( ) R1 ( ) R2 (X) R3 ( ) PG0 ( ) PG1 ( ) Estagiário ( ) Tecnólogo ( ) PIBIC

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Service (sector)
Refractive Surgery (RS)

Nº CEP
01565/07

Fluorescence Confocal Microscopy of Porcine Corneas Crosslinked with Riboflavin and Ultraviolet-A

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Purpose: to assess ultrastructural stromal modifications in porcine corneas after riboflavin and ultraviolet-A (UVA) exposure using fluorescence confocal imaging.
Method: Fifteen freshly enucleated porcine eyes enrolled the study. Five eyes served as control (Group 1). Five eyes had its epithelial removed (group 2) and five eyes had its epithelial intact (Group 3). Groups 2 and 3 were crosslinked with riboflavin 0,1% solution (10 mg riboflavin-5-phosphate in 10 mL 20% dextran-T-500) and UVA (365nm, 3 mW/cm2) for 30 minutes. Ultrathin sections (10um) of the corneas were stained with anti-collagen-I (Seikagaku) and DAPI (4´6-diamidino-2-fenilindole, dihidrocloride) and its fluorescence was revealed under confocal microscopy (LSM 500 meta – Zeiss).
Results: The porcine treated corneas (group 2) showed an anterior pronounced fluorescence zone of 180 um, divided into a superficial zone of 130 um, with collagen fibers hightly organized and a posterior zone of 50um partially organized. This fluorescence anterior zone was not founded in the control group, neither in the corneas that wasn't previously deepithelialized (group 3). In order to check if the number and location of keratocytes was affected, the groups were analyzed by fluorescence microscopy for nuclei (DAPI staining). A reduction in the number of cell nuclei seems to occur after crosslink (group 2).
Conclusion: it was possible, for the first time in the literature, to observe the riboflavin/UVA treatment effect using confocal fluorescence imaging, allowing a direct quantitative study to be performed in order to establish the safety of such procedure in new devices to come. Crosslinked corneas showed a pronounced and limited zone of organized collagen fibers, strongest in the anterior stroma. Treatment of the cornea with riboflavin and UVA without previous deepithelization did not induce any crosslinking effect, thereby, to facilitate diffusion of riboflavin throughout the corneal stroma, the epithelium should be removed as an important and initial step of the treatment.