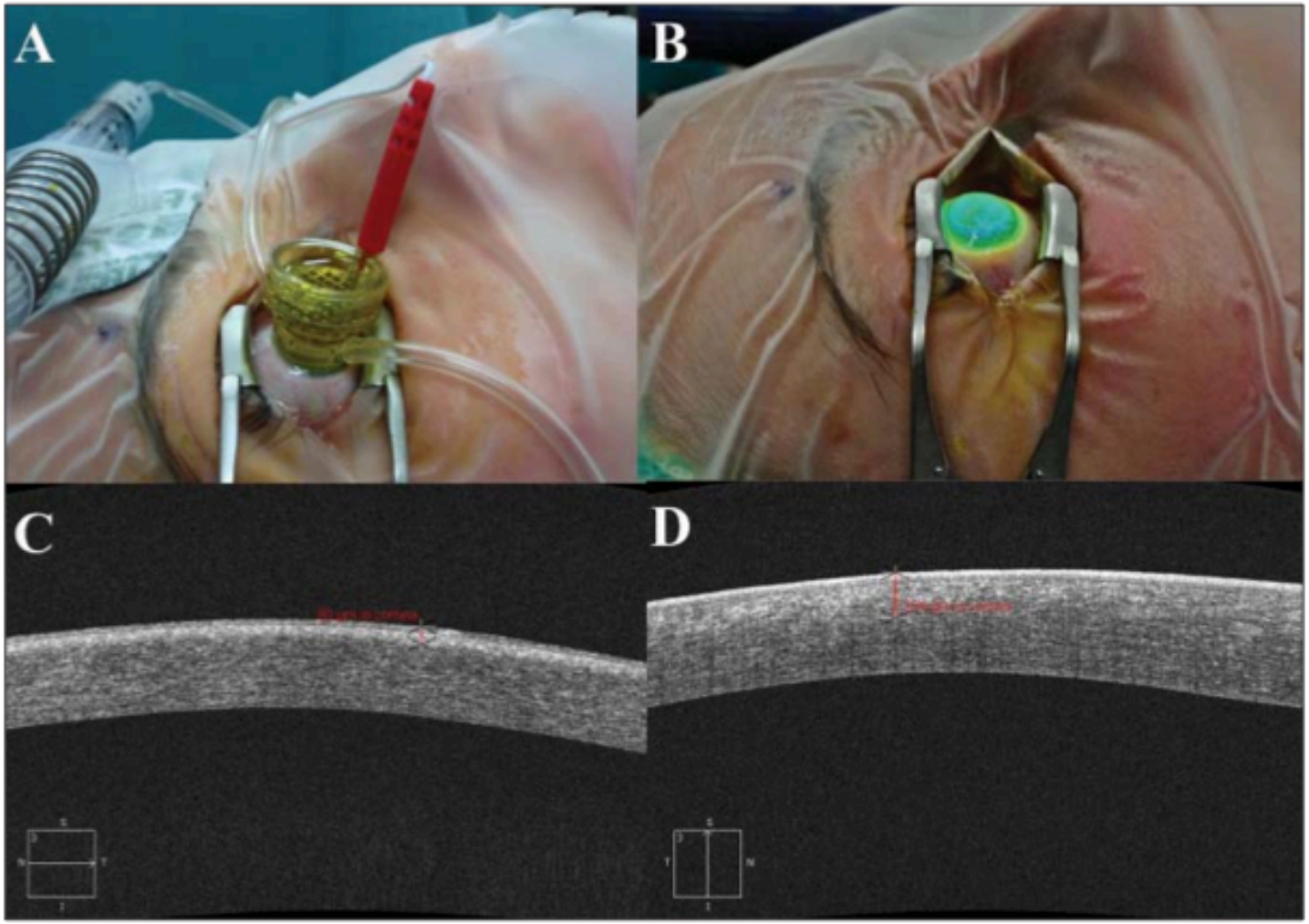
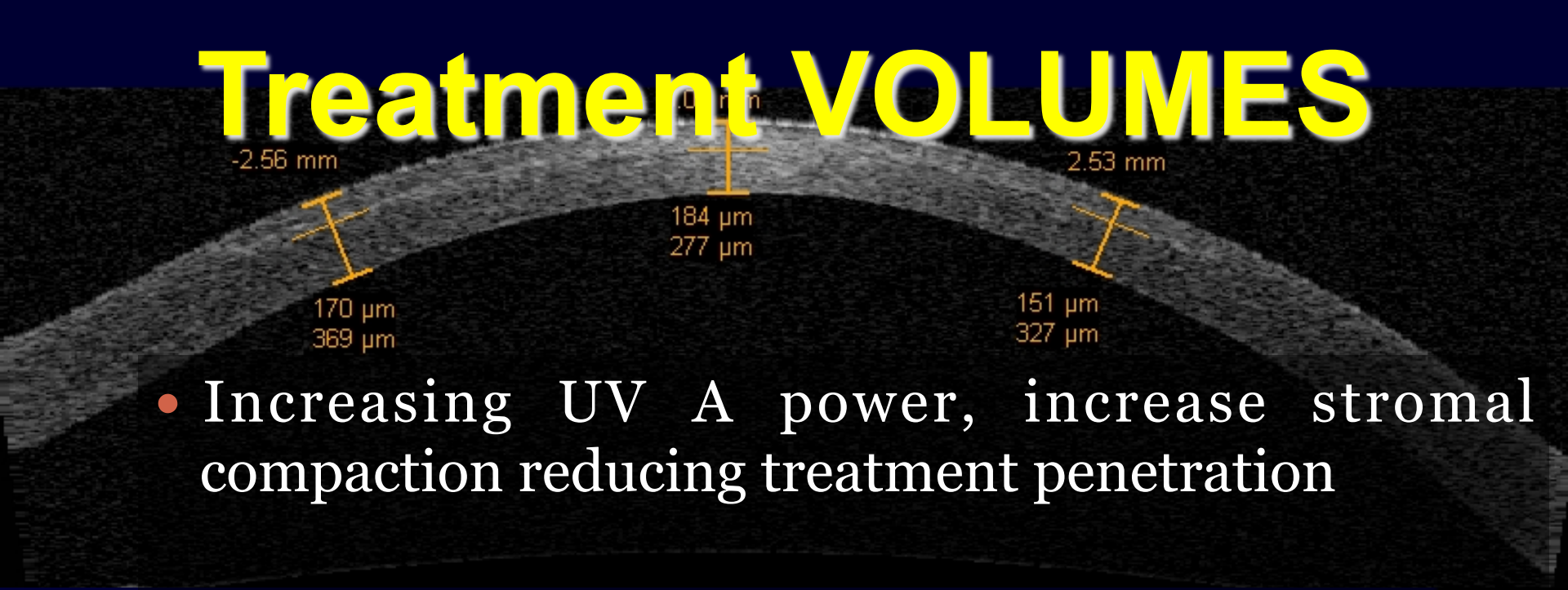


Figure 1. Iontophoresis impregnation phase (A) throughout an intact epithelium and (B) the irradiation phase in which it is possible to see a intense fluorescence. Intraoperative high-resolution optical coherence tomography images show (C) a homogeneous hyperreflective band at a mean depth of 80 μm in the epi-off group and (D) a less uniform band with a fading effect extending through the anterior 200 μm of the cornea.

