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

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Service (sector)
Cornea and External Disease

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Gene Transfer to Primary Corneal Epithelial Cells Ex Vivo

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Purpose: To evaluate the transfer of heterologous genes to cultured corneal epithelial cells expanded in vitro.

Methods: Freshly enucleated rabbit corneoscleral tissue was used to obtain corneal epithelial cell suspensions via double enzymatic digestion (dispase followed by trypsin). Cells were plated at a density of 5×103/cm2 and allowed to grow for 5 days (to 70-80% confluency) prior to transduction with 5 dilutions of a GFP expressing lentiviral vector. Gene transfer was monitored using fluorescence microscopy and fluorescence activated cell sorting (FACS). At 4 days post-transduction, one set of P0 cells were dual sorted by FACS for GFP expression as well as Hoechst dye exclusion. A second set of cells was serially passaged to P2 and the levels of GFP expression monitored at each passage.

Results: GFP expressing lentiviral vectors (titers of 105cfu/ml) were able to effectively transduce rabbit primary epithelial cell culture in vitro. Live cell imaging 4 days post-transduction demonstrated GFP-positive cells with normal epithelial cell morphology and growth. When double sorted by FACS to isolate both GFP positive and side population cells, transduced side population cells were identified. Moreover, the percentage of side population cells that were transduced was similar to the percentage of putatively more mature cells in the main population. The number of cells transduced at P0 was markedly dose-dependent, and at the highest concentrations of lentivirus approached 9%. FACS sorting allowed for the isolation of a pure population of GFP positive transduced cells which were readily expanded to confluency at P1. After serial passages, transduced epithelial cells maintained a quantitatively similar GFP positive phenotype from P0 to P2, even as cell morphology became more mature. Furthermore, the side population cells were not only transduced, but the ratio of infected cells was found to approach the ratio of infected non-side population corneal epithelial cells.

Conclusions: Lentiviral vectors can effectively transfer heterologous genes to corneal epithelial cells expanded in vitro. Genes were stably expressed through

three cell passages and could be transferred to mature corneal cells as well as putative stem cells.