Factors associated with thickness of eye bank-prepared DSAEK graft tissue

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Purpose: To identify factors associated with thickness of eye bank-prepared DSAEK grafts.

Methods: Data from all corneal tissues processed for DSAEK at a single eye bank from 2008-2012 were included (n=7,668). Multivariate linear regression modeling was used to identify factors contributing to thickness of DSAEK grafts.

Results: Eye bank-prepared DSAEK grafts have become thinner over time, on average 48 microns thinner in 2012 than in 2008. Decreased pre-processing corneal thickness and higher pre-processing endothelial cell density were associated with thinner lamellar grafts (p<0.0001), but post-processing cell density and graft diameter were not associated with graft thickness (p=0.70 and p=0.88). Older donor age was associated with thinner grafts (p=0.001). There was no significant interaction between donor age and endothelial cell density in relation to final graft thickness (p=0.161). Death-to-procurement time and death-to-processing time were not associated with graft thickness (p=0.94 and p=0.93). DSAEK grafts processed during the “work-day” (8a-5p) were on average 3.8 microns thinner than those processed “after-hours” (5p-8a; p<0.0001). In comparing technician experience, no significant difference was found between number of tissues processed and post-processing thickness.

Conclusions: Eye bank-prepared DSAEK graft thickness has decreased over time, and thinner grafts were associated with thinner corneal tissue, increased endothelial cell density, increased donor age, and daytime tissue preparation. There was no association between graft thickness and death-to-procurement time, death-to-processing time, or technician experience.

Commercial Relationships: Grace M. Wang, None; Maria A. Woodward, None; Michael O’Keefe, Midwire (E); Indu Vadakkepattath, Midwire (E); Michael Titus, Saving Sight (E); Taylor Blachley, None; David C. Musch, None; Roni M. Shtein, None

Program Number: 2697
Presentation Time: 8:30 AM–8:45 AM

Pre-Descemetetic Automated Endothelial Keratoplasty (PDAEK), towards an automated DMEK preparation technique

Mor Dickman1, Jurriaan Brekelmans1, Petra Steiger-Vermaat2, Teja Wesseling2, Friso W.F. van Marion1, Frank Van den Biggelaar2, Tos TJM Berendschot1, Rudy M. Nuijs1. 1University Eye Clinic Maastricht, Maastricht University Medical Center, Maastricht, Netherlands; 2Euro Cornea Bank, Euro Tissue Bank, Beverwijk, Netherlands.

Purpose: In the main limitations to wide-spread adoption of Descemet Membrane Endothelial Keratoplasty (DMEK) is the lack of a standardized automated technique for graft preparation. In the current study we set out to establish a proof of concept for automated harvesting of planar pre-Descemetetic (~50μm) endothelial grafts using a novel vacuum assisted microkeratome system.

Methods: Sixty-nine organ cultured human donor corneas unsuitable for transplantation were dissected using a prototype linear microkeratome system (Gebauer SLC Expert; Gebauer; Germany). Following epithelial removal, donor corneas were aspirated using vacuum (600 mbar) against a porous rigid-reference-member (RRM) designed to correct for central to peripheral thickness differences in the donor cornea (figure 1). Thickness measurements were obtained by Fourier domain Anterior-Segment Optical-Coherence-Tomography (Casia SS-1000; Tomey; Japan). Endothelial cell density was determined by Trypan blue exclusion, followed by

France) (n=6), or 300μm SLC (n=9) single use heads (Gebauer GmbH, Germany). Artificial anterior chamber pressure in both systems was set to 95cm H2O. The CBN passage time was kept at 5 seconds. Preparation was conducted after 21-28 days of culture in 100ml modified minimal essential medium (MEM), followed by 24-48 hours deswelling in the same medium containing 5% Dextran (CM 2). After dissection the anterior lamella (AL) was placed back on the posterior lamella (PL) and then incubated in CM2 for another 6 days. Corneal thickness (CT) was measured by pachymetry (SP-100, Tomey GmbH, Germany) and spectral domain optical coherence tomography (AS-OCT, Heidelberg Engineering, Germany) before preparation (0h) and 1 and 24 hours after dissection with AS-OCT. The endothelial cell density was evaluated at 0, 1, 24 and 144 hours. PL surface roughness was evaluated by Field Emission Scanning Electron Microscopy in cryo-mode (S4800, Hitachi Ltd., Japan) and assessed by 3 masked observers.

