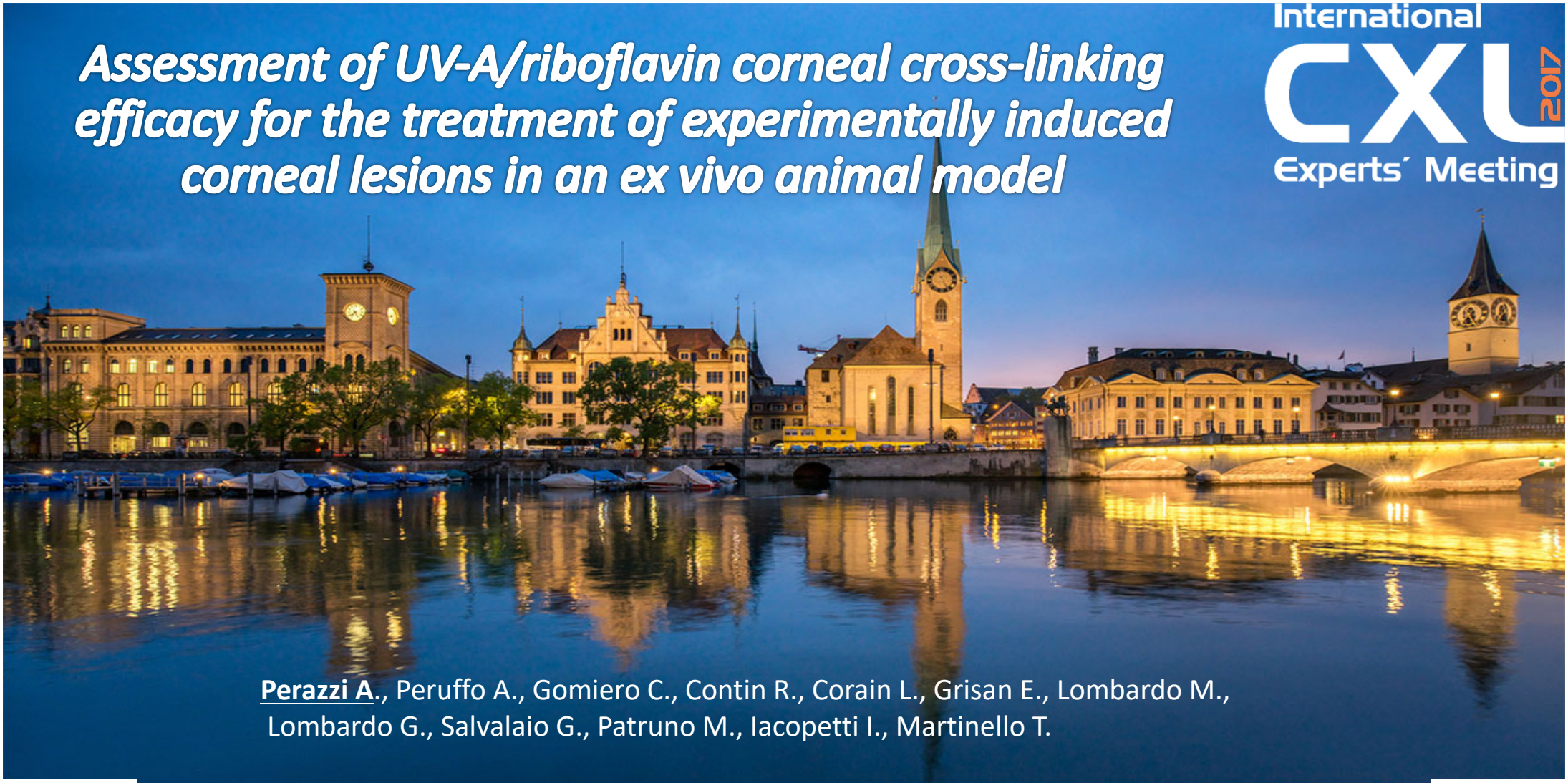
Assessment of UV-A/riboflavin corneal cross-linking efficacy for the treatment of experimentally induced corneal lesions in an ex vivo animal model

International CXL 2017 Experts´ Meeting



Perazzi A., Peruffo A., Gomiero C., Contin R., Corain L., Grisan E., Lombardo M., Lombardo G., Salvalaio G., Patruno M., Iacopetti I., Martinello T.

