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Ex vivo gene transfer to primary corneal epithelial cells.

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Purpose: To develop a lentiviral vector system to transfer heterologous genes to primary cultures of corneal epithelium. **Methods:** Corneal epithelial cells from rabbits were obtained following surgical excision and subsequent isolation using enzymatic digestion. Cells were allowed to grow for 7 days prior to transduction with a GFP expressing lentiviral vector. Gene transfer was monitored using fluorescence microscopy. The epithelial lineage of these cells was evaluated via immunocytohistochemistry for p63 and cytokeratin 3 markers. **Results:** Viable corneal epithelial cultures were obtained, and at 7 days were approximately 90% confluent and beginning to form multiple layers. A transduction efficiency of 10% was observed by fluorescence microscopy following infection with a GFP expressing lentiviral vector. Infected cells were almost exclusively p63 and/or cytokeratin 3 positive, indicating that they were of an epithelial lineage of varying degrees of differentiation. **Conclusion:** We have demonstrated the feasibility of heterologous gene transfer to primary corneal epithelial cells. Given previously established techniques utilizing cultured epithelium for the re-population of stem cell depleted ocular surfaces, the ability to stably transfer genes to primary epithelial cells holds promise for ex vivo gene therapy.