Abstract

Introduction: Recent advances in posterior lamellar keratoplasty necessitated the preparation of posterior corneal lamellae even in the Czech Republic. The aim of this work was to introduce and standardize a novel method of manual preparation of corneal lamellae for Descemet Membrane Endothelial Keratoplasty with a Stromal rim (DMEK-S) in an ocular tissue bank. After setting the criteria for endothelial quality, we obtained a licence to provide the tissues for transplantation purposes. The obtained results were analysed after two years. Furthermore, a novel lamellar insertion technique using a cartridge was assessed. The potentials for the long-term storage of posterior corneal lamellae by the vitrification in liquid ethane was studied using human amniotic membrane as a model tissue.

Material and Methods: Corneoscleral buttons stored in tissue cultures were used to prepare lamellae consisting of a central zone of endothelium and Descemet membrane supported by a stromal rim at the periphery. The manual preparation was performed on an artificial anterior chamber using the big bubble technique. Endothelial quality was assessed before storage, before and immediately after preparation as well as after 2 days of storage at 31°C. A group of 12 corneas with a live endothelial cell density $\geq$ 2500 cells/mm^2 and a group of 10 corneas with a lower density were assessed to establish the tissue quality criteria. All 65 successfully prepared pre-cut posterior corneal lamellae provided for grafting during a two-year period were analyzed retrospectively. The damage to endothelial cells induced by the experimental insertion technique using a cartridge was assessed. Two groups of amniotic membrane specimens differing in their preparation method were vitrified in liquid ethane (-183°C). The epithelial viability was assessed 1, 3 and 7 days after thawing using calcein and ethidium homodimer-1 fluorescence.

Results: A live endothelial cell density $\geq$ 2500 cells/mm^2 was set as the main criterion for acceptable lamellar quality. Immediately after lamellar preparation, we found on average 1.8 % of dead cells, decreasing to 1.0 % after two days of subsequent storage. The damage to endothelial cells induced by the experimental insertion through the cartridge was 3.3 % on average. After vitrification, the presence of live epithelial cells was observed in both tested groups. Mechanical pressure used to push out the excess culture medium between the holder and the membrane resulted in a higher cell survival rate.

Conclusion: A novel method of corneal preparation for posterior lamellar keratoplasty was successfully introduced, and the tissue quality criteria were established. Altogether, 65 corneal lamellae were prepared under the conditions of an ocular tissue bank and used for grafting during a two-year period. The cartridge-based lamellar insertion technique induced only mild damage to the endothelial cells and therefore can be recommended for clinical use. A method for the vitrification of amniotic membranes in liquid ethane was introduced as a model for the long-term storage of posterior corneal lamellae.

Key words: cornea; corneal endothelium; posterior lamellar keratoplasty; amniotic membrane; vitrification.