Treatment VOLUMES

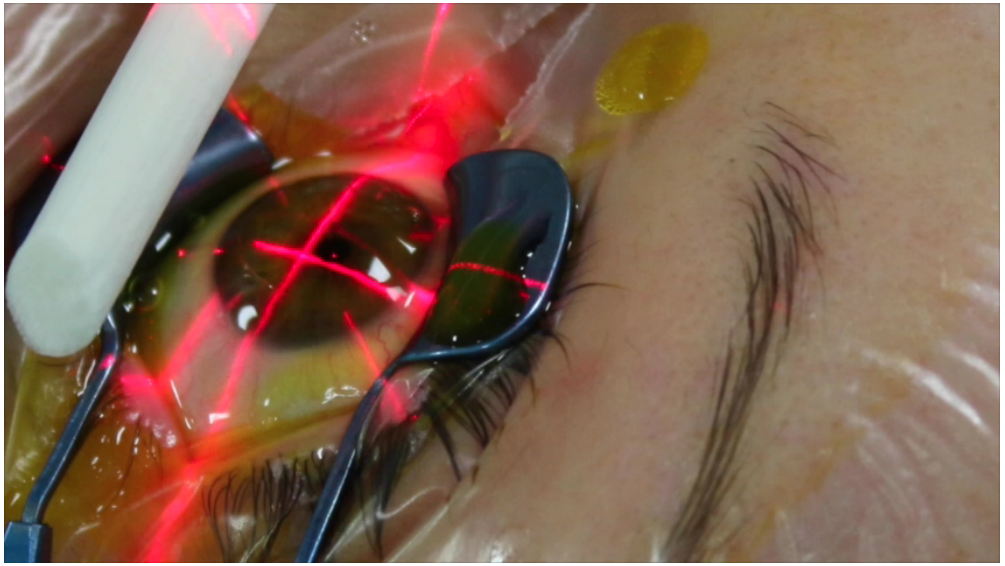


- Increasing UV A power, increase stromal compaction reducing treatment penetration

- Reducing UV A power and increasing exposure time, increase the depth of treatment penetration reducing tissue compaction due to relative riboflavin consumption

It is possible to increase CXL efficacy

by



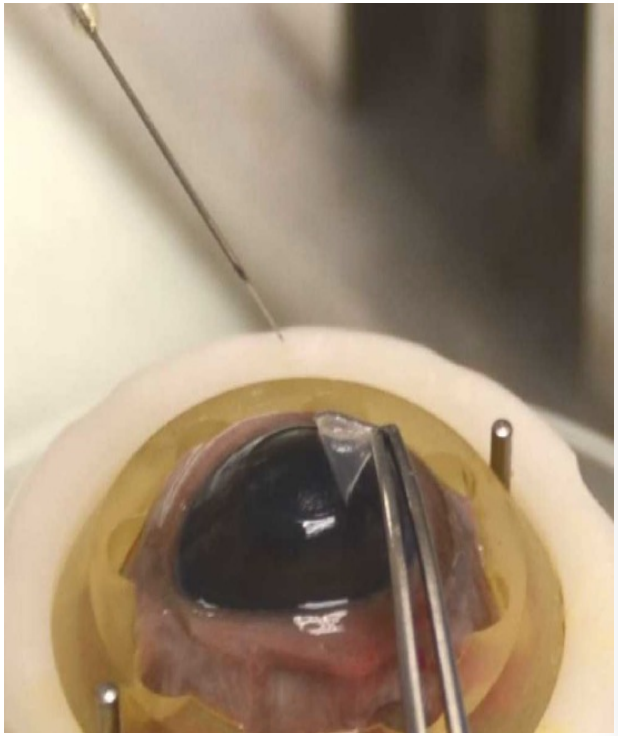
1. Pulsed light (re-oxigenation of tissue)
2. additives (oxigen, glucose)

Cornea

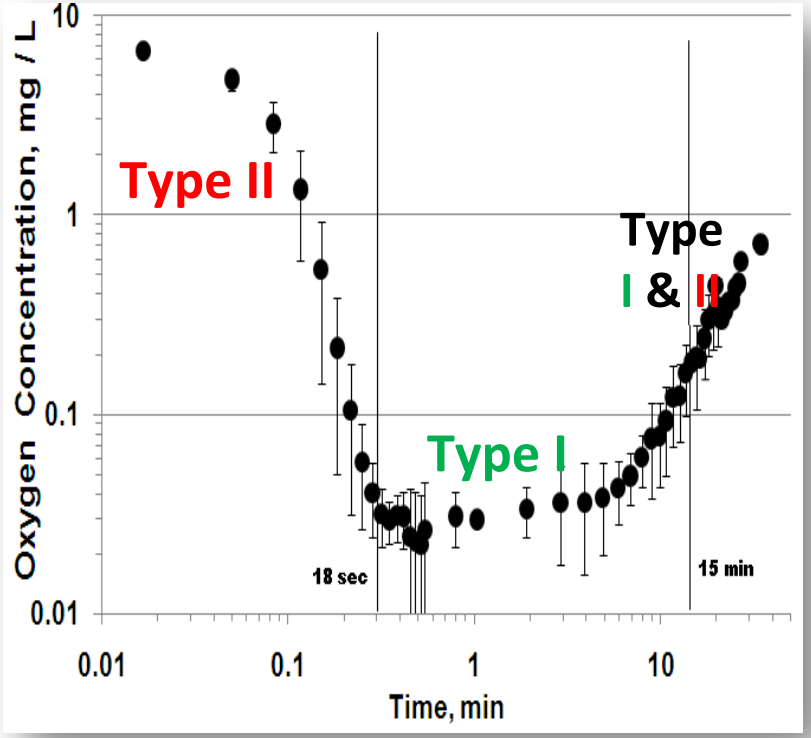
Photochemical Kinetics of Corneal Cross-Linking with Riboflavin

Pavel Kamaev, Marc D. Friedman, Evan Sherr, and David Muller

Pulsing the light (re-oxigenating the tissue)



