

Use of a rabbit cornea model for the development of a cell transfer system for limbal epithelial cells

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INTRODUCTION: After cataracts, the major causes of blindness worldwide are diseases affecting the cornea. These diseases in particular have an adverse effect on the limbus: the stem cell factory of the cornea. Procedures have been developed to prevent blindness from occurring by transplantation of donor corneas or by transfer of limbal cells using a carrier, but these methods depend on the availability of the donor cornea and the carrier. Also it has been seen that the results maybe good, but the transparency of the cornea is not completely regained. The aim of this work is to develop a contact lens system for transferring laboratory expanded limbal epithelial cells for treatment of the cornea. The project uses a chemically defined plasma polymer surface which supports both the initial attachment of epithelial cells and their subsequent transfer onto the denuded cornea.

METHODS: A rabbit organ culture model has been established to examine the transfer of cells onto the cornea. Initial studies identified the most appropriate plasma polymer coating for support of epithelial cells. We are also examining the contribution of stromal cells to the survival of epithelial cells under serum-free conditions in this model. First we examined the culture of a human corneal epithelial cell line (HCEC) and primary rabbit limbal epithelial cells on a range of plasma coatings. Acrylic acid (AAc), allyl amine (AAM) and allyl alcohol (AIA) plasma polymer surfaces were synthesised at different powers and flow rates and their surface chemistry examined by XPS analysis. From these, the surface which best supported epithelial cell culture (both human and rabbit cells) was identified. Cells were then cultured on contact lenses coated with this surface. Rabbit corneal organ cultures with the intact epithelium were then denuded of epithelial cells (Fig 1a & b). Lenses with cells were placed onto the cornea and kept in place for 4 days. Transfer of cells from lenses was examined by pre-staining cells with CellTracker™ Red CMTPX and also by subsequent staining of cells on the cornea with DAPI and phalloidin FITC.

RESULTS: The surface that best supported the epithelial cell culture was acrylic acid. Preliminary results using this model show that the primary rabbit limbal epithelial cells and the human corneal cell line will transfer from the lens onto the cornea (Fig 1c & d).

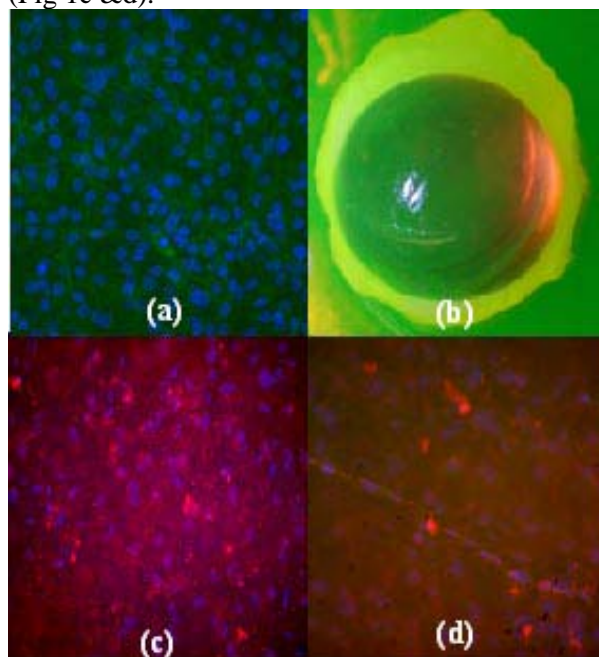


Fig 1: Culture model with (a) intact epithelium (labelled with DAPI and phalloidin FITC), (b) a denuded epithelium (stained with fluorescein), (c) rabbit limbal epithelial cells and (d) human corneal epithelial cell line after transfer from ppAAc coated lens (labelled with CellTracker™ Red).

CONCLUSIONS: Results show that this model can be used to develop a culture and transfer protocol which we hope to use for future clinical studies.

REFERENCES: Notara M, Bullett NA, Deshpande P, Haddow DB, Macneil S, Daniels JT (2007) Journal of Materials Science: Materials in Medicine, **18** (2): 329-338

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