Viscoelastic Bubble Dissection: A New Method for Tissue Preparation in Descemet Membrane Automated Endothelial Keratoplasty

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Purpose
Selective transplantation of the corneal endothelium and bare Descemet membrane (DM), as in DM endothelial keratoplasty (DMEK), may have advantages over the more widely used Descemet stripping automated endothelial keratoplasty (DSAFEK), which produces a thicker graft that includes an additional layer of overlying stromal tissue. The exclusion of donor stroma may serve to improve visual outcomes (Price, Kruse) and decrease rejection rates (Price). However, this approach presents a unique challenge to eye banks and surgeons, as it is difficult to isolate, manipulate and insert such a delicate layer of tissue without causing endothelial cell damage. Providing peripheral stromal support to the endothelial layer, as has been previously described for DM automated endothelial keratoplasty (DMEK), has the theoretical advantage of safer manipulation of tissue and greater ease of deployment than has been encountered during traditional DMEK techniques, while providing the optical interface desired for better visual outcomes (Price). Methods to create DMEK tissue employ an air bubble dissection of DM from the overlying central stroma, but air can be unpredictable, as it is highly compressible and can expand rapidly leading to bubbles that are too large, or cause extensive stromal oedema, or rupture of DM. Here we examine a new technique, in which we use trypan-stained cyscoelastic viscoelastic to create a controlled central bubble. Our aim is to compare controlled central bubble success rate between air bubble DMEK (DMEK), and viscoelastic bubble DMEK (vDMEK), and endothelial cell loss (ECL) between DMEK, aDMEK and vDMEK.

Methods

Results
Controlled central bubble formation was successful in 88% (14/16) of tissues prepared by vDMEK, compared to 64% (7/11) for DMEK. Tissues prepared by DMEK were 100% (8/8) successful in yielding intact central DM keratocytes. These differences in success rate were not statistically significant (aDMEK vs. vDMEK p = 0.72; DMEK vs aDMEK p = 0.81; DMEK vs vDMEK p = 0.59). OCT imaging of DMEK tissues dissected with viscoelastic and air showed centrally barred DM with residual, micromin, stromal remnants measuring between 30 and 100 microns in thickness. Trypan-stained viscoelastic was easily removed from tissue during preparation. By inspection, areas of negative staining fell into 2 patterns: 1) small zones of acute cell loss and 2) larger zones of possible DM stretching. Tissues prepared by standard DMEK resulted in a mean ECL of 22% (95% Confidence interval (CI): 16-29%). By comparison, grafts prepared by aDMEK yielded a mean ECL of 28% (CI: 24-31%), not a statistically significant difference (p = 0.28). ECL was significantly higher in DMEK grafts (39% (CI: 31-48%), p = 0.03), than in grafts prepared by DMEK and aDMEK (p = 0.04 and p = 0.03, respectively).

Conclusions
• Viscoelastic more predictably dissects central DM than air and can easily be washed away from grafts after preparation.
• Endothelial attenuation by calcein AM staining is significantly higher in vDMEK tissue than DMEK and aDMEK.
• The reticular and geographic pattern of cell loss in the DMEK grafts may reflect OM stretching with breaks in intercellular adhesion vs. absolute cell loss.

References

Disclosures
• None

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Table 1: Reagents.

Table 2: Donor tissue characteristics. All values expressed as mean ± standard deviation. * p = 0.10 p = 0.04. ‡ p = 0.03.

Figure 2: Viscoelastic bubble dissection. a) SD-OCT image of successful controlled central bubble. b) SD-OCT of failed bubble with dissection to limbus. c) Photomicrograph of successful controlled central bubble with white arrow indicating the plane of section depicted in panel B. d) Photomicrograph of failed bubble dissection with white arrow indicating the plane of section depicted in panel B.

Figure 4: % Endothelial cell loss. aDMEK vs vDMEK.

Figure 3: Calcein AM stained graft images with corresponding binary images and NEC.

Figure 1: Corneal stromal rims, not suitable for transplantation with endothelial cell counts ranging from (1850-3320) cells/mm² from adult donors aged 35 to 75 years were obtained. 27 tissues used in DMEK preparation were prepped with a Mora microkeratome to generate a deep lamellar incision. DM was barred in the central 0.5mm zone of 16 DMEK tissues with an injected mixture of 1% sodium hyaluronate, trypan blue and balanced salt solution, and in 11 aDMEK tissues with injection of air. Controlled central bubbles less than 0.5mm in size were considered successful in barrowing central DM. Additional tissues were prepared using conventional DMEK technique with manual DM peeling. Centrally implanted tissue from the three techniques were examined for endothelial damage with calcein AM viability dye. Color images were captured with an inverted light microscope, digitally stitched together (Photoshop Elements, Adobe) and converted to black and white as binary images (FALSE) image II. Endothelial cell loss (ECL) was calculated from the number of black pixels divided by the total number of pixels.