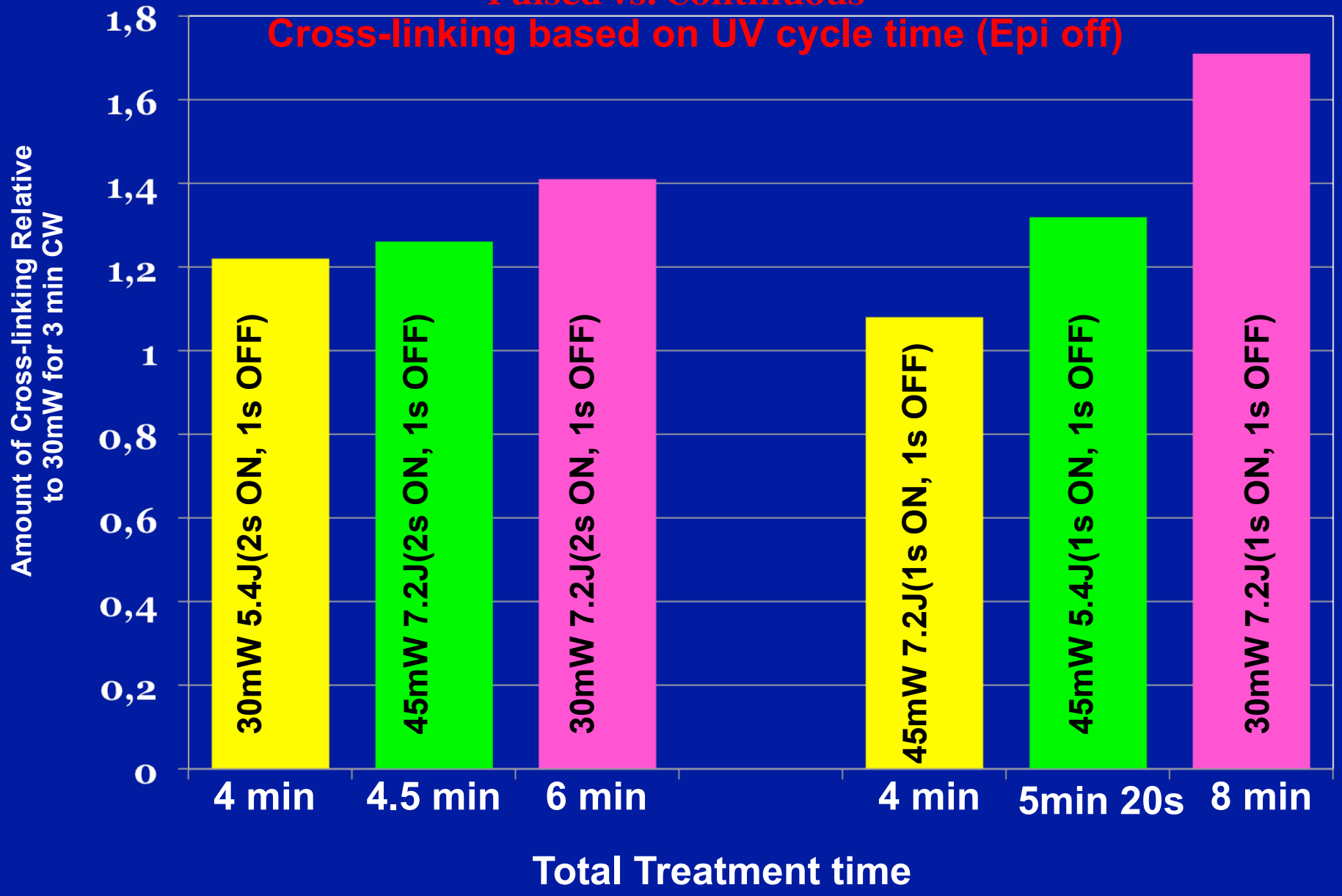
O_2 sensor being inserted under flap



Oxygen Depletion Over 30 Minutes

WHY PULSED Cross-linking?

