An Experimental Investigation of the Biomechanical Effects of Epi-On Corneal Collagen Crosslinking in Porcine and Human Models

Anna Pia Bonuomo[1,2], Ibrahim Seven[3], Pat Lopath[3], Paolo Fogagnolo[4], Federica Boschetti[2], Anna Pandolfi[1]

[1] Civil and Environmental Engineering Department, Politecnico di Milano, Milan, Italy,
[2] Chemistry, Materials, and Chemical Engineering Department, Politecnico di Milano, Milan, Italy,
[3] TECLens, Inc. (9 Riverbend Dr S, Stamford, CT 06907, USA),
[4] Eye Clinic, ASST Santi Paolo e Carlo, University of Milan, Health Sciences Department.

Background: Corneal collagen crosslinking (CXL) is an established therapy for halting the progression of corneal ectasia by strengthening the stromal architecture. This study investigated the biomechanical effects of transepithelial (epi-on) CXL through two separate experimental series: one conducted on porcine corneas and the other on human donor tissue. Porcine corneas were primarily used to develop and validate a consistent experimental protocol, due to their accessibility and anatomical similarity to the human cornea. Epi-on CXL offers several clinical advantages over the conventional epi-off approach, such as reduced risk of infection, faster epithelial healing, and improved patient comfort. Although the epithelium presents challenges in riboflavin diffusion and UV-A penetration, advancements in delivery techniques have enhanced its efficacy, making epi-on a promising alternative in corneal crosslinking therapies.

Methods: Fresh porcine corneas were mounted on a custom anterior inflation chamber and preconditioned with cyclic pressurization between 4 and 20 mmHg. Riboflavin was delivered using a reservoir lens for 30 minutes under 15 mmHg pressure, which is considered the physiological intraocular pressure (IOP) in a healthy human eye. The baseline hydration level was carefully controlled by adjusting the NaCl concentration in both the riboflavin and saline solutions at different stages to maintain tissue biomechanics. Epi-on corneal crosslinking (CXL) was then performed with UV-A irradiation at 4 mW/cm² for 10, 20, or 30 minutes. Corneal shape changes were tracked in real time via 3D optical coherence tomography (OCT). A theoretical model based on linearized shell theory was used to estimate the increment of the corneal stiffness. The same experimental workflow was applied to human donor corneas, with adaptations to account for their increased variability. Preconditioning pressures were extended up to 32 mmHg, and hydration control proved more challenging due to post-mortem changes in tissue permeability.

Results and Discussion: The linearized shell theory established a quantitative relationship between corneal stiffness and the UV-A irradiation dose. All experiments showed a significant increase in stiffness post-treatment compared to pre-treatment values, with longer UV-A exposure times resulting in a more pronounced stiffening. In porcine corneas, the most significant stiffening occurred with the longest UV-A exposure. This observation will be verified in human donor corneas in future experiments. The ultimate goal is to develop customized corneal crosslinking (CXL) treatments, tailoring parameters like UV-A exposure time to the biomechanical profiles of individual patients. Future developments will also focus on verifying the influence of the post-UV exposure period on the stiffening process in human corneas, as already observed in porcine corneas.

Keywords: Corneal Collagen Crosslinking (CXL), Transepithelial (Epi-On) CXL, Corneal Stiffening, UV-A Irradiation, Optical Coherence Tomography (OCT).