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University of Padua, Department of Animal Medicine, Production and Health (Italy)

…. In literature…

Human medicine

Acta Ophthalmologica 2015

Review Article

Corneal collagen cross-linking for infectious keratitis: an update of clinical studies

Tommy C. Y. Chan,[1,2] Tiffany W. S. Lau,[1,2] Jacky W. Y. Lee,[3] Ian Y. H. Wong,[3] Vishal Jh Raymond L. M. Wong[1,2,3]

Original Article

Corneal Collagen Cross-linking for Treatment of Non-healing Corneal Ulcers

Mitra Zamani, MD; Mahmoodreza Panahi-Bazaz, MD; Mona Assadi, MD

Ophthalmic Infections Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Veterinary medicine

Veterinary Ophthalmology (2013) 1–11 DOI:10.1111/vop.12027

Corneal collagen cross-linking (CXL) for the treatment of melting keratitis in cats and dogs: a pilot study

Bernhard M. Spiess,* Simon A. Pot,* Marion Florin*,[1] and Farhad Hafezi†

Veterinary Ophthalmology (2013) 1–11

Corneal collagen cross-linking as treatment for infectious and noninfectious corneal melting in cats and dogs: results of a prospective, nonrandomized, controlled trial

Simon A. Pot,* Nicolin S. Gallhöfer,* Franziska L. Matheis,* Katrin Voelter-Ratson,* Farhad Hafezi† and Bernhard M. Spiess*

Hellander-Edman *et al. BMC Veterinary Research* 2013, **9**:128
http://www.biomedcentral.com/1746-6148/9/128

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Corneal cross-linking in 9 horses with ulcerative keratitis

Anna Hellander-Edman[1*], Karim Makdoumi[2], Jes Mortensen[2] and Björn Ekesten[3]

…. And from the histological point of view?…

EVALUATION OF THE

HISTOLOGICAL AND **IMMUNOHISTOCHEMICAL** CHANGES

INDUCED BY UV-A/RIBOFLAVIN CORNEAL CROSS-LINKING

IN **EXPERIMENTALLY INDUCED CORNEAL LESIONS**

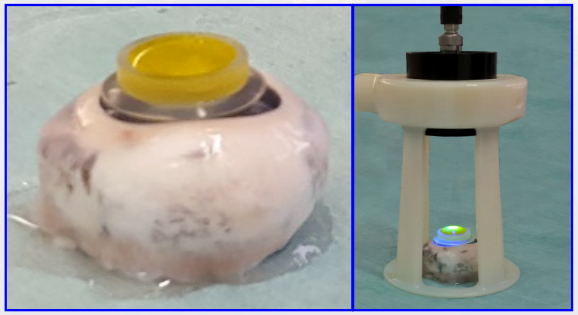
IN AN **EX VIVO ANIMAL MODEL**

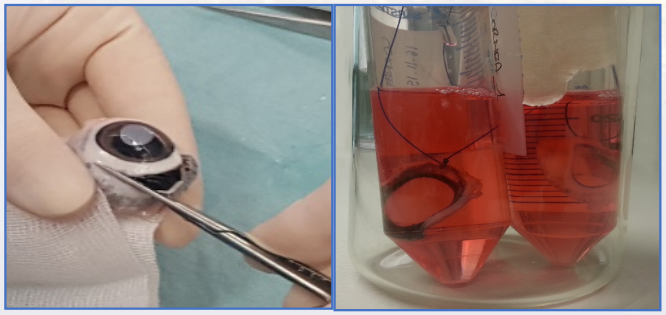
3 populations of coltured cornea: **10 HEALTHY**, **10 INJURED** (only lesion), **10 TREATED** (lesion+treatment)