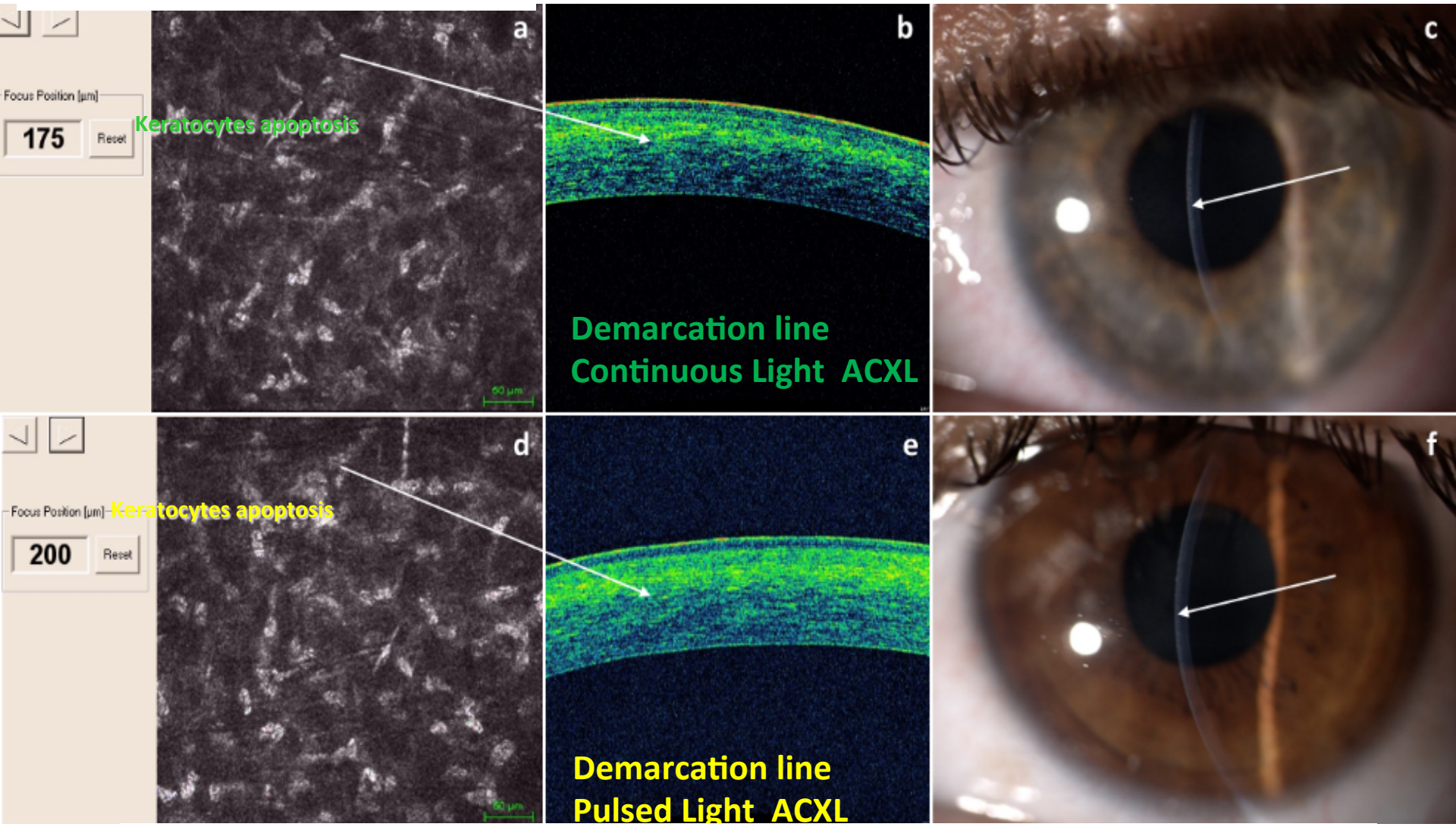
Pulsed vs. Continuous



Eye
Accepted May 2014, In Press

Pulsed vs Continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT

The Royal College of Ophthalmologists



[1]Cosimo Mazzotta MD, PhD, [1]Claudio Traversi MD, [1]Stefano Caragiuli MD, [2]Miguel Rechichi MD, PhD

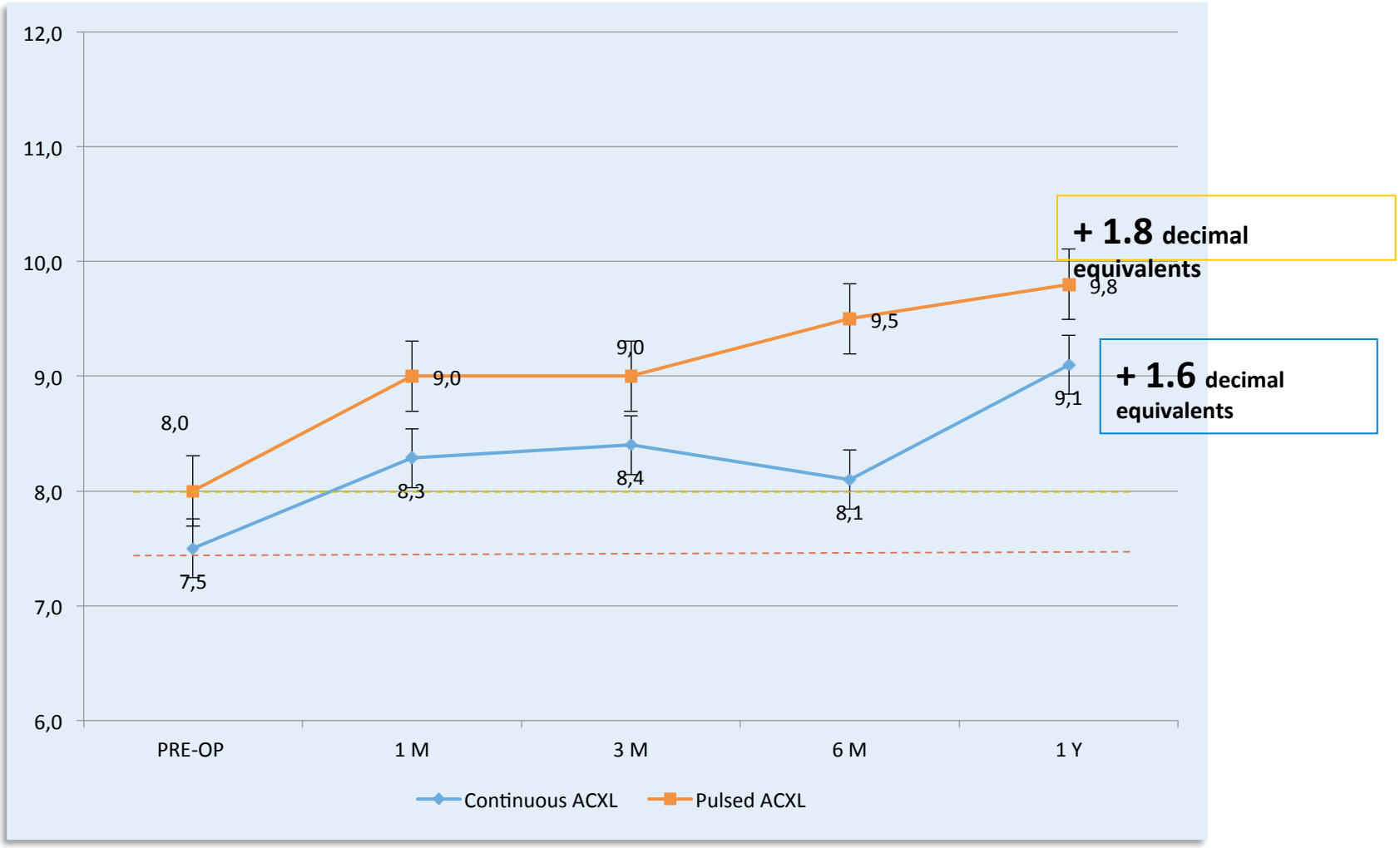
C. Mazzotta, C. Traversi, AL Paradiso, ME Latronico, M Rechichi

