Immediate Effect of Ultraviolet-A Collagen Cross-linking Therapy on the Biomechanics and Histology of the Human Cornea

Wollensak et al. introduced corneal collagen cross-linking (CXL) using riboflavin and ultraviolet-A for the treatment of progressive keratoconus.1 Empirical in vitro studies evaluated the stiffening effect of CXL and some used human corneas to assess the stiffness achieved,2,3 but they did not present a histological analysis of the treated human cornea.

To learn the immediate mechanical and histological effect induced by CXL in human corneal tissue, we performed tensile stress tests and histological comparative observation of cross-linked and untreated healthy human corneas. The corneas were retrieved from the local tissue bank. They were clear with normal epithelium, endothelium, and corneal thickness. One sample received a standard epithelium–off-riboflavin/ultraviolet-A CXL treatment and the other was only deepepithelialized. The stress-strain test showed a stiffer response of the treated tissue than that of the untreated tissue. The treated cornea resulted in 1.8, 1.6, 1.7, and 1.5 folds stiffer than the untreated sample at the 6%, 8%, 10%, and 12% stretches, respectively. Stiffness of the tissue after treatment was increased by 64% (factor of 1.64). Although Wollensak et al.2 initially found a 350% increase of stiffness in the treated tissue (greater than that of the current study), this value was later corrected and an increase in stiffness by a factor of 1.5 was given by Spoerl in the 7th Cross-linking Congress. The study by Raiskup and Spoerl4 gave a value of 70% increase (factor of 1.7), which matches our result. Besides increasing the biomechanical rigidity of the human cornea, CXL induces secondary effects such as keratocyte apoptosis.1 Therefore, the safety of the procedure must be guaranteed. The cytotoxicity of the riboflavin/ultraviolet-A treatment on keratocytes and endothelium cells was studied by Wollensak et al.,2 who established a cytotoxic threshold of 5.4 J/cm². However, the significance of the damage is not yet completely known and, to our knowledge, it has not been reported in in vivo studies with human corneas in the literature.

Our histological analysis of the treated sample showed an immediate effect of the outermost stroma compacting with increased fibrillar density and decreased interlamellar space in comparison with the control. Dehydration reached approximately 65% of the thickness. Regarding the cellular population, the treated sample showed absence of keratocyte nuclei in the stroma even at a deep level, whereas the control sample showed intact keratocyte nuclei (Figure 1). Therefore, keratocyte apoptosis happens immediately after treatment in the cross-linked cornea and at a deeper level than expected, possibly due to immediately performing the histological analysis. Wollensak5 observed keratocytes in the posterior stroma 24 hours after CXL. We suppose keratocytes could have repopulated the deeper cornea during these hours.

According to our results, CXL has an immediate biomechanical and histological effect. Stiffening of the tissue, stromal compacting, and a keratocyte apoptosis deeper than compacting were observed.

REFERENCES

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