

## ASSESSMENT OF UV-A/RIBOFLAVIN CORNEAL CROSS-LINKING EFFICACY FOR THE TREATMENT OF EXPERIMENTALLY INDUCED CORNEAL LESIONS IN AN EX VIVO ANIMAL MODEL

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**Purpose** – The aim of this pilot study was to evaluate the histological changes induced by UV-A/riboflavin corneal cross-linking in experimentally induced corneal lesions in an ex vivo animal model.

**Methods** – Three groups of long-term (7 days) cultured porcine corneas were used, such as the control group (healthy), the injured group (alkali-induced corneal stromal melting) and the treated group (injured and treated with Vetuvir, 30 mW/cm<sup>2</sup> for 3 min). All samples were kept in an organ culture medium for a week and then processed for histological analysis characterization. By using automated-image analysis of HE-stained sections, we collected quantitative data on histological features across the 3 group. Differences were evaluated using both parametric and nonparametric ANOVA-type inferential methods.

**Results** – Statistically significant differences ( $P < 0.001$ ) were found among the three groups in the mean of “brightness” (the proxy feature we used as measure of the relative extension of the injured areas in the corneal stroma). Specifically, the treated group showed a significant effect on the repair process after cross-linking treatment in comparison with the injured group (Tukey’s tests,  $P < 0.001$ ) and an equal level of brightness as in healthy (Tukey’s tests,  $P = 0.359$ ). Multi-aspect-type nonparametric analysis confirmed the effects in the mean of brightness showing also a significant difference in scatter between healthy and treated groups ( $P < 0.001$ ). These findings suggest that the tissue was recovered ‘in mean’ while keeping a higher heterogeneity.

**Conclusions** – The obtained results prove the effectiveness of cross-linking on the repair process from a histological point of view. The results support the rationale of the study and encourage further investigation in terms of extending both the sample size and the evaluating the repair process from a cellular and molecular point of view. One of the future aspects of this project could be the implementation of clinical trials of cross-linking on small domestic animals in order to evaluate whether technique may provide a valid alternative and /or complement conventional therapy also in the everyday clinical practice.

[1] Pot et al. Corneal collagen cross-linking as treatment for infectious and noninfectious corneal melting in cats and dogs: Results of a prospective, nonrandomized, controlled trial. *Veterinary Ophthalmology*, 17(4), 250–260, 2014. [2] Spiess et al. Corneal collagen cross-linking (CXL) for the treatment of melting keratitis in cats and dogs: a pilot study. *Veterinary Ophthalmology*, 17(1), 1–11, 2014. [3] Chan et al.

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