Int Ophthalmol 2014

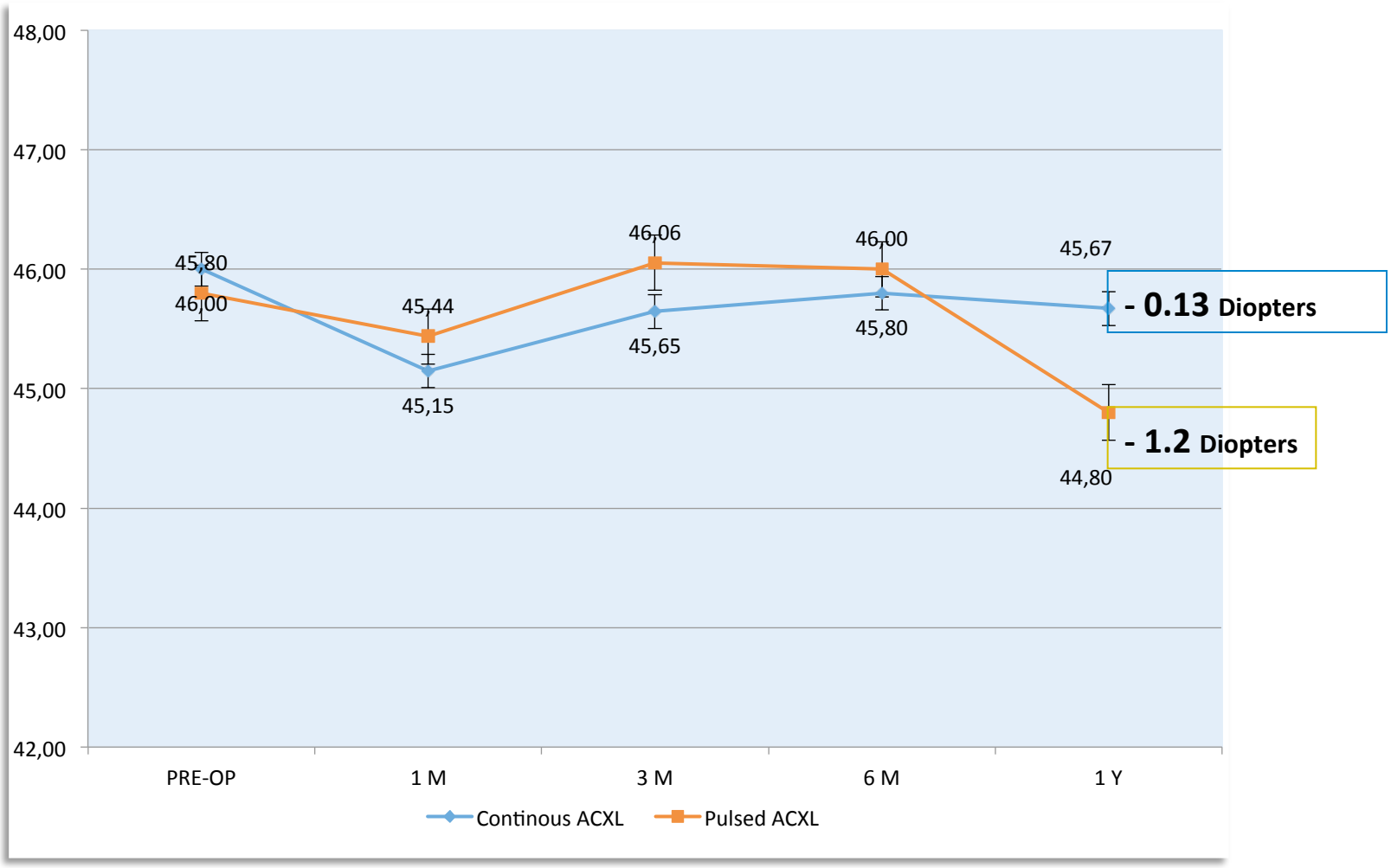
CXL Special Issue

accepted june 2014, in Press

CDVA



K average


48,00
47,00
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44,00
43,00
42,00
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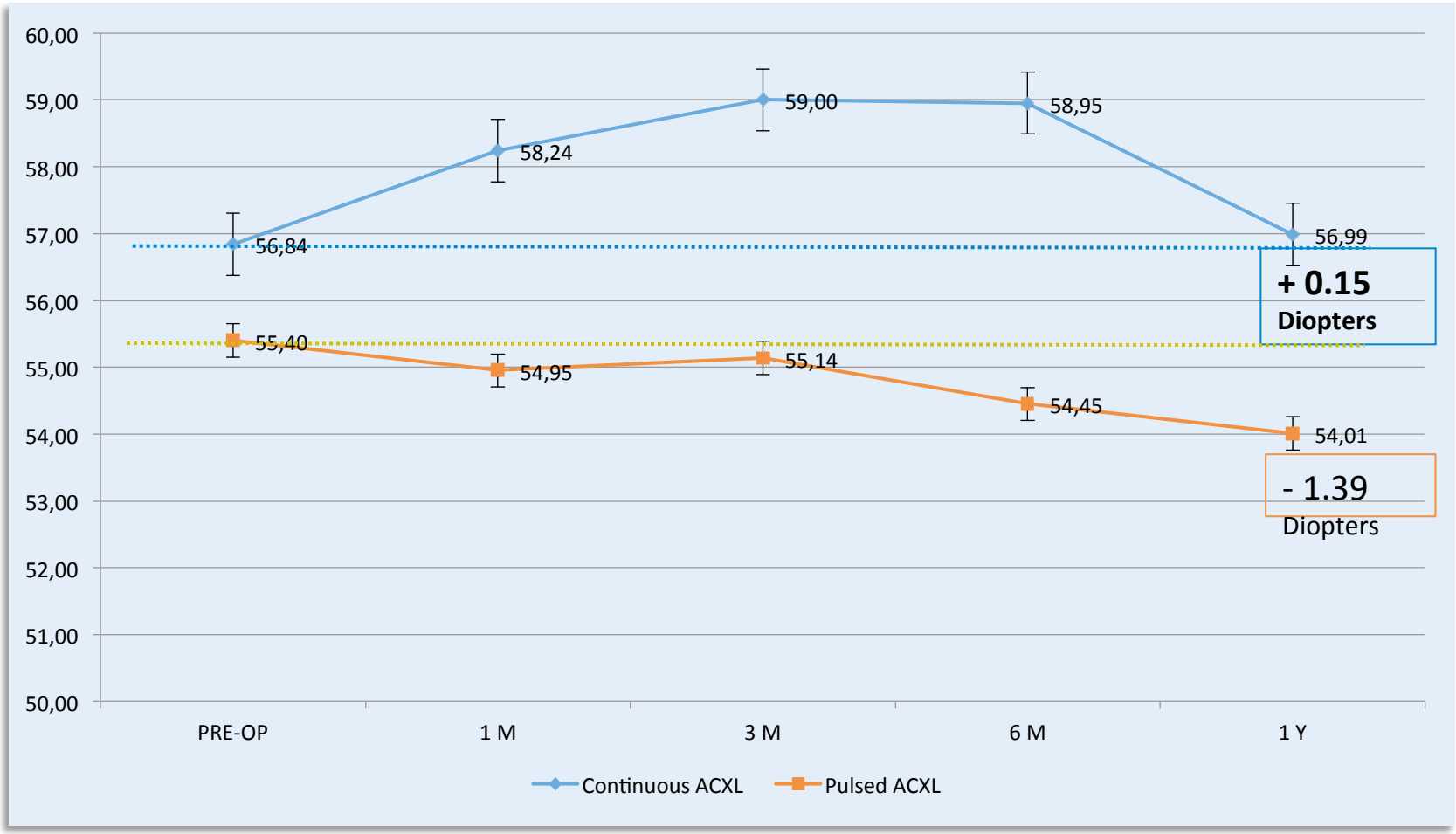
46,00
45,44
45,15
46,06
45,65
46,00
45,80
45,67
44,80
PRE-OP
1 M
3 M
6 M
1 Y
Continous ACXL
Pulsed ACXL
- 0.13 Diopters
- 1.2 Diopters

AK: apical kurvature



Semi-thin sections

*Caliper control 150 µm

Group A (control) showing high lamellar compaction in the first 150 µm (stiff cornea).