- **induction of lesion**: ALKALI-INDUCED CORNEAL STROMAL MELTING
  (filter Whatman paper with NaOH 1N for 1 minute)

- **treatment**: APPLICATION of isoosmolar (dextran 20%) 0.1% riboflavin drops for 30 minutes
  IRRADIATION with UVA 30 mW/cm^2 for 3 minutes (5.4 J/cm^2) - Vetuvir®

- **isolation and colture**: 7 days in a colture medium (Carry-C®)

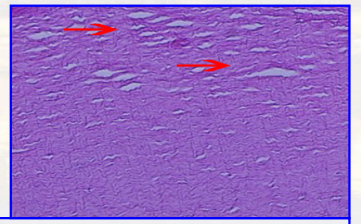


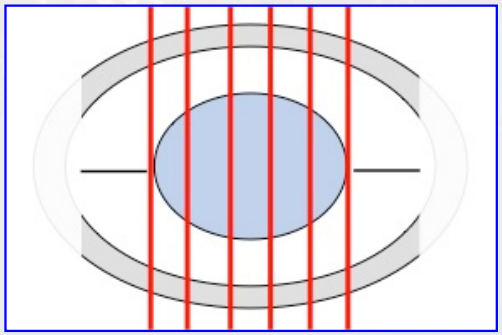


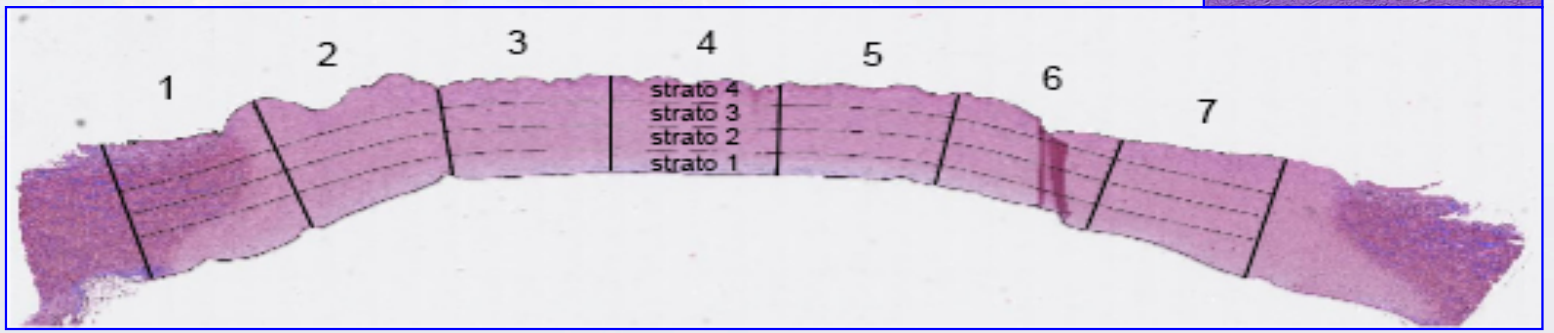
- **histological and immunohistochemical characterization**: serial 5-µm cryostat sections: evaluation in the healed area of 1) "cellularity" of the new tissue 2) orientation and diameter of collagen fibres 3) type of collagen expressed

- **image analysis**: lesioned fibers were quantified measuring the **indicator density (intensity of white area)** by using a specific software tool (developed in Matlab™). Density was recorded on **28 regions of interest (ROI)** defined by **7 radial section** and **4 layers** (identified dividing the cornea slide along the minor and the major)

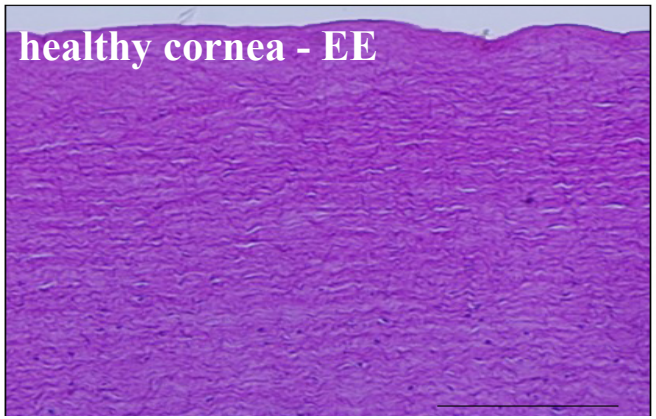
- **statistical analysis**: dotplot graphs, multiway ANOVA method and Tukey method

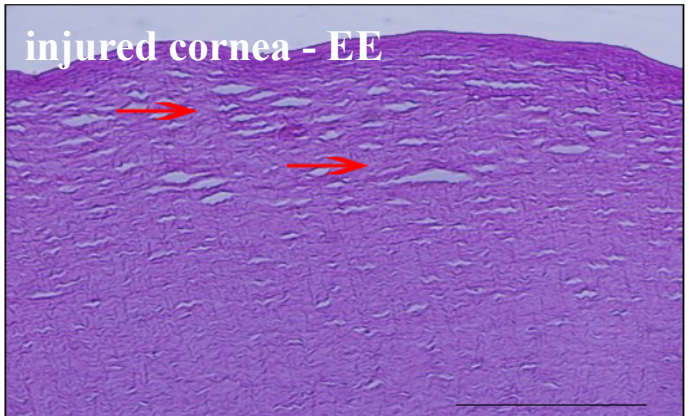


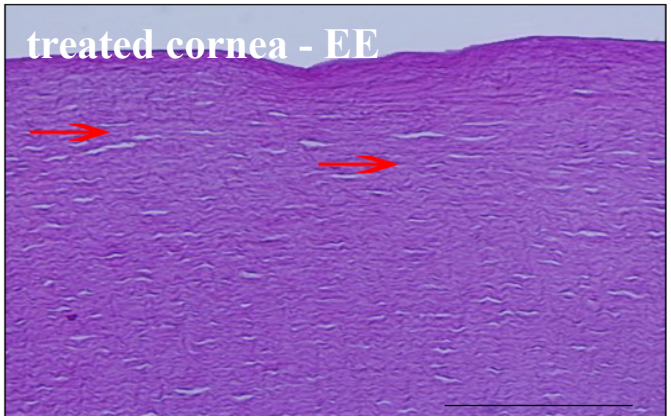




✧ **histological and immunohistochemical characterization**:







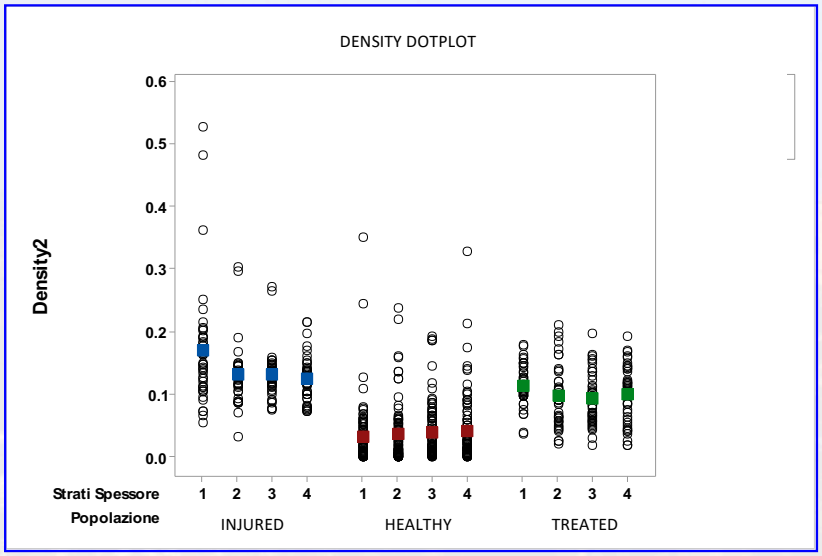
INJURED CORNEA → alterations of the stroma in the central area:

