Comparison of Swollen and Dextran Deswollen Organ-Cultured Corneas for Descemet Membrane Dissection Preparation: Histological and Ultrastructural Findings

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PURPOSE. To compare the use of swollen tissue versus tissue deswollen by addition of dextran to the medium in the dissection of organ-cultured Descemet membrane (DM) with regard to preparation-related characteristics and ultrastructural findings to optimize transplantation in Descemet membrane endothelial keratoplasty (DMEK).

METHODS. DMs of 20 corneoscleral rims were separated using organ-cultured groups, one immersed in culture medium without dextran (group A) and the other in medium with added dextran for 24 hours (group B). The preparation details were noted. The difficulty of preparation was analyzed using a scoring system (0 = impossible, 10 = easy). By means of a micrometer, ultrathin sections of endothelial layer were obtained. Presence of any residual stroma, thickness of the DM, thickness of the endothelial cell layer, and the smoothness of the lamella were analyzed.

RESULTS. In both group A and group B, all 10 usable DMs were available. Mean preparation time was 6.5 ± 1.4 minutes in group A and 6.1 ± 0.9 minutes in group B (P = 0.399). The difficulty score was 7.9 ± 1.9 in group A and 8.0 ± 2.0 in group B (P = 0.726). The total mean thickness of the DM (without the endothelial cell layer) was 13.58 ± 2.81 μm in group A and 12.69 ± 2.06 μm in group B (P = 0.474). The total mean thickness of the endothelial cell layer was 3.99 ± 0.62 in group A and 3.98 ± 0.52 in group B (P = 0.989). Light microscopy and transmission electron microscopy revealed no evidence of any adherent remnants of corneal stroma on any specimen in each group.

CONCLUSIONS. DM-endothelium grafts for transplantation in DMEK procedures can be surgically prepared from organ-cultured corneal rims in swollen and deswollen conditions. Both separation methods seem to be equivalent in regard to the preparation characteristics of the obtained DM. Nevertheless, clinical studies are still necessary in order to confirm these findings.

Keywords: Descemet membrane endothelial keratoplasty, Descemet membrane dissection, electron microscopy, Descemet membrane, organ culture

Recently, endothelial keratoplasty has been shown to offer a promising alternative to penetrating keratoplasty and has become a popular procedure for the management of corneal endothelial failure.1–4 Melles et al.5 introduced a new technique for transplanting isolated Descemet membrane (DM) through a self-sealing tunnel incision, which they referred to as Descemet membrane endothelial keratoplasty (DMEK). Compared with penetrating keratoplasty, DMEK leaves a secure eye with less postoperative astigmatism, faster visual rehabilitation, and better optical quality.5,6 Lie et al.8 obtained donor material by stripping off the DM with a fine forceps technique. Meanwhile, different types of dissection techniques and instruments were described.9–11 Today, organ culture is the most commonly used corneal storage method in Europe.12,13 It allows long storage times of more than 4 weeks.14 However, prolonged storage times may lead to increased endothelial cell loss and decreased graft survival.15,16 A disadvantage of organ culture is the need for a deswelling process before corneal transplantation, since all organ culture media rapidly trigger significant corneal swelling, resulting in doubled corneal thickness, reduced corneal transparency, and increased stromal and DM folding.16,17

For optimal surgery, the corneal graft thickness must be similar to that of the recipient’s cornea when performing a penetrating keratoplasty procedure. Deswelling is usually performed by applying a hyperosmolar medium (i.e., 5% dextran-containing medium) for 24 hours.18 A maximum endothelial cell density at the time of transplantation is necessary to ensure long-term graft survival, but the corneal endothelial cell number declines during the deswelling process.19,20 With the new developments of lamellar surgery techniques, the requirements for corneal banking will most likely change. For preparation of the DM for DMEK surgery, it is not mandatory to deswell the corneas. To our knowledge no data about the preparation details in swollen and deswollen corneas are available to date. The purpose of the current study was to compare DM preparation characteristics in both swollen and deswollen corneas to evaluate the culture conditions used and evaluate the superiority of one over the other.
MATERIALS AND METHODS

Organ Culture Storage Conditions

After preparation of the corneoscleral buttons, they were stored in culture medium I (Culture Medium I; Biochrom AG, Berlin). The medium was first changed after 3 days of incubation. In group A, cell culture settings were kept the same until the preparation of the DMs, whereas in group B, the corneoscleral buttons were immersed in dextran-containing culture medium II (Culture Medium II; Biochrom AG) 24 hours before preparation. The thickness of the DM was measured with the AxioVision 4.8.2 program (Version 15, 06-2010; Carl Zeiss, Göttingen, Germany). Three pictures were taken from each DM at ×630 magnification, and the thickness was measured in each picture at four different positions.

