Cell viability assay in corneal endothelium

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Introduction

Endothelium is the inner layer of the cornea, which must be viable for transplantation. The limited availability of corneas makes necessary the developing of preservation techniques that allow a long storage without losing endothelial viability.

Objectives

Determination of the presence of live cells in cryopreserved corneas after thawing.

Methods

Two corneas were cryopreserved by classical cryopreservation while in other two corneas, vitrification was realised. Extracted endothelia from thawed corneas and on endothelium from a cornea which was storage in hypothermic conditions (control) were stained with LIVE/DEAD imaging kit and Hoechst. Corneal endothelia were imaged using a fluorescence microscope.

Results

Viable cells were found in corneal endothelium of cryopreserved corneas by classical cryopreservation (Figure 1, A and B) and in corneal endothelium of cornea which was storage in hypothermic conditions (Figure 2).

Endothelia form vitrificated corneas showed cells with low esterase activity, and any viable cell (Figure 1, C and D).

Non viable cells, intermediate cells, and cells with positivity for Hoechst and for the two components of the kit was observed, but in a small number (Figure 2 and 3).

Conclusions

Classical cryopreservation offers viable endothelial cells in thawed corneas, while vitrification cause the lost of viability of endothelial cells.

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