Automated Donor Tissue Preparation for Descemet Membrane Automated Endothelial Keratoplasty (DMAEK): An Experimental Study

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■ BACKGROUND AND OBJECTIVE: Graft harvesting for Descemet membrane endothelial keratoplasty (DMEK) occurs manually and demonstrates surgical difficulties. In the current study, a mechanical automated technique for preparing a donor Descemet membrane (DM) carrying autologous endothelium for DMEK was evaluated.

■ MATERIALS AND METHODS: Ten rabbit corneas were placed on an artificial anterior chamber and mechanical separation of the DM was conducted using an Epi-keratome (Senturium; Norwood Abbey EyeCare, Victoria, Australia). All DM specimens were properly fixated and optical microscopy was performed.

■ RESULTS: DM separation was achieved in a controlled fashion in 7 of 10 eyes. As demonstrated by optical microscopy of the specimens, no corneal stroma was attached on the DM, whereas the endothelial layer was preserved in several areas.

■ CONCLUSION: Automated separation of the DM can be achieved using an Epi-keratome. Additional studies and improvements of the current technique are needed to draw final conclusions.


INTRODUCTION

Penetrating keratoplasty was the standard treatment option for rehabilitation of corneal blindness and visual impairment in the second half of the 20th century. Today, approximately 30% to 50% of all corneal transplants occur for the treatment of posterior corneal disorders and endotheliopathies and the development of new sutureless techniques that overcome many of the disadvantages of penetrating keratoplasty have been popularized. Descemet stripping automated endothelial keratoplasty (DSAEK) is such a treatment option for corneal endothelial disease. Although DSAEK has been demonstrated to have satisfactory results, the technique has several disad-
vantages such as interface abnormalities that may decrease best spectacle-corrected visual acuity; induced hyperopia, graft detachment, graft failure, graft rejection, and pupillary block. Transplantation of only the Descemet and endothelial layers without the presence of corneal stroma on the graft lenticule may overcome these limitations.

Descemet membrane endothelial keratoplasty (DMEK) was described in 1998 and the feasibility of an isolated Descemet membrane transplantation was demonstrated in 2007. Descemet membrane stripping from the donor cornea occurs manually and although several different techniques have been proposed, they are time consuming and fraught with several difficulties such as Descemet’s tearing.

In our study, we evaluated an automated mechanical technique for preparing a donor Descemet membrane carrying autologous endothelium for DMEK in rabbit corneas.

**MATERIALS AND METHODS**

**Animal Population**

Ten eyes of five male pigmented rabbits weighing 2.5 to 3.5 kg were used in this study. All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research, and the design of the study was approved by the Animal Care Committee.

**Surgical Technique**

The animals were killed by intravenous injection of an overdose of pentobarbital sodium (80 mg/kg body weight) and the eyes were enucleated. After enucleation, a corneal-scleral donor tissue button of 13 to 15 mm in diameter was prepared. Donor tissues with the endothelial cell layer facing up were placed on a modified Barron artificial anterior chamber (Katena Products, Inc., Denville, NJ) (Fig. 1); intrachamber pressure was controlled by injecting balanced salt solution and the endothelium was stained using trypan blue. Mechanical separation of the Descemet membrane was conducted using the Centurion SES epikeratome (Norwood Abbey EyeCare, Victoria, Australia). Only one pass of the epikeratome was performed for each button.

**Histology**

The specimens were prefixed in cold glutaraldehyde 2.5% in 0.1 M cacodylate buffer (pH 7.4). Immediately after separation, the tissue samples were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 1 hour at 4°C, dehydrated in a series of alcohols and in propylene oxide, and then imbedded in epoxy resin. For light microscopic examination, 1- to 3-µm sections were prepared and stained with a modified trichrome stain. Optical microscopy of the specimens followed.

**RESULTS**

Of the ten corneal-scleral tissue donor buttons used in this study, separation of the Descemet membrane in a controlled fashion was achieved in seven eyes. In the other three buttons, either no separation was achieved (2 buttons) or separation was incomplete (epikeratome stopped, 1 button). Optical microscopy of the seven successful separations demonstrated no corneal stroma attached on the Descemet membrane graft (Fig. 2), whereas the endothelial layer was preserved in several areas (Fig 2B).

**DISCUSSION**

The evolution and development of new surgical techniques for posterior corneal transplantation commenced in the late 1990s, with posterior lamellar keratoplasty being the first. Posterior lamellar keratoplasty was later modified and deep lamellar endothelial keratoplasty was introduced in the United States;
both posterior lamellar keratoplasty and deep lamellar endothelial keratoplasty require the creation of a stromal pocket. This surgical step was avoided after Melles introduced a technique for stripping Descemet membrane from the recipient cornea (removal of diseased endothelium), commonly known as Descemet membrane automated endothelial keratoplasty (DSAEK).

DSAEK is now the procedure of choice for many surgeons for the treatment of posterior corneal disorders demonstrating satisfactory outcomes. The main drawbacks of DSAEK are graft detachment (dislocation), graft failure, graft rejection, pupillary block, and interface (between the stroma of the donor and the recipient cornea) irregularities, which may decrease best spectacle-corrected visual acuity. DMEK may overcome these limitations, especially concerning the incidence of interface irregularities due to the absence of corneal stroma on the donor graft.

DMEK is a new and promising technique that is minimally invasive when compared with penetrating keratoplasty. Descemet membrane stripping from both the donor and recipient cornea occurs manually and is surgically difficult. Several different techniques have been proposed for harvesting the Descemet membrane and endothelial layer from the donor cornea. They all are time consuming and can be complicated by Descemet tearing. Furthermore, manual harvesting does not offer reproducible tissue quality or graft creation consistency.

In our study, we evaluated a mechanical automated technique for separating the Descemet membrane (DMAEK) using an epikeratome (used for Epi-LASIK) in rabbit corneas. Mechanical separation was achieved in a controlled fashion in seven eyes; however, in three cases either no separation was achieved or it was incomplete. No corneal stroma was attached on the Descemet membrane whereas the endothelial cell layer was preserved in several areas. The Centurion epikeratome was used in this study due to two major advantages it demonstrates: (1) no corneal applanation is necessary prior to separation (avoiding endothelial cell damage) and (2) the separated Descemet membrane sheet overlies the polymethylmethacrylate block of the epikeratome blade. The described advantages contribute to endothelial cell survival because the cells do not undergo minimal mechanical strain and trauma.

Our study is limited by the small number of rabbit corneas used, the intra-chamber pressure, which was not constant and stable during (or between) separations, and the lack of endothelial cell viability evaluation after separation. Furthermore, we used a preselected series of settings (same as those used for epithelial separation) both for blade oscillation and blade forward speed.

Automated mechanical separation of the Descemet membrane can be achieved using an epikeratome. Additional studies using electron microscopy for further evaluation of the viability of the endothelial cell layer are needed. Improvements of the current technique with the use of different epikeratome settings, which could further minimize endothelial cell damage, are also necessary to draw final conclusions regarding this technique.

REFERENCES

3. Ida T, Yoo SH, Kymionis GD, Goldman JM, Perez VL, O’Brien TP. Descemet-stripping automated endothelial keratoplasty: effect of an-