Descemet Membrane Preparation. A total of 20 human corneas with a mean donor age of 78.6 years, not suitable for therapeutic transplantation, were provided by the Tuebingen Eye Bank. A paired design was applied to allocate the corneas. The right cornea was used for group A and the left cornea for group B. The donor corneoscleral rim was placed on a sterile oval surface and stained with trypan blue to highlight the scoring mark. Thereafter, it was placed in a corneal viewing chamber containing corneal storage solution. While holding the donor rim with a toothed forceps, the scored edge of DM was grasped with a curvilinear forceps type and stripped slowly off the stroma. Afterwards, it was placed back, the 8.5-mm central area was trephanned, and then both electron microscopical and light microscopical analyses were done (Fig. 1). All preparation details, for example, time, difficulty of DM preparation using a scoring system 0 to 10 (0 being impossible, 10 being easy dissection), and details about tears were noted accurately. All preparations concerning lamellar techniques of keratoplasty were performed by the same experienced surgeon (EY).

Light and Electron Microscopy

After preparation of the DMs, they were fixed overnight at 4°C in a 0.1 M cacodylate buffer (pH 7.4, 2% glutaraldehyde, 100 mM sucrose). After washing with the cacodylate buffer, DMs were postfixed with 1% osmium tetroxide in a 0.1 M cacodylate buffer at room temperature for 1 hour. Dehydration was then started by a series of 10-minute incubations in 30%, 50%, and 70% ethanol. The samples were stained with saturated uranyl acetate. Dehydration was continued by incubations in 70%, 80%, 96% ethanol (10 minutes each), absolute ethanol (two times for 15 minutes each), and propylene oxide (two times for 15 minutes each). The samples were then embedded in Epon (SPI-Pon812 Epoxy Embedding Kit; SPI Supplies, West Chester, PA). Semi-thin
sections were stained with toluidine blue and examined by light microscopy (Axioplan2 imaging; Carl Zeiss). For electron microscopy the sections were cut ultrathin, stained with uranyl acetate and lead citrate and observed using an electron microscope (Model 902; Carl Zeiss). Electron microscopy was used to assess the presence, localization, and thickness of residual stroma. For light microscopy hematoxylin and eosin, Alcian blue, and periodic acid Schiff staining were performed and observed under microscope (Axiovert 135; Carl Zeiss).

**Expression of Results and Statistics**

Data from group A and group B were combined for the overall comparisons and correlations. Comparisons were assessed by using a paired t-test when data were distributed normally.
otherwise a Wilcoxon nonparametric test was used applied. All data that was expressed with the mean ± SD, and $P < 0.05$ was considered as statistically significant. All analyses were performed using commercial software (version 19.0, SPSS, Inc., Chicago, IL).

**RESULTS**

**Preparation and Endothelial Cell Count**

In group A 10 usable DMs were available, but one of the DMs suffered a peripheral tear during preparation (donor age 80 years). However, by preparing the DM from the opposite side, a transplantable lamella (central DM of 8.5 mm in diameter) could be salvaged. In group B 10 usable DMs were available. One DM suffered a peripheral tear during preparation similar to the one in group A (donor age 80 years). By preparing the DM from the opposite side a transplantable lamella (central DM of 8.5 mm in diameter) could be salvaged. Mean preparation time was 6.5 ± 1.4 minutes in group A and 6.1 ± 0.9 minutes in group B, demonstrating that there was no significant difference observed in this manner ($P = 0.399$). The difficulty score was 7.9 ± 1.4 in group A and 8.0 ± 2.0 in group B ($P = 0.726$). The subjective difficulty was the same in both groups resulting in a nonaffecting difficulty grade. The mean total thickness of the DM (without endothelial cell layer) was 13.58 ± 2.81 μm in group A and 12.69 ± 2.06 μm in group B ($P = 0.474$). The mean total thickness of the endothelial cell layer was 3.99 ± 0.62 in group A and 3.98 ± 0.52 in group B ($P = 0.989$). As expected, a decrease of the DM thickness was observed in the culture medium with added dextran. However, no significant difference between the two groups was observed, and the endothelial cell layer remained nearly constant in both groups (Fig. 2).