Results: Pre-cut donor cornea thickness did not differ significantly measured by AS-OCT or pachymetry with a high correlation of both devices (r²=0.92, p<0.0001). One hour after preparation the AL showed a significantly higher dissection depth in CBm (417.39±14.55μm) than SLC (344.26±54.44μm, p=0.0019), with the variance of SLC being higher (p=0.047). AL and PL thickness increased slightly in the subsequent culture period in CM2. Initial and final central endothelial cell density differed between the 2 groups (CBm pre-cut=2200±505cells; CBm post-cut=1913±165cells / SLC pre-cut=2744±202cells; SLC post-cut=2344±459cells). Evaluation of the surface of the PL by cryo-electron microscopy showed comparable surface structures for both systems.

Conclusions: The Gebauer SLC system agrees more with the designated cutting depth than the Moria CBm. However, the variance may be higher in the automatic SLc system. Endothelial cell loss and posterior lamella surface structure were comparable.

Commercial Relationships: Matthias Fuest, None; Ansgar Flammersfeld, None; Sabine Salla, None; Peter Walter, None; Martin Hermel, None

Program Number: 2698
Presentation Time: 9:00 AM–9:15 AM

Comparison of Gebauer SLC and Moria CBm ALK

Microkeratomes for DSAEK lamella preparation and storage

Matthias Fuest, Ansgar Flammersfeld, Sabine Salla, Peter Walter, Martin Hermel. Ophthalmology, RWTH Aachen University, Aachen, Germany.

Purpose: In this study we compared preparation accuracy, endothelial cell loss and lamellar surface structures of the hand-guided ALK Microkeratome (CBm) by Moria and the Gebauer SLC (SLc), a fully automatic preparation system, for lamellar donor preparation in Descemet’s Stripping Automated Endothelial Keratoplasty (DASKA).

Methods: 15 human donor corneas unsuitable for transplantation were dissected with 300μm CBm multiuse heads (Moria S.A., France) (n=6), or 300μm SLC (n=9) single use heads (Gebauer GmbH, Germany). Artificial anterior chamber pressure in both systems was set to 95cm H2O. The CBN passage time was kept at 5 seconds. Preparation was conducted after 21-28 days of culture in 100ml modified minimal essential medium (MEM), followed by 24-48 hours deswelling in the same medium containing 5% Dextran (CM 2). After dissection the anterior lamella (AL) was placed back on the posterior lamella (PL) and then incubated in CM2 for another 6 days. Corneal thickness (CT) was measured by pachymetry (SP-100, Tomey GmbH, Germany) and spectral domain optical coherence tomography (AS-OCT, Heidelberg Engineering, Germany) before preparation (0h) and 1 and 24 hours after dissection with AS-OCT. The endothelial cell density was evaluated at 0, 1, 24 and 144 hours. PL surface roughness was evaluated by Field Emission Scanning Electron Microscopy in cryo-mode (S4800, Hitachi Ltd., Japan) and assessed by 3 masked observers.

Results: Pre-cut donor cornea thickness did not differ significantly measured by AS-OCT or pachymetry with a high correlation of both devices (r²=0.92, p<0.0001). One hour after preparation the AL showed a significantly higher dissection depth in CBm (417.39±14.55μm) than SLC (344.26±54.44μm, p=0.0019), with the variance of SLC being higher (p=0.047). AL and PL thickness increased slightly in the subsequent culture period in CM2. Initial and final central endothelial cell density differed between the 2 groups (CBm pre-cut=2200±505cells; CBm post-cut=1913±165cells / SLC pre-cut=2744±202cells; SLC post-cut=2344±459cells). Evaluation of the surface of the PL by cryo-electron microscopy showed comparable surface structures for both systems.

Conclusions: The Gebauer SLC system agrees more with the designated cutting depth than the Moria CBm. However, the variance may be higher in the automatic SLc system. Endothelial cell loss and posterior lamella surface structure were comparable.

Commercial Relationships: Matthias Fuest, None; Ansgar Flammersfeld, None; Sabine Salla, None; Peter Walter, None; Martin Hermel, None

Program Number: 2699
Presentation Time: 9:00 AM–9:15 AM

Pre-Descemetetic Automated Endothelial Keratoplasty (PDAEK), towards an automated DMEK preparation technique

Mor Dickman1, Jurriaan Brekelmans1, Petra Steiger-Vermaat2, Teja Wesseling2, Friso W.F. van Marion1, Frank Van den Biggelaar2, Tos TJM Berendschot1, Rudy M. Nuijs1. 1University Eye Clinic Maastricht, Maastricht University Medical Center, Maastricht, Netherlands; 2Euro Cornea Bank, Euro Tissue Bank, Beverwijk, Netherlands.

