Matrix-metalloprotease activation in cultured corneas

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Corneas that are maintained in tissue culture medium prior to grafting shed their epithelial cells and their repopulation by host epithelial cells following surgery is an essential facet of the healing process. Failure to do so may be a result of structural damage to the epithelial basement membrane of a donor cornea and apparently occurs more frequently following epikeratophakia rather than penetrating keratoplasty. For these reasons, and because corneas destined to be cryo-lathed are often held in medium for periods exceeding 4weeks, we have investigated the possibilty that activation of the enzyme that exhibits specificity towards basement membrane type IV collagen (Gelatinase A or MMP-2) occurs during storage. The results obtained from a study of the Gelatinase A activities present in corneas that had been maintained in culture for varying lengths of time and were of varying donor age support this hypothesis and are as follows:-

(i) There is no correlation between total protease and corneal culture time or age.

(ii) The stromal tissue of fresh, normal corneas produce a single inactive gelatinase A of apparent Mr 65000

(iii) Cultured corneas readily produce a second inactive Gelatinase A of apparent Mr 61,000. The appearance of a third Gelatinase of Mr 43,000 correlates with corneal culture time but not age, and with the selective ability to cleave Type IV collagen.

APPLICATION OF ARTIFICIAL NEURAL NETWORKS FOR IMAGE ANALYSIS OF ORGAN CULTURE PRESERVED DONOR CORNEAS: A PILOT STUDY.

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Purpose: There has been a recent resurgence of interest in the study and application of computerized neural networks (NN) within the broad field of artificial intelligence. One approach of NN application aims at pattern recognition and pattern classification. This present study reports for the first time on the use of NN , Synapse I (SIEMENS), for morphometry of donor corneal endothelium in organ culture preserved transplants. Methods: Donor corneal endothelium was photographed with a color chilled 3CCD camera (HAMAMATSU) attached to an inverted phase contrast microscope (IMT-2 OLYMPUS) and scanned into a personnal computer. Experienced investigators evaluated the obtained image by defining and teaching Synapse I what is considered a cell. Results: Several models of NN have been trained, either adjusting some components of the architecture of the networks or combining different neural networks. The preliminary results confirm the potential and promising performance of the connectionist's approach and will be discussed

CONSTITUTIVE EXPRESSION OF THE VEGF RECEPTOR KDR/FLK-1 IN CORNEAL ENDOTHELIAL CELL MEDIATES THEIR PROLIFERATION.

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Purpose. Vascular endothelial growth factor (VEGF) binds to corneal endothelial cells (BCE). We screened BCE for the expression of the VEGF receptors KDR/FLK-1 and FLT-1 and analysed their functions.

Methods. The expression of KDR/FLK-1 and FLT-1 mRNA was analysed by northern-blot. BCE were also transfected with expression vectors carriing the VEGF receptors sequences. Their ability to proliferate upon VEGF or specific KDR ligands was studied.

Results. BCE cells express FLT-1, migrate but do not proliferate upon VEGF addition. Constitutive expression of KDR/FLK-1 confers to BCE cells the ability to proliferate in response to VEGF. Moreover the oligomerization of KDR/FLK-1 obtained by bivalent ligands leads to higher mitogenic response than that obtained with VEGF, whereas monovalent ligand have no effect.

Conclusion. These results emphasize the requirement of KDR/FLK-1 oligomerization for BCE proliferation.