Impact of Donor Age on Endothelium-Descemet Membrane Layer Harvesting and Roll Formation

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ABSTRACT

Purpose: Descemet membrane endothