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TECHNOLOGY FOR OBTAINING AN ULTRATHIN POSTERIOR LAMELLAR CORNEAL GRAFT AT THE EYE TISSUE BANK

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Objective: to develop technologies for preoperative preparation of the posterior lamellar corneal graft based on our own formulation of the preservation medium for optimal dehydration of the donor cornea and a technique for cutting out an ultrathin flap using an optimized method at the Eye Tissue Bank. **Materials methods.** In a series of experimental studies, we obtained data on the hydration level of cadaveric donor corneas that were preserved in various solutions at different observation periods. Using 16 corneas, analytical weighing and pachymetry were performed via optical coherence tomography in the experimental (n = 8) and control (n = 8) groups. Morphological and functional characteristics of the corneal endothelium were then assessed. At the next stage of work, ultrathin grafts were formed from 16 corneas after hypothermic preservation in the experimental (n = 8) and control (n = 8) solutions by single-pass microkeratome, followed by microscopy of the samples using a scanning electron microscope. **Results.** After the first days of preservation in the proposed solution, there was dehydration of 9% cornea in the experimental group in comparison with the samples of the control group. After 4 days of preservation, there was no reliable difference found between the groups ($p > 0.05$) in the study of the endothelial cell viability of ultra-thin corneal grafts by immunofluorescent microscopy using the "Live and dead" marker. Scanning electron microscopy revealed that corneal stromal collagen fibers, preserved in the proposed medium, retained their integrity. **Conclusion.** The proposed technology can be recommended for use at eye banks for formation of an ultra-thin corneal graft at the preoperative stage.

Keywords: ultrathin lamellar corneal graft, preservation solution, eye bank, keratoplasty.

INTRODUCTION

Penetrating keratoplasty (PK) has long been considered the gold standard for surgical treatment of patients with epithelial endothelial dystrophy (EED) of the cornea. In order to improve the biological and functional results of engraftment of large corneal grafts, various PK modifications have been proposed at different times, namely: mushroom, conical, and stepped keratoplasty [1]. However, PK and its modifications still do not exclude the emergence of a number of significant problems: the operation is accompanied by a volumetric and prolonged depressurization of the eyeball, which leads to the risk of hemorrhagic and infectious complications; in the post-transplant period, immunobiological reactions of tissue incompatibility often develop and induced ametropias of varying severity appear. leading to unsatisfactory optical results [2, 3]. To eliminate the above problems associated with PK and the leading role of the endothelial layer in the development of EED, various techniques for replacing the posterior corneal layers have been proposed since the middle of the last century, but in 2001 M.A. Terry developed a technique of layer-by-layer replacement of damaged posterior layers of the cornea, called endothelial keratoplasty and performed using high-precision, high-tech microsurgical equipment and instrumentation [4]. To date, several modifications of endothelial keratoplasty have been proposed, one of which is descemet stripping automated endothelial keratoplasty (DSAEK), which is most widely used in the clinic for the treatment of patients with EED of the cornea of various origins [5–7]. The essence of the DSAEK operation is to remove Descemet's membrane with a layer of the affected corneal endothelium of the recipient and replace the cut-out graft of the posterior layers of the donor cornea through a 3 mm incision using a modified Busin slider and then pressing the flap against the posterior surface of the cornea with sterile air [8, 9]. At the same time, all stages of automated cutting out of the posterior corneal graft are carried out exclusively in the operating room, in parallel with manipulations on the patient's eye, which delays the operation time. In addition, the forced haste of the surgeon when performing intraoperative sections of the donor cornea often ends with the perforation of the ultrathin graft and the cancellation of the operation. Until now, both in Russia and abroad, there are no formulations of conservation media for nominal dehydration of the donor cornea and the optimal technique for cutting out ultrathin grafts (50–145 microns, on average 127 microns) using a single-pass microkeratome under

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the conditions of the Eye Tissue Bank at the stage of preoperative preparation [10–14]. The urgency of the problem and the lack of resolution of the above provisions in the DSAEK technology determined the purpose of thy present study.

The **purpose** of the present study was to develop a technology for preoperative preparation of the posterior layer-by-layer corneal graft based on our own formulation of the preservation medium for optimal dehydration of the donor cornea and the technique of cutting out an ultrathin flap using an optimized method in the conditions of the Eye tissue bank.

MATERIALS AND METHODS

Viable cadaveric human corneas used from the Eye Tissue Bank of the Fyodorov Eye Microsurgery Federal State Institution of the Ministry of Health of Russia (32 from 16 donors). One cornea from each donor was used as a control, the other, a steam room, served as a prototype. The control corneas were placed in vials with a basic preservation medium [15]. The corneas included in the experimental group were preserved in vials with the previously declared "agent for the preservation of the posterior layer-by-layer graft of the donor cornea" [16], which, due to its pharmacological components, has a membrane-stabilizing and membrane-restoring effect. At the first stage, the level of hydration of the stroma of preserved donor corneas in the experimental (n = 8) and control groups (n = 8) was studied by analytical weighing (Sartorius BP 210S, Germany) and pachymetry using an optical coherence tomograph (Optovue, iVue 100, USA) on 0, 1, 2, 3 and 4 days of the study. Upon completion of conservation, a morphofunctional study of the viability of endothelial cells of the obtained ultrathin corneal grafts was performed using immunofluorescence microscopy (Olympus FV 10i, Japan) using the Live and dead marker (Abcam, Great Britain). Based on the data obtained, the preservation period was determined at which the optimal dehydration of donor corneas is achieved. Then we proceeded to the second stage of the study, in which the corneas of the control (n = 8) and experimental (n = 8) groups were preserved for 2 days in the indicated solutions. On the 2nd day of conservation, ultrathin posterior layered grafts were formed from the corneas of both groups under the control of an optical coherence tomograph (Optovue, iVue 100, USA) using a microkeratome (Moria, France) by a single pass with a 550 μm cutting head (Moria, France). Then the samples of both groups were subjected to microscopy using a scanning electron microscope (JEOL JCM-6000, Japan) to assess the ultrastructure of the endothelial cell layer, orientation and damage of stromal collagen fibers, and the section profile.

DATA PROCESSING

To count the endothelial cells of ultrathin posterior layered grafts, CellProfile software was used, which allows for a quantitative analysis of images of stained cells.

Statistical analysis of the data obtained was performed with Graph Pad Prizm7 software.

RESULTS

According to the results of the first stage of the study, measuring the weight and pachymetry of donor corneas, dehydration and a decrease in thickness by 9% from the initial in the experimental group on the 1st day of conservation with a gradual increase and achievement of the nominal value (weight 0.195 ± 15 g, thickness 648 ± 35 μm) by the 3rd day, in the control group hydration (increase in thickness and weight) was observed from the first day of conservation. Pachymetric assessment using an optical coherence tomograph at all periods of observation in the experimental and control groups is shown in Fig. 1.

Thus, a correlation was found between the weight and thickness of the studied corneas during the conservation process. Determined the degree of dehydration in the first two days in the experimental group and hydration in the control from the first day of conservation. In addition, it was found that starting from the 3rd day, hydration in the experimental group reached nominal values with a tendency to increase, so further observation became inappropriate. Thus, the experimental preservation medium developed in this study [16] provides a more pronounced dehydration of the cornea during the first 2 days of storage compared to the standard preservation medium used in the control group.

At the end of 4 days of conservation in the study of the viability of endothelial cells of ultrathin corneal grafts by immunofluorescence microscopy using "Live and dead" marker, there was no significant difference between the groups ($p > 0.05$): 88.4% of living cells, 11, 6% of dead cells in the experimental group (Fig. 2, a) and 87.9% of living and 12.1% of dead cells in the control group (Fig. 2, b). Thus, despite the dehydration in the experimental group, the study of the morphofunctional preservation of endothelial cells of the ultrathin graft did not reveal a significant difference with the control group, which excludes the toxic and damaging effect of the conservation medium created according to the proposed recipe.

At the second stage of the study, ultrathin posterior layered grafts in both groups were formed using a longitudinal microkeratome by a single pass with a 550 μm cutting head (Moria, France). It was noted that in the experimental group, due to the lower hydration of the donor

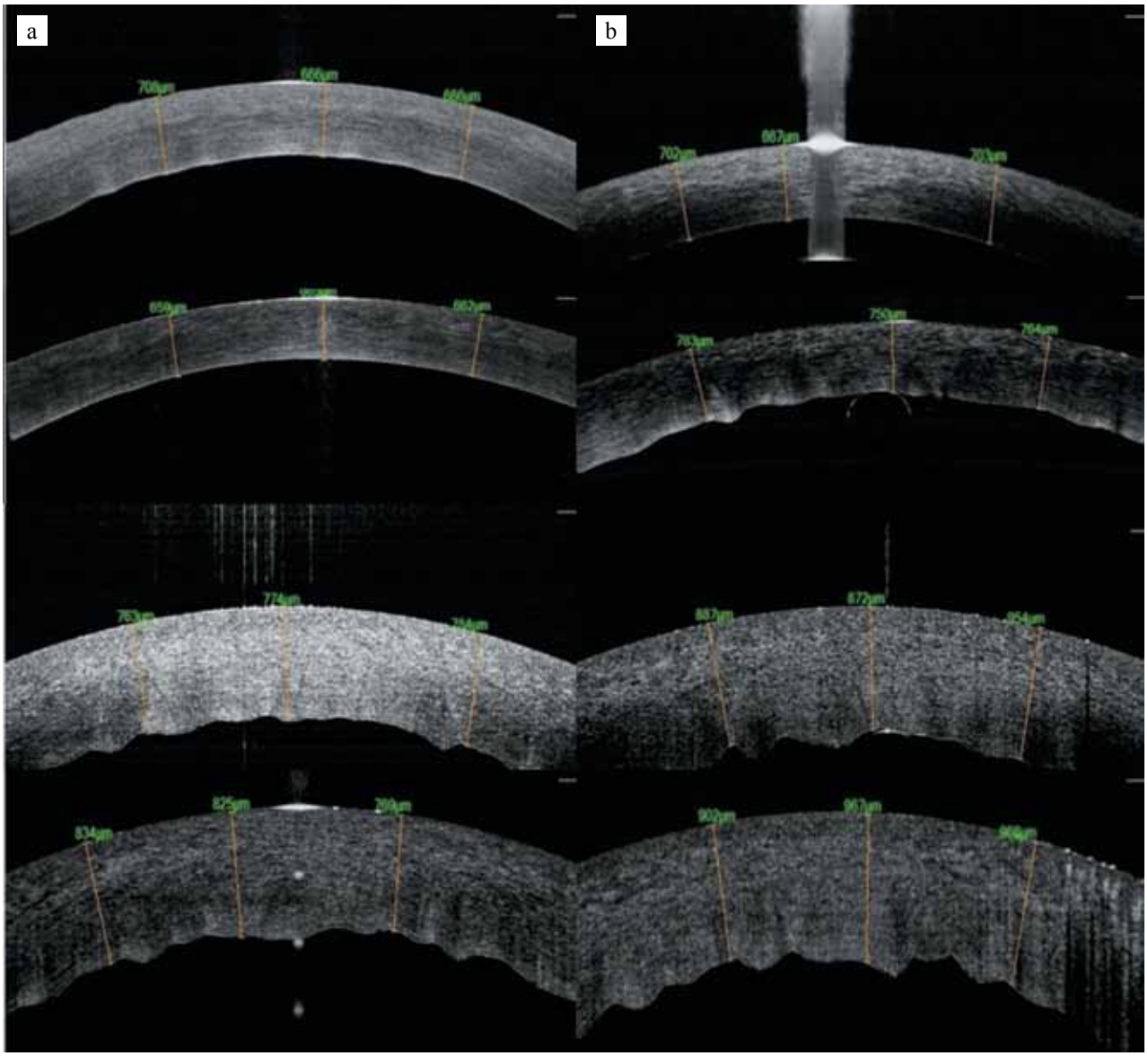

Fig. 1. OCT-picture of changes in the thickness of the donor cornea in the experimental (a) and control (b) group on the 0th (1), 1st (2), 3rd (3) and 4th (4) day of the study

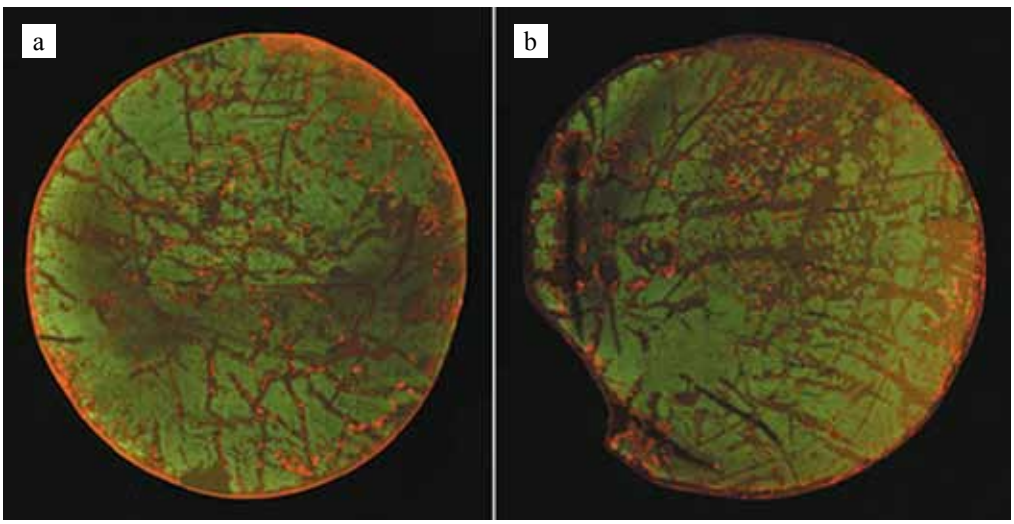

Fig. 2. Fluorescence staining of endothelial cells of the posterior corneal graft on the 4th day of conservation, Live and dead, in the experimental (a) and control (b) group. Laser scanning microscopy, ×100. Staining: green – living cells, red – dead

corneas, a single passage with the cutting head makes it possible to form statistically significantly ($p < 0.05$) thinner posterior layered grafts (123 ± 27 μm) compared to the control (190 ± 35 μm) (Fig. 3, a–b).

The results of scanning electron microscopy of ultrathin grafts formed according to the proposed technique demonstrate the preservation of the architectonics of corneal collagen fibers preserved in the proposed medium (Fig. 4, a), and signs of hydration of corneal fibers from the control group on the 2nd day of conservation (Fig. 4, b). Additionally, an image of the ultrastructure of the graft of the experimental group is shown in Fig. 5.

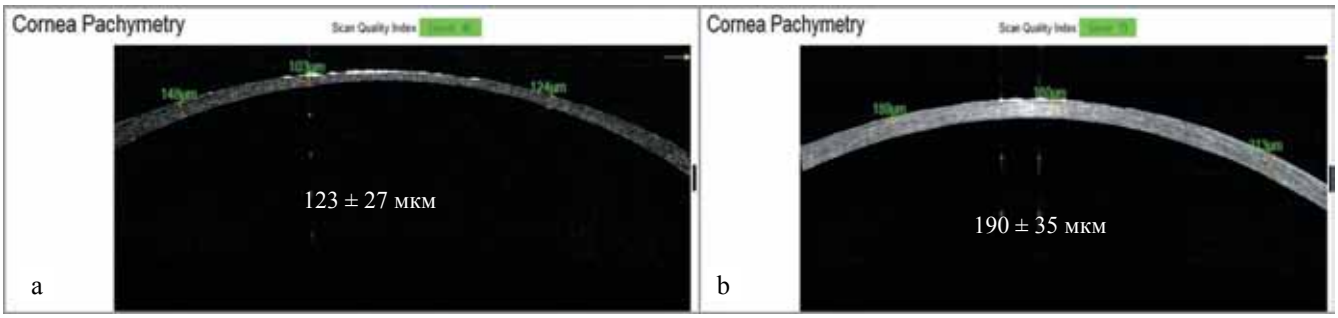

Fig. 3. OCT picture of an ultrathin posterior transplant of a donor cornea from the experimental (a) and control (b) groups

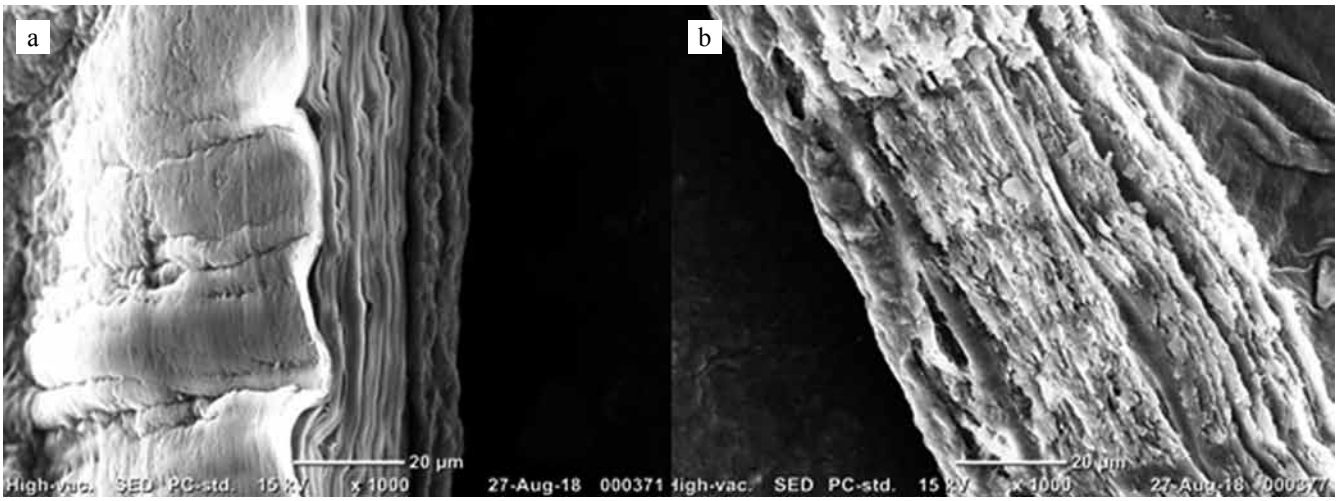

Fig. 4. The picture of the slice of the ultrathin posterior transplant on the 2nd day of conservation in the experimental (a) and control (b) group. Scanning electron microscopy, ×1000