Purpose: One of the main limitations to wide-spread adoption of Descemet Membrane Endothelial Keratoplasty (DMEK) is the lack of a standardized automated technique for graft preparation. In the current study we set out to establish a proof of concept for automated harvesting of planar pre-Descemetetic (~50μm) endothelial grafts using a novel vacuum assisted microkeratome system.

Methods: Sixty-nine organ cultured human donor corneas unsuitable for transplantation were dissected using a prototype linear microkeratome system (Gebauer SLC Expert; Gebauer; Germany). Following epithelial removal, donor corneas were aspirated using vacuum (600 mbar) against a porous rigid-reference-member (RRM) designed to correct for central to peripheral thickness differences in the donor cornea (figure 1). Thickness measurements were obtained by Fourier domain Anterior-Segment Optical-Coherence-Tomography (Casia SS-1000; Tomey; Japan). Endothelial cell density was determined by Trypan blue exclusion, followed by

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triple Hoechst-Ethidium-Calcein (HEC) labeling for pan-corneal endothelial viability assessment.

**Results:** Mean post-cut endothelial thickness measured 40 (±7) μm, significantly thinner than 50μm (p<0.05). RRM application resulted in significantly planar graft thickness profiles compared with the meniscus shape observed without RRM application (p<0.05) (figure 2). Mean post-cut endothelial cell density measured 2245 (±175) cells/mm² with an average 70% (±10%) pan-corneal endothelial viability.

**Conclusions:** This study established a proof of concept for reproducible automated vacuum assisted harvesting of planar pre-Descemetic endothelial grafts with satisfactory endothelial cell density and viability, addressing many of the limitations of current mechanical microkeratomes and introducing a novel technique for endothelial graft preparation (PDAEK).

**Figure 1. Gebauer SLc Expert Illustration.**
Vacuum assisted corneal applanation against a porous profiled rigid-reference-member (RRM), designd to allow dissection of 30-950 μm thick planar endothelial grafts.

**Figure 2. RRM Assisted Corneal Profiling.**
Anterior-Segment Optical-Coherence-Tomography (CASIA SS-1000; Tomey; Japan) images of 2 donor corneas prior to (upper row) and following (lower row) dissection of ~50μm grafts with (left) and without (right) profiled rigid-reference-member (RRM) correction for central to peripheral thickness differences.

**Commercial Relationships:** Mor Dickman, None; Jurriaan Brekelmans, None; Petra Steijger-Vermaat, None; Teja Wesseling, None; Friso W.F. van Marion, None; Frank Van den Biggelaar, None; Tos TJM Berendschot, None; Rudy M. Nuijts, Gebauer Medizintechnik GmbH (C)

**Program Number: 2700**
**Presentation Time:** 9:15 AM–9:30 AM

**Preparation of large grafts for Endothelial Keratoplasty**
Vito Romano, Adrian Tey, Natalie Hill, Jern Chen, Claire Britten, Sajjad Ahmad, Mark Batterbury, Colin E. Willoughby, Stephen Kaye. Royal Liverpool University Hospital, University of Liverpool, Liverpool, United Kingdom.

**Purpose:** To describe a technique to achieve a large thin posterior lamellar graft for endothelial keratoplasty in order to increase the amount of peripheral endothelium that is transplanted

**Methods:** After thinning of the donor cornea, the anterior 350um of corneal stroma was removed with a microkeratome. A manual lamellar dissection was then performed to remove the remaining anterior peripheral circumferential margin of donor tissue. A 9.5mm graft was then trephined from the endothelial surface, inserted into the anterior chamber and tamponaded against the recipient’s bared posterior cornea with air. Cord lengths and sag heights of the transplanted tissue were measured using anterior segment OCT.

**Results:** Twenty-four patients underwent this modified technique. All patients had well-attached endothelial grafts with the graft edge achieving good clearance from the peripheral iris and anterior chamber angles. Cord lengths of the graft inside the eye were reduced due to the increased radius of curvature of the posterior corneal surface. Central and peripheral graft thickness were 100-150um and 180-200um, respectively.