- **disorganization of the collagen lamellae**
- **many white fissures between the collagen fibers**
- **lack of cell nuclei**

TREATED CORNEA → better morphology of the stroma in the central area:

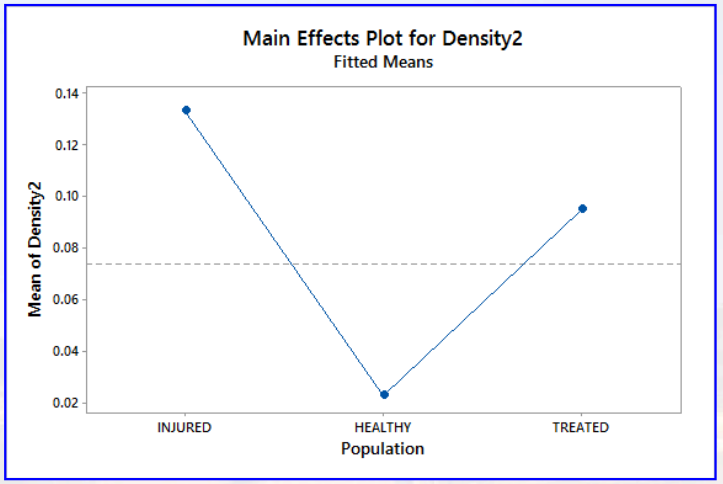
- **minor disorganization of the collagen lamellae (more tidy and compact)**
- **few white fissures between the collagen fibers**
- **presence of cell nuclei in the peripheral area with centripetal direction**

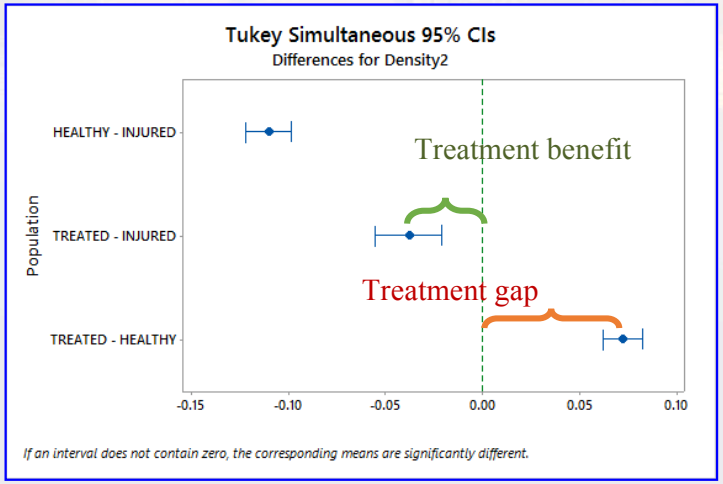
✧ **image and statistical analysis**: dotplot graphs, multiway ANOVA method, and Tukey method



This dotplot show the **DESCRIPTIVE ANALYSIS** for the 4-layer in each population **ACCORDING TO THE DENSITY VALUES**. The different dots represent the density values in the 4 layers, while the squares in blue, red and green represent the sample averages referring respectively to the injured, healthy and treated corneal populations.

SIGNIFICANT CHANGE of the MEAN OF DENSITY (5% significance p-value, six p-values (POPULATION, RADIAL SECTION, LAYER, HEALTHY, INJURED and TREATED)





At 95% confidence level we can finally conclude that the **TREATED CORNEAS HAS MEAN DENSITY LAYING IN BETWEEN THOSE OF THE CONTROL AND INJURED CORNEAS**.

- A **repeteable** and quantificable ex vivo model of corneal melting lesion

- An **appropriate image analysis** for the study of the healing process of the cornea

- A **statistically significant effect of cross-linking** on the induced lesions

The data obtained suggest an interesting continuation of the research project which will focus on:

- the evaluation of the repair process from a **cellular and molecular point of view**
- the evaluation of the repair process following a **longer time colture**
- the application of the **treatment on canine and feline patients** affected by melting ulcers and stromal cheratopathies.

Thank you for your attention

...and thanks to my precious co-author