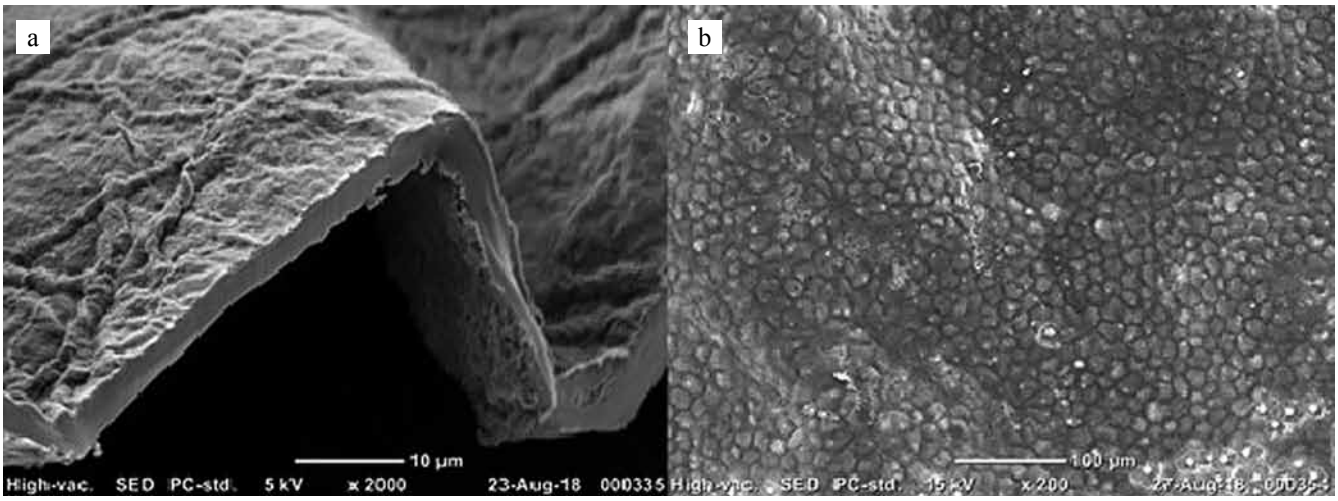

Fig. 5. Pictures of the profile of the slice (a) and endothelial layer of cells (b) of the ultrathin posterior graft on the 2nd day of conservation in the experimental group. Scanning electron microscopy, a – ×2000, b – ×200

DISCUSSION

The advantage of selective endothelial keratoplasty at the moment is doubtless. Operation in a closed palate significantly reduces the risk of developing dangerous intraoperative complications; in addition, the absence of an extensive through scar and complete preservation of the stroma allows maintaining the biomechanical resistance of the cornea to injury, innervation and trophism of the recipient's cornea, and minimizing induced astigmatism. In general, this contributes to a reduction in the period of clinical and visual rehabilitation of patients after transplantation of the donor cornea. The descemet stripping automated endothelial keratoplasty (DSAEK) is currently the most commonly used surgical method for the rehabilitation of patients with epithelial endothelial corneal dystrophy of various origins in developed countries. However, the question of the possibility of improving the clinical and functional results of DSAEK by using grafts of the posterior layers of the donor cornea with a minimum residual stroma thickness remains unresolved. In this regard, on the basis of eye tissue banks, the proposed technology for the preparation of an ultrathin flap of a cadaveric donor cornea based on the created preservation medium for dehydration and an optimized cutting technique can be used.

CONCLUSION

The conservation medium developed in this study [16] provides a more pronounced dehydration of the cornea during the first 2 days of cultivation as compared to the standard conservation medium. It was shown that this fact makes it possible to obtain a graft of the posterior corneal layers with a thickness of 123 ± 27 μm by a single passage with a microkeratome with a 550 μm head, compared with a thickness of 190 ± 35 μm obtained in the control group. The study of the morphofunctional preservation of endothelial cells of the ultrathin corneal graft did not reveal a significant difference with the control group, which excludes the toxic and damaging effect of the conservation medium created according to the proposed recipe. Thus, the proposed conservation medium can be used to obtain ultrathin transplants of the posterior layers of the cornea and thereby improve the functional results of the operation.

The authors declare no conflict of interest.

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