**Conclusions:** The modified technique reduces the thickness of the peripheral cornea outside of the region removed by the automated trephine enabling larger trephine sizes to be used. A larger graft is expected to provide approximately 10-20% more transplanted endothelial cells.

**Commercial Relationships:** Vito Romano, None; Adrian Tey, None; Natalie Hill, None; Jern Chen, None; Claire Britten, None; Sajjad Ahmad, None; Mark Batterbury, None; Colin E. Willoughby, None; Stephen Kaye, None

**Program Number: 2701**
**Presentation Time:** 9:30 AM–9:45 AM

**Comparison of corneal endothelial change post DSAEK using paired grafts from the same donor to two recipients**

**Purpose:** To investigate the prognosis of corneal endothelium and factors affecting endothelial cell loss post Descemet’s Stripping Automated Endothelial Keratoplasty (DSAEK) using paired grafts from the same donor to two recipients.

**Methods:** Retrospective review of 20 pairs of eyes treated by DSAEK using donor corneal grafts from the same donor. All paired donor grafts were pre-cut and used for DSAEK on the same day by the same surgeon. The pull-through technique using a Busin glide was applied for all cases. The mean patient age was 72.0±9.0 years, and the mean follow-up period was 35 months (range: 12-51 months). The primary diseases were argon laser iridotomy-induced bullous keratopathy (BK) in 17 eyes (42%), Fuchs’ corneal dystrophy in 7 eyes (18%), glaucoma surgery-related BK in 3 eyes (8%), pseudophakic BK in 3 eyes (8%), aphakic BK in 3 eyes (8%), and other disorders in 7 eyes (16%). Patients with surgical complications or graft rejection were excluded from the study. Noncontact specular microscopy (SP-3000; TOMEY Corp., Nagoya, Japan) was used to evaluate corneal endothelium at the central region.

**Results:** The mean donor endothelial cell density (ECD) was 3022±134 cells/mm² (range: 2504-3386 cells/mm²) and the mean difference of ECD in the paired corneas was 4.2% (range: 0-12%).

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After 1 year postoperative, 12 pairs (Group A; 24 eyes, 60%) showed similar endothelial cell loss and 8 pairs (Group B; 16 eyes, 40%) showed a more than 10% difference of endothelial cell loss. The mean difference of CED in the paired cases in Group A and Group B are 2.9% and 22.1%, respectively. In Group A, 4 pairs (33%) showed matching primary diseases. However, in Group B, 7 paired (88%) showed a mismatch of the primary diseases. Six preexisting glaucoma cases in 5 pairs showed accelerated endothelial cell loss. 

Conclusions: Donor endothelial cell viability is essential for the long-term survival of corneal endothelium post DSAEK. However, the primary diseases affect the prognosis of endothelial cell survival and preexisting glaucoma accelerates post-DSAEK endothelial cell loss.

Commercial Relationships: Tsutomu Inatomi, None; Hiroko Nakagawa, None; Kanae Miyamoto, None; Chie Sotozono, None; Shigeru Kinoshita, None

Program Number: 2702
Presentation Time: 9:45 AM–10:00 AM
Relationship between Hyperopic Shift after DSAEK and Corneal Anterior Curvature, Posterior Curvature, and Thickness


Purpose: A hyperopic shift is known to occur after corneal endothelial keratoplasty (EK). Possible sources for the postoperative refractive shift include anterior curvature flattening, corneal deswelling, and/or posterior curvature steepening. The purpose of this study was to determine the relationship between hyperopic shift after Descemét’s stripping automated EK (DSAEK) and corneal curvatures and thickness.

Methods: Subjects undergoing DSAEK alone (17 eyes) or with concurrent cataract surgery (DSAEK triple procedure) (13 eyes) at the Duke Eye Center were enrolled under IRB approval. Pentacam (Oculus, Wetzlar, Germany) imaging and manifest refraction (MRx) were performed preoperatively and at least 6 months postop. Pre to post DSAEK changes in corneal posterior curvature (delta Kp), anterior curvature (delta Ka), and central thickness (delta CCT) were calculated and each was t-tested for significance. Pre to post DSAEK changes in spherical equivalent MRx were calculated (delta MRx). For eyes undergoing DSAEK triple, target MRx was used for preop MRx. Univariable and multiple-regression analyses of delta MRx as a function of delta Kp, delta Ka, and/or delta CCT were performed.