Group B (conventional CXL at 3 mW/cm2) placed at the top right (2 b) showing the deeper keratocytes apoptosis associated with lamellar compaction approximately at 300 µm depth;

Group C (epi-off A-CXL at 30 mW/cm2) placed at the mid left (2 c) showing apoptosis and collagen compaction approximately at 100 µm;

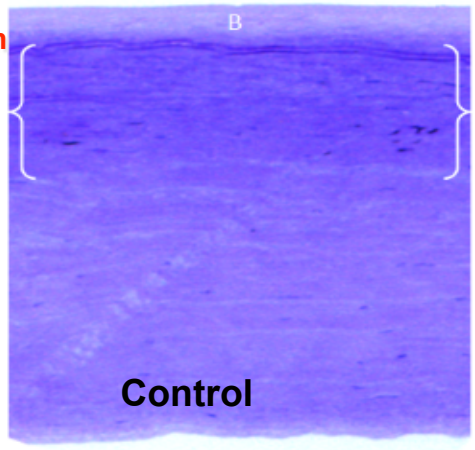
Group D (epi-off A-CXL at 12 mW/cm2) placed at the mid right (2 d) showing keratocytes apoptosis and collagen compaction approximately at 180 µm;

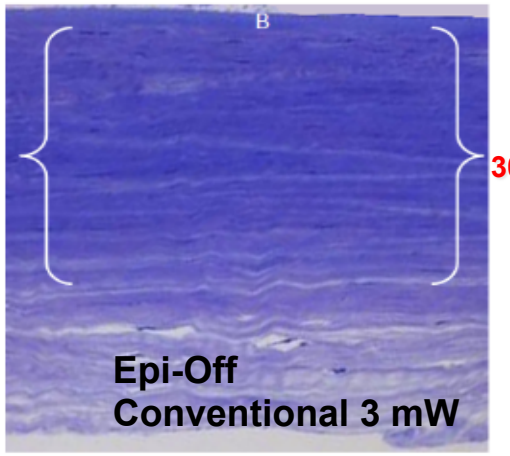
Group E (epi-on TE at 10 mW/cm2) showing a collagen compaction and keratocytes apoptosis approximately at 60 µm.

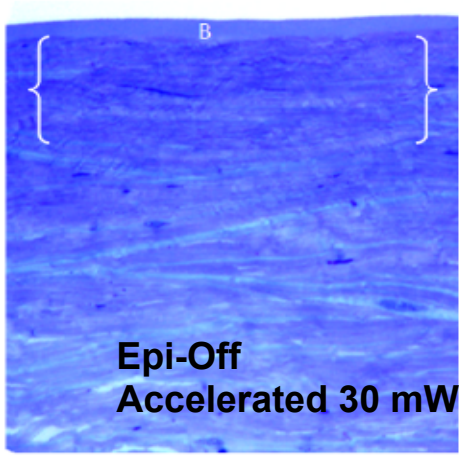
Group F (CXL at 45 mW/cm2) is placed at the bottom left (2 e) showing a collagen compaction and keratocytes apoptosis approximately at 50 µm;

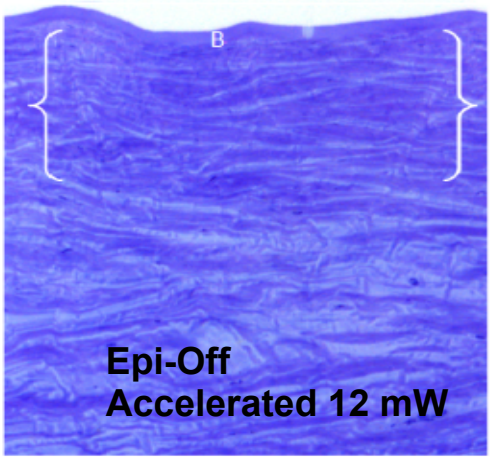
Legend:
E: Epithelium; B: Bowman's layer; Curly brackets define the stromal area and depth where the tissue is denser.