**Light and Electron Microscopy**

Light microscopy did not reveal any evidence of adherent remnants of corneal stroma on specimen in either group (Fig. 3). Transmission electron microscopy showed no stromal remnants in any specimen, demonstrating a stromal free separation. Many cytoplasmic vacuoles were present in the endothelial cells of culture medium with added dextran, some of which contained dense materials that were not observed before deswelling. (Without dextran deswelling process: left image; with dextran deswelling process: right image). Magnification: ×2000.

**Discussion**

DMEK has dramatically evolved into an alternative to penetrating keratoplasty in cases of a compromised endothelial cell layer without irreversible stromal damage. For optimized conditions of corneal transplantation, a graft with a maximum number of vital endothelial cells and a thickness similar to the recipient’s cornea is required. Organ culture offers several advantages over cold storage such as allowing for valid assessment of the number of vital endothelial cells and higher sterile conditions.

To balance the onset of modifications triggered by the culture medium, facilitate suturing, and accelerate postoperative visual recovery, the cornea must be first thinned before surgery through immersion in the same organ culture medium supplemented with a water-soluble macromolecule that produces colloid osmotic pressure to extract excess water accumulated in the stroma. The deswelling process should be reduced to the shortest time possible in order to obtain a graft thickness similar to that of the recipient and to ensure the highest possible number of endothelial cells. An average of an 8.4% endothelial cell loss was reported after 1 to 4 days of deswelling in an organ culture medium supplemented with 5% dextran T500. Thuret et al. reported a 15% cell loss after 2 days of deswelling in commercial media containing 5% dextran T500. A result consistent with that of Rieger et al. in similar conditions using a home-made medium containing 5% dextran T500. In contrast to the daily endothelial cell loss of 0.9% in dextran-free media, the cell loss in dextran-containing media is much higher than the daily average.

In summary, a short deswelling time is associated with a decreased corneal graft survival rate in organ culture. On the other hand, the deswelling process causes endothelial cell loss, which is more pronounced with longer deswelling times. In contrast to the procedure for penetrating keratoplasty, storage in a dextran-containing medium is theoretically not obligatory for preparing the DM for DMEK surgery. For DMEK surgery, the DM must have a smooth area and a viable cell layer. A high and functional cell layer adheres rapidly to the underlying stroma and reduces the possibility of dislocation.

The present study analyzes for the first time, to our knowledge, the preparation of swollen and deswollen organ-cultured corneas by means of light and electron microscopic analysis also showed, besides healthy endothelial cells, signs of apoptosis and necrosis with condensed nuclei, ruptured plasma membranes, and swollen organelles. Many cytoplasmic vacuoles were present in the endothelial cells, some of which contained dense materials that were not
techniques, the difficulties in DM preparation, and the issue of DM tearing.

Our results suggest a nonsignificant decrease of the DM in dextran-supplemented storage for 24 hours, with little decrease in the smoothness of the DM area. The difficulties in the preparation and the complications were similar in dissection with and without dextran. Ultrastructural findings confirmed complete removal of the DM alone, with the endothelial cell layer not having any adherent stromal remnants, using both dissection techniques. In this regard media with and without were likewise equivalent. On the whole, transmission electron microscopy analysis showed healthy endothelial cells surrounded by signs of apoptosis and necrosis, some of which (e.g., granules containing dense materials) were undetectable before deswelling.

To conclude, DM-endothelium grafts for transplantation in DMEK procedures can be surgically prepared from organ-cultured swollen and deswollen corneal rims. Both separation methods seem to be equivalent in regard to the quality of the obtained DM. Clinical studies are necessary in order to come to definite conclusions.

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References