CORNEA: KERATOPLASTIE B, KERATOPROTHESIS

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CORNEAL TRANSPLANTATION CAUSES RECURRENT HERPES SIMPLEX IN RATS

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Purpose To study HSV reactivation and recurrent disease after corneal transplantation in rats.
Methods Female PVG rats were inoculated on the cornea with HSV-1 McKrae. At least 4 weeks after inoculation, they received either allogeneic (DA or LEW) or syngeneic corneal grafts and were examined for signs of recurrent HSV disease for up to 30 days.
Results All allografts were rejected. Virus was shed in the tear film of 4/36 rats (11%) receiving allografts and 7/55 rats (13%) receiving syngeneic grafts. This was comparable to that after UV-irradiation of the cornea, a known stimulus of recurrent disease. Characteristic epithelial lesions were seen on the recipient cornea of 3 animals. One syngeneic graft became completely opaque. Neither removal of sutures nor rejection provoked recurrent disease. Histological examination of 4 eyes that shed virus revealed HSV antigens in all at the graft host junction. HSV-expressing cells were numerous in the stroma and extended to the endothelium. Infiltrating cells in diseased areas were mainly HIS48$^+$ granulocytes and many expressed HSV antigens.
Conclusions Surgical trauma appears to be the most potent stimulus of recurrent disease after transplantation. Virus occasionally recurs in the recipient epithelium, but does not penetrate the basement membrane to the stroma. The graft-host junction appears to be a 'weak spot' where antigen readily reaches the stroma, perhaps from nerve endings severed in the operation. Infiltrating cells then act as a conduit to the endothelium, which may become infected and prejudice the graft.

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CONFOCAL MICROSCOPIC AND HISTOLOGICAL EVALUATION OF LONG-TERM CORNEAL ENDOTHELIAL CELL TRANSPLANTATION ((J. Mohay,[1] J.V. Jester,[2] W.M. Petroll,[2] H.D. Cavanagh,[2] X. Chen,[1] T.O. Wood,[3] B.J. McLaughlin,[1])) Depart. of Ophthal. and Visual Sciences, Univ. of Louisville School of Med., Louisville, KY[1]; Depart. of Ophthal., Univ. of Texas, Dallas TX.[2]; Assoc. Ophthalmic Spec., Inc., Memphis, TN.[3]

Purpose: The present study determines the in vivo morphologic characteristics of long-term corneal endothelial cell grafts which can be correlated with clinical observations and ex vivo histological evaluation.
Methods: Transplantation of corneal endothelial cells using a cell/carrier device was performed in 19 rabbits. All grafts in this study were clear at the time of evaluation. Two grafts with 12 and 24 month survivals were evaluated in vivo by 3D confocal microscopy. The same transplants were processed for electron microscopy (EM) and light microscopy. Bromo-deoxy-uridine (BrdU) labeling of the grafted cells was also performed in order to distinguish between host and graft endothelium.
Results: Confocal microscopy of the grafts demonstrated a regular hexagonal pattern of the transplanted endothelial cell monolayer without polymegathism. EM of these same grafts showed identical ultrastructure with the unoperated corneal endothelium. Confocal microscopy detected endothelial "cracks", stress lines in the stroma and a 35% decrease in endothelial cell density. Confocal and EM microscopy confirmed a time-dependent increase in thickness of the newly formed Descemet's membrane in the grafted corneas. BrdU labeling showed a homogenous labeling of the transplanted, endothelial cell nuclei, which demonstrated that the original donor endothelial cells remained on the grafted carrier. Light microscopy showed a well positioned carrier with an intact endothelial monolayer in all 19 corneas.
Conclusion: This study demonstrates that corneal endothelial cells can be replaced and remain functional with few side effects. This may be a feasible alternative to current keratoplasty procedures. Supported by Baptist Memorial Research Foundation, Memphis, TN.

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ADENOVIRAL GENE TRANSFER INTO HUMAN ENDOTHELIAL CORNEAL CELLS OF CORNEAL GRAFTS EX VIVO

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Purpose To determine the efficiency of gene transfer into human endothelial cells of corneal button, preserved in culture media by an adenoviral vector.
Methods 10 human corneal button preserved in specific media at 31°C were selected for gene transfer. A recombinant adenoviral vector containing a marker gene encoding the enzyme activity β-galactosidase was added to the conservation media using doses up to 10^{10} pfu/ml. Efficiency, stability and toxicity of adenoviral particles were analysed after *ex vivo* transfer.
Results β-galactosidase staining of the corneal button shows a high efficiency of gene transfer into the endothelial cells. At the titers used no cytopathic effect was observed on the endothelial layer of grafts. No evidence for morphological changes was noted on the endothelial cell layer.
Conclusion Adenoviral vectors are suitable for gene transfer strategies into corneal endothelial cells and could open new avenues in the therapeutic approach of physiological and primary or secondary endothelial loss anddiseases.

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ULTRASTRUCTURAL AND BIOCHEMICAL ANALYSIS OF A FLUOROCARBON POLYMER IMPLANTED AT THE POSTERIOR SURFACE OF THE RABBIT CORNEA

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Purpose : It has been shown that an implant of porous expanded polytetrafluoroethylene (PTFE) in the corneal stroma allows fast cell colonization and can become translucent. We studied the behaviour of the same fluorocarbon polymer implanted in the anterior chamber of the rabbit eye and sutured to the posterior surface of the cornea.
Methods : The expanded tetrafluoroethylene was provided as 200 microns thick sheets in 3 pores diameters (2O, 5O and 8O microns). Discs (5 mm) were implanted in the anterior chambers of 18 rabbits and sutured to the posterior surface of the cornea. Histological and ultrastructural studies were performed after 3 and 4 months. Quantimetry was done on TEM images to analyse the fibrillar structure of the intercellular matrix inside and around the implanted polymer. Biochemical analysis was performed on postoperative day 120. After pepsin solubilization collagen content was determined by OH-proline assays.
Results : The material was well tolerated. There was mild central corneal edema in all cases, which disappeared after 1 month. Mild neovascularisation occurred in 5O %, decreasing after one month. The polymer became translucent after 8 days. Keratocytes from the corneal stroma colonized the implant via breaks in Descemet's membrane along the sutures. Quantimetry showed 3 types of fibrils inside and beside the polymer. Corneal endothelial cells regenerated over the fibroblasts and over the polymer. Collagen content was consistent with ultrastructural findings and similar to the results observed when the polymer was implanted in the corneal stroma.
Conclusion : Fluorocarbon polymer implanted in the anterior chamber and sutured to the posterior surface of the cornea was well tolerated and there was real incorporation, with keratocytes producing collagen inside the polymer and endothelial cells forming a posterior cell monolayer. This is promising for the development of a keratoprothesis with posterior fixation.