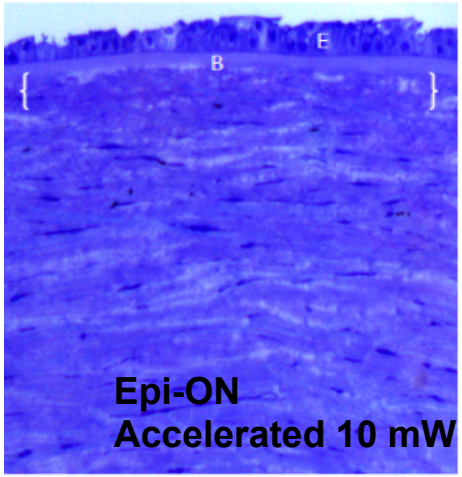
Caliper control 150 µm

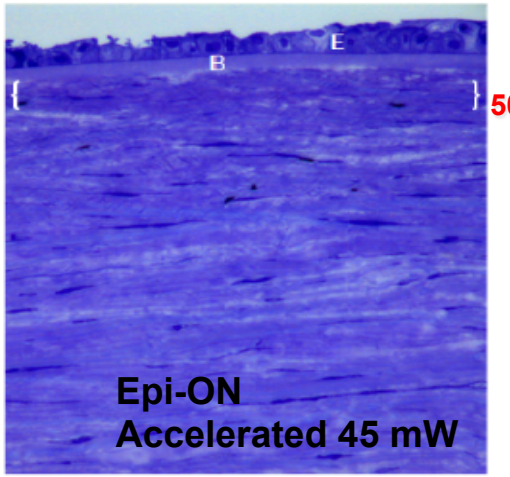




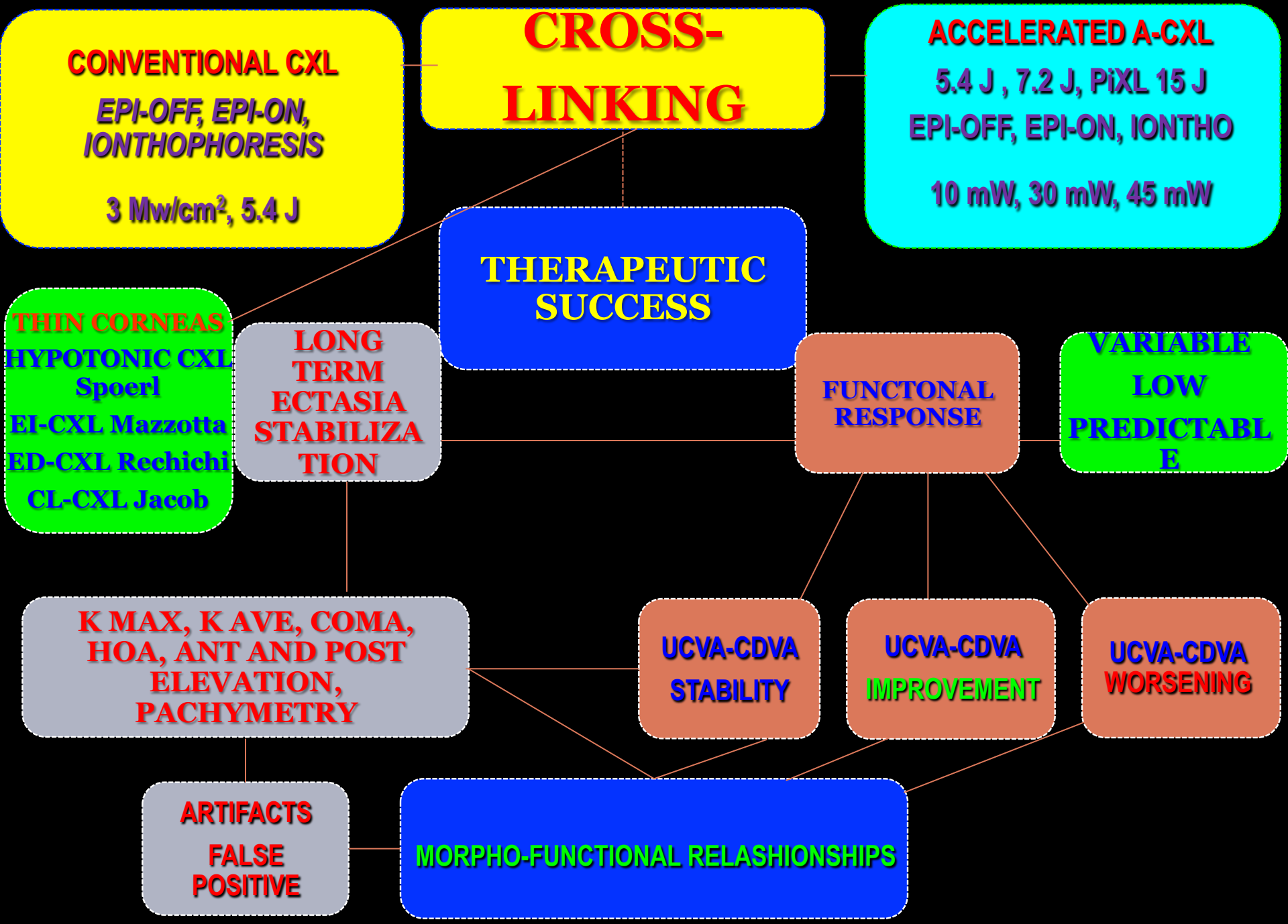








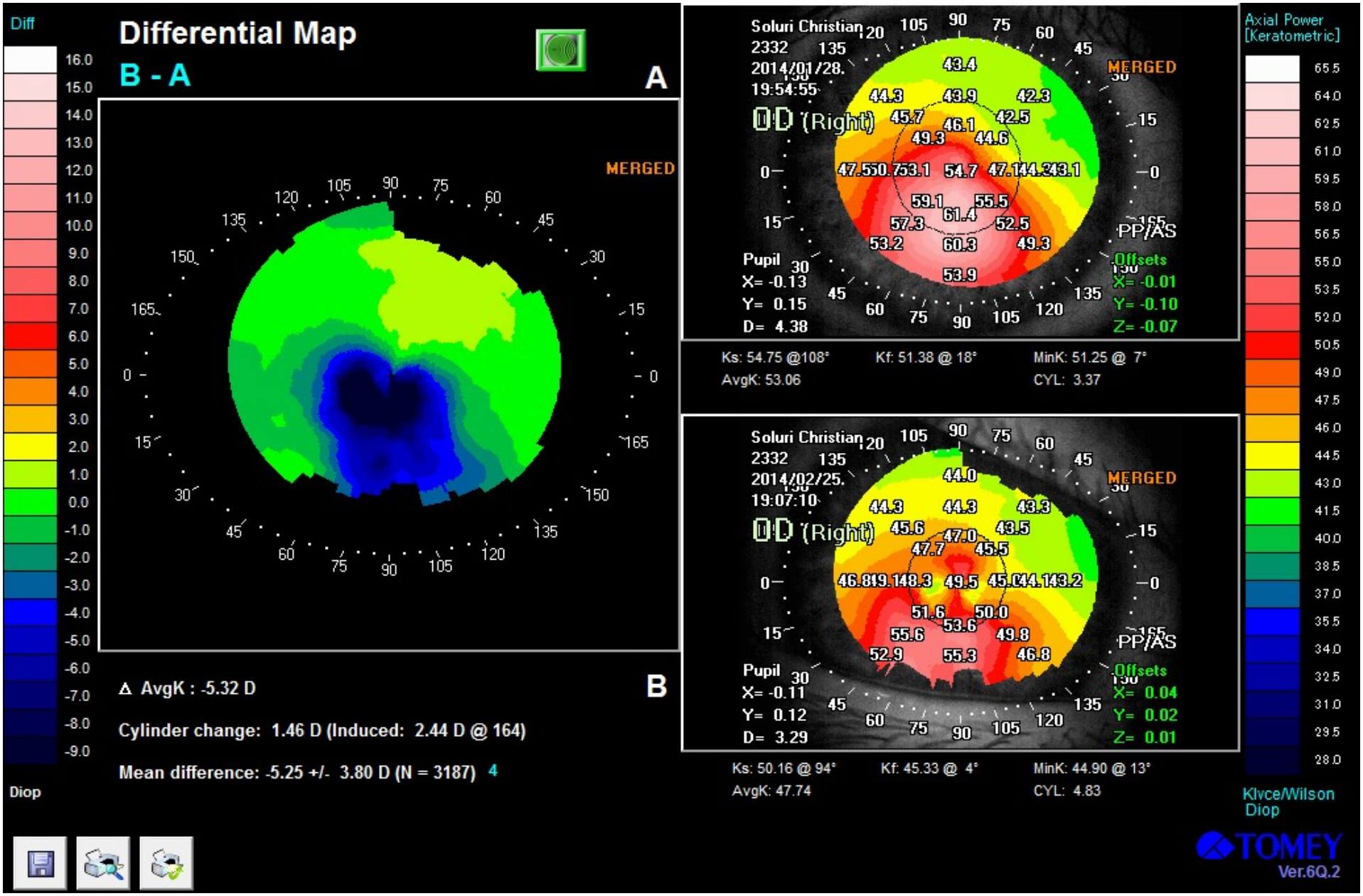
C. Mazzotta, M. Rechichi submitted under review