Results: Mean delta MRx from DSAEK was +0.670 ± 0.158 D (hyperopic shift), p=0.003. Mean delta Kp was -0.665 ± 0.132 D (hyperopic shift), p=0.0003. Mean delta Ka was -0.107 ± 0.077 D, p=0.184.

A univariable linear regression model of delta MRx as function of delta Kp had correlation coefficient R= -0.54 and a significantly nonzero slope, p=0.002. A regression of delta MRx as a function of delta Ka had R= -0.40 with p=0.03. A regression of delta MRx as a function of delta CCT had R= -0.03 with p=0.87.

A multiple-regression analysis of delta MRx as function of delta Kp, delta Ka, and delta CCT had R=0.64. For this model, delta Kp had a statistically significant nonzero slope, p=0.005. The slopes for delta Ka (p=0.086) and delta CCT (p=0.13) were not significantly different from zero.

Conclusions: DSAEK causes a significant change in corneal posterior curvature that is associated with the postoperative hyperopic shift. There was no significant change in corneal anterior curvature after DSAEK. Deturgescence of the cornea from DSAEK also had no significant relationship to the hyperopic shift. Posterior curvature changes should be prioritized in developing clinical nomograms to account for post DSAEK refractive shifts.

Commercial Relationships: William B. Wainright, None; Ryan McNabb, Bioptigen (P); Alan Carlson, None; Terry Kim, Ocular Systems Inc (C); Natalie A. Afshari, None; Anthony N. Kuo, Bioptigen (P)

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Program Number: 2703
Presentation Time: 10:00 AM–10:15 AM
Quality of Life Before and After Keratoplasty for Fuchs Endothelial Dystrophy

Eleanor Trousdale, David Hodge, Keith H. Baratz, Leo J. Maguire, William M. Bourne, Sanjay V. Patel.

Purpose: To assess health-related quality of life (HR-QOL) in Fuchs endothelial corneal dystrophy (FECD), and changes in HR-QOL after three types of keratoplasty (penetrating keratoplasty, PK; deep lamellar endothelial keratoplasty, DLEK; and Descemet stripping endothelial keratoplasty, DSEK) for FECD.

Methods: Patients with corneal edema due to FECD were enrolled in two consecutive prospective studies at Mayo Clinic, Rochester, MN. In a randomized controlled trial, 12 eyes (12 patients) received PK and 11 eyes (11 patients) received DLEK. In a prospective observational study, 40 eyes (40 patients) received DSEK. All subjects were examined before keratoplasty and at 6 months, 1 year, and 3 years after keratoplasty. At each examination, HR-QOL was assessed by using the 25-item National Eye Institute Visual Functioning Questionnaire; best-spectacle-corrected and uncorrected visual acuities were measured by using the electronic Early Treatment of Diabetic Retinopathy Study protocol; and disability glare was measured with a straylight meter (C-Quant, Oculus, Inc.).

Results: The mean HR-QOL composite score for all eyes with FECD before keratoplasty was 72 ± 11 (n=63). HR-QOL improved by 6 months with all treatments when compared to preoperative (PK, p=0.008; DLEK, p=0.03; DSEK, p<0.001). At 6 months, HR-QOL was higher after DSEK than after PK (p=0.006), whereas at 3 years, there were no significant differences in HR-QOL between PK, DLEK, and DSEK (p=0.33; mean minimum detectable difference, 8 [α=0.05, β=0.20]). After keratoplasty, HR-QOL was correlated with uncorrected visual acuity at 1 year (r= -0.38, p=0.001) and at 3 years (r= -0.36, p=0.02), with disability glare at 3 years (r= -0.41, p=0.02), and with best-corrected visual acuity at 6 months (r= -0.34, p=0.03), but not thereafter.

Conclusions: HR-QOL is impaired in patients with FECD (compared to subjects with healthy eyes who typically have composite scores >90), but improves after keratoplasty, irrespective of the technique. The improvement in HR-QOL is faster after DSEK compared to after PK, and might be explained in part by rapid improvement in uncorrected visual acuity after DSEK.

Commercial Relationships: Eleanor Trousdale, None; David Hodge, None; Keith H. Baratz, assessing the likelihood of developing Fuchs corneal dystrophy (P); Leo J. Maguire, None; William M. Bourne, None; Sanjay V. Patel, None

Support: Research To Prevent Blindness, New York, NY; Mayo Foundation, Rochester, MN

Clinical Trial: NCT00346138