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 (DSAEK), 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 (DMAEK), 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 DMAEK 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 crepitus, or rupture of DM. Here we examine a new technique, in which we use trypan-stained cohesive viscoelastic to create a controlled central bubble. Our aim is to compare controlled central bubble success rate between air bubble DMAEK (aDMAEK), and viscoelastic bubble DMAEK (vDMAEK), and endothelial cell loss (ECL) between DMEK, aDMAEK and vDMAEK.

Methods

DONOR CORNEAL SCLERAL RIM

Reagent	Manufacturer
ProVisc (1% Sodium Hyluronate)	Alcon Laboratories
0.4% Trypan Blue	MP Biomedicals LLC
Calcein, AM	Invitrogen
Balanced Salt Solution	Alcon Laboratories
Dimethyl Sulfoxide	Fisher Scientific Inc

Table 1: Reagents.

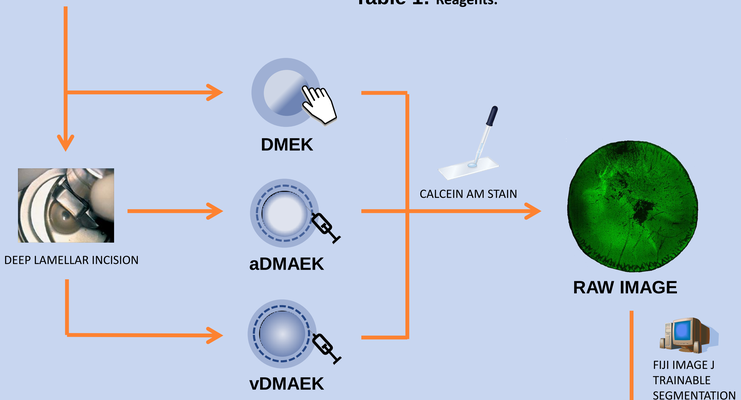

Figure 1: Corneal scleral rims, not suitable for transplantation with endothelial cell counts ranging from (1183-3205 cells/mm^2), from adult donors aged 35 to 75 years were obtained. 27 tissues used in DMAEK preparation were precut with a Moria microkeratome to generate a deep lamellar incision. DM was barred in the central 6.5 mm zone of 16 vDMAEK tissues with an injected mixture of 1% sodium hyaluronate, trypan blue and balanced salt solution, and in 11 aDMAEK tissues with injection of air. Controlled central bubbles less than 6.5 mm in size were considered successful in barring central DM. 8 additional tissues were prepared using conventional DMEK technique with manual DM peeling. Centrally trephined 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 binary images (Fiji Image J). Endothelial cell loss (ECL) was calculated from the number of black pixels divided by the total number of pixels.

	Donor Age (yr)	Death to Preservation Interval (h)	Preservation to Processing Interval (d)	Endothelial Cell Count
DMEK	55.9 ± 11.6	9.89 ± 4.10	18.1 ± 5.7†	2098 ± 502
aDMAEK	62.4 ± 4.8*	9.63 ± 2.50	15.6 ± 6.2‡	2649 ± 377
vDMAEK	52.9 ± 11.0*	8.24 ± 2.29	10.4 ± 4.3†‡	2504 ± 291

Table 2: Donor tissue characteristics. All values expressed as mean averages +/- standard deviation. * p = 0.03. p = 0.04. ‡ p = 0.005.

Results

Controlled central bubble formation was successful in 88% (14/16) of tissues prepared by vDMAEK, compared to 64% (7/11) for aDMAEK. Tissues prepared by DMEK were 100% (8/8) successful in yielding intact central DM lenticule. These differences in success rate were not statistically significant (aDMAEK vs vDMAEK p = 0.71, DMEK vs aDMAEK p = 0.81, DMEK vs vDMEK p = 0.59). OCT imaging of DMAEK tissues dissected with viscoelastic and air showed centrally barred DM with residual, noncompact, 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 discrete 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 aDMAEK yielded a mean ECL of 28% (CI: 24-31%), not a statistically significant difference (p =0.28). ECL was significantly higher in vDMAEK grafts 39% (CI: 31-48%, p = 0.03), than in grafts prepared by DMEK and aDMAEK (p = 0.004 and p = 0.03, respectively).

Figure 2: Viscoelastic bubble dissection . A) sdOCT image of successful controlled central bubble. B) sdOCT of failed bubble with dissection to limbus. C) Photomicrograph of successful controlled central bubble with white arrow indicating the plane of section for the OCT image depicted in panel A. D) Photomicrograph of failed bubble dissection with white arrow indicating the plane of section depicted in panel B.

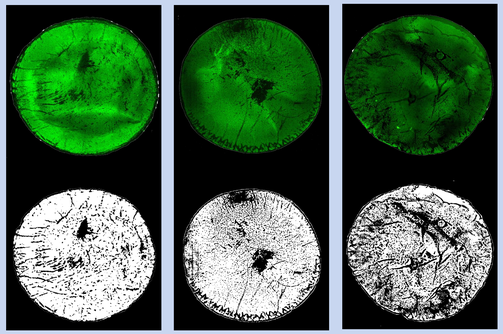

Figure 3: Calcein AM stained graft images with corresponding binary images and %ECL.

	Successful Dissection	Failed Dissection	Success Rate
DMEK	8	0	100%
aDMAEK	7	4	64%
vDMAEK	14	2	88%

Table 3: Dissection success rate.

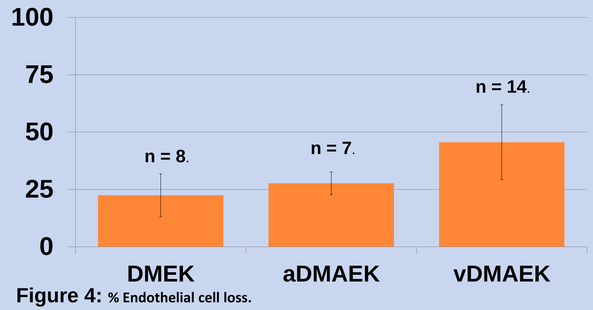

Figure 4: % Endothelial cell loss.

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 vDMAEK tissue than DMEK and aDMAEK.
- The reticular and geographic pattern of cell loss in the DMAEK grafts may reflect DM stretching with breaks in intercellular adhesion vs. absolute cell loss.

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Disclosures

- None

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