CONVENTIONAL CXL
EPI-OFF, EPI-ON, IONTHOPHORESIS
3 Mw/cm², 5.4 J
CROSS-LINKING
ACCELERATED A-CXL
5.4 J , 7.2 J, PiXL 15 J
EPI-OFF, EPI-ON, IONTHO
10 mW, 30 mW, 45 mW
THERAPEUTIC SUCCESS
THIN CORNEAS
HYPOTONIC CXL Spoerl
EI-CXL Mazzotta
ED-CXL Rechichi
CL-CXL Jacob
LONG TERM ECTASIA STABILIZATION
FUNCTONAL RESPONSE
VARIABLE LOW PREDICTABLE
K MAX, K AVE, COMA, HOA, ANT AND POST ELEVATION, PACHYMETRY
UCVA-CDVA STABILITY
UCVA-CDVA IMPROVEMENT
UCVA-CDVA WORSENING
ARTIFACTS
FALSE POSITIVE
MORPHO-FUNCTIONAL RELASHIONSHIPS

NEXT FUTURE OF CXL

KXLII FEATURES

- *Topography guided cross-linking*
- *Programmable illumination pattern*
- *Real-time eye tracking*
- *Integrated Scheimpflug imaging*
- *Real-time riboflavin dosimetry measurements*


Diff
Differential Map
B - A
A
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14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
-7.0
-8.0
-9.0
Diop
Soluri Christian
2332
2014/01/28.
19:54:55
OD (Right)
MERGED
43.4
44.3
43.9
42.3
45.7
46.1
42.5
49.3
44.6
47.5
50.7
53.1
54.7
47.1
44.2
43.1
59.1
55.5
61.4
57.3
52.5
53.2
60.3
49.3
53.9
PP/AS
Pupil
X= -0.13
Y= 0.15
D= 4.38
Offsets
X= -0.01
Y= -0.10
Z= -0.07
Ks: 54.75 @108°
Kf: 51.38 @ 18°
MinK: 51.25 @ 7°
AvgK: 53.06
CYL: 3.37
Soluri Christian
2332
2014/02/25.
19:07:10
OD (Right)
MERGED
44.0
44.3
44.3
43.3
45.6
47.0
43.5
47.7
45.5
46.8
49.1
48.3
49.5
45.0
44.1
43.2
51.6
50.0
53.6
55.6
49.8
52.9
55.3
46.8
PP/AS
Pupil
X= -0.11
Y= 0.12
D= 3.29
Offsets
X= 0.04
Y= 0.02
Z= 0.01
Ks: 50.16 @ 94°
Kf: 45.33 @ 4°
MinK: 44.90 @ 13°
AvgK: 47.74
CYL: 4.83
B
Δ AvgK : -5.32 D
Cylinder change: 1.46 D (Induced: 2.44 D @ 164)
Mean difference: -5.25 +/- 3.80 D (N = 3187) 4
Axial Power
[Keratometric]
65.5
64.0
62.5
61.0
59.5
58.0
56.5
55.0
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52.0
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Diop
TOMEY
Ver.6Q.2

NEXT FUTURE OF

- Epi-Off ACXL with Vibex Rapid™ represents the faster A-CXL technique availalble at the moment to perform a safe and efficacious corneal CXL treatment (under 20 minutes)
- Epithelium should be always removed to allow an efficient UV A penetration and CXL
- CUSTOM ACXL with pulsed light plus intraoperative oxygenation may optimize CXL efficacy according to our preliminary data in press
- ZXL (Zonal Cross-Linking) customization and, Lasik XTRA and Refractive XL are the upcoming challenging topic in ectasia treatment
- Photo-refractive intrastromal XL: PiXL

Sharply defined edges

- Eye Tracking

Complex patterning

- DMD Technology

More stiffening than current technology in the shortest time

- PO_2 (Pulsed and Oxygen) Accelerated Cross-linking

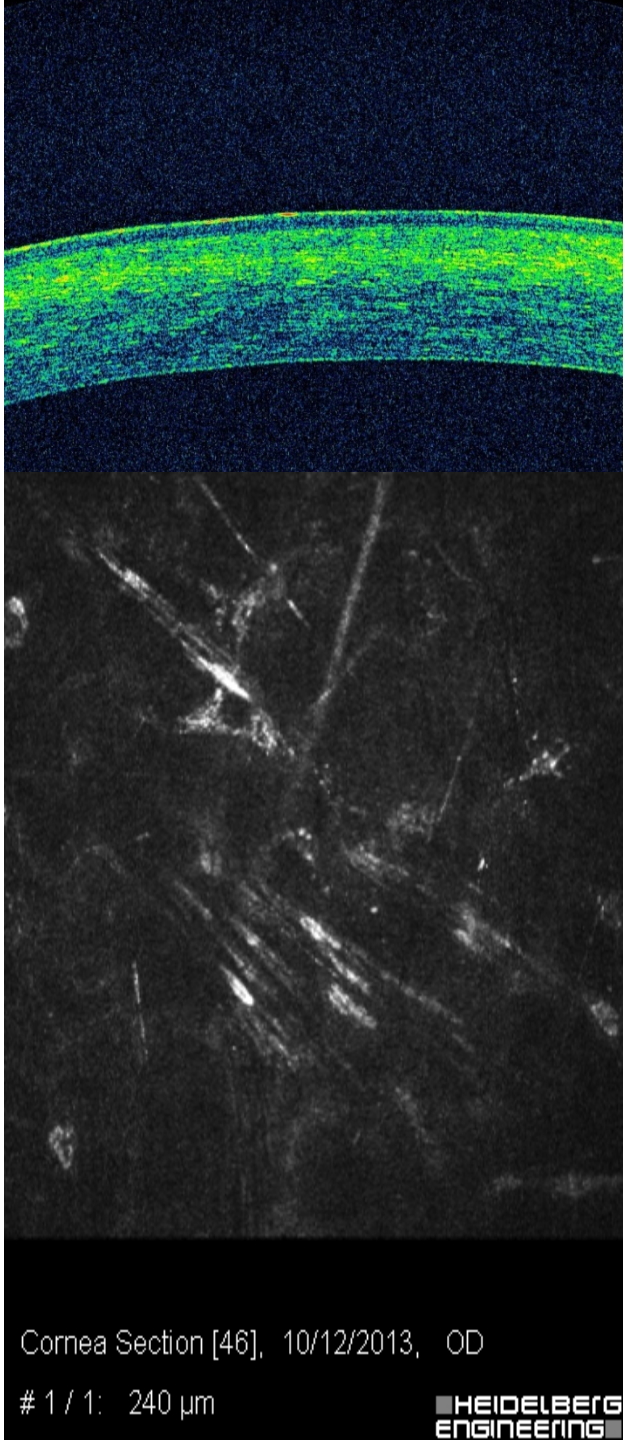
Conclusion

Optimizing CXL factors:

- **High-Fluence (up to 10 mW/cm2)**
- **Enhanced kinetic (pulsing the light)**
- **Customized Zonal Exposure Times**
- **Customized Zonal Energy Deliver (J/cm2)**

High-fluence, customized treatment time, intraoperative oxygen re-uptake influence a deeper penetration of photo-oxidative process that may improve CXL results

M.Rechichi MD, PhD
Magna Graecia University, Italy



Thank you…