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In vitro biocompatibility testing during early-stage development of ophthalmic formulations using a 3D corneal epithelial model

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Biocompatibility screening is an essential early step in preclinical studies of topical ophthalmic formulations. Given the increasing awareness of the cytotoxic effects of certain ophthalmic preservatives, it is crucial to assess the risks associated with topical application of the tested formulations. *In vitro* 3D cell models offer a physiologically relevant and ethically preferable approach for biocompatibility testing under well-controlled experimental conditions. Formulation biocompatibility should be evaluated following both single- and multiple-dose treatment.

In our study, biocompatibility testing was conducted during the development of a preservative-free latanoprost formulation. For this purpose, an extended-throughput 3D corneal epithelium model was employed, based on an immortalized human corneal epithelial cell line (HCE-T) cultured on polycarbonate inserts in a 96-well plate format. Biocompatibility screening was performed using complementary viability assays (MTT and CellTiter-Glo® 3D) and a cytotoxicity assay (LDH-Glo™). While viability assays determine the number of viable cells in a 3D cell culture, the LDH assay assesses plasma membrane integrity by measuring the release of the soluble, stable cytosolic enzyme lactate dehydrogenase (LDH).

The results demonstrated that benzalkonium chloride, used as a preservative, significantly reduced cell viability and caused plasma membrane damage, whereas preservative-free latanoprost formulations did not exhibit cytotoxic effects on cells following either single- or multiple-dose exposure.

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