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Effect of trypsin-EDTA on expression of DNA damage repair enzyme OGG1 in human limbal- and conjunctival- epithelial cells.

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Enzymatic cell dissociation may provide cells for experimental cultures and tissue engineering. We hereby examine corneo-scleral ring-associated limbal- and conjunctival- epithelial cell morphology, as well as expression of DNA base-excision repair (BER) enzyme OGG1 during incubation in trypsin-EDTA.

All experiments were conducted in accordance with the Declaration of Helsinki and Local Committees for Medical Research Ethics. Post-mortem samples of organ cultured human corneo-limbal rings were incubated in 0.05% trypsin-EDTA for 0 (control) or 3 hours, fixed, embedded, sectioned and stained with H&E and processed for immunohistochemistry (IHC) for protein expression of OGG1.

In sections stained with H&E, limbal and conjunctival epithelium in control samples showed regular bi- or multilayered morphology. Incubation in trypsin-EDTA for 3 hours induced detachment of the superficial, as well as the basal limbal and conjunctival cells. Complete detachment of epithelial cells from the basement membrane was also observed. A low-level expression of OGG1 was detected by IHC in control samples, and any noticeable alteration in expression could not be observed during incubation in trypsin-EDTA.

Damage to DNA may, when unrepaired, interfere with cell function, differentiation, proliferation and viability. Dissociation of cells by trypsin-EDTA may induce molecular stress and damage. In vivo, oxidized DNA bases are normally repaired by BER enzymes including OGG1. Noticeable changes in the protein levels of this particular repair enzyme could not be observed during incubation in trypsin-EDTA in the present study.

Keywords:
DNA damage, repair enzyme, trypsin-EDTA, limbal epithelial cells, conjunctival epithelial cells.