

# ABSTRACT

Corneal quality and its preparation for keratoplasty can determine significantly its functionality and survival of the graft. Corneas are generally stored in media at reduced temperature (hypothermic storage) or at 31-37 °C (tissue culture storage). Among the important factors for corneal graft survival is the presence of Fas ligand in the corneal endothelial cells. The quality of the storage medium could be significantly influenced by metabolites of the stored tissue. Nitric oxide produced by corneal cells can have cytoprotective or cytotoxic effects depending upon its concentration.

The aims of this study were to examine the behaviour of injured corneas (by the induction of a lesion in the corneal endothelium) under tissue culture or hypothermic conditions, and to characterize the repair process and its rate using histological, microscopic and morphometric approaches. Real-time RT-PCR and immunohistochemistry were used to detect the changes in the expression and localization of Fas ligand depending on time of storage and storage conditions. Nitric oxide production by corneas stored up to 3 weeks in tissue culture or hypothermic media was followed using the Griess reaction.

Our results show that the repair process occurs only during tissue culture storage. A lesion area of 1 mm<sup>2</sup> was fully repaired within 5 days; however, the endothelial cell density in the wounded area continues to increase up to 3 weeks post-injury. These results indicate that corneas with presence of dead cells or with endothelial defects should be stored under tissue culture conditions. The repair kinetics enable us to estimate the storage times that are sufficient for repairing larger defects of the corneal endothelium.

We discovered that Fas ligand is maintained on the plasma membrane for at least 3 weeks of tissue culture storage. Fas ligand is expressed in the endothelium of corneas stored under hypothermic conditions, as well, but partial inhibition of protein transport to the plasma membrane occurs.

We determined the level of nitric oxide in corneas stored under tissue culture or hypothermic conditions and found that these concentrations are lower than those that could cause cytotoxic effects, especially in the endothelial cells.

The results of this work have practical consequences that can be useful for further improving of tissue quality for keratoplasty and increasing our understanding of the behaviour of corneas stored under different conditions and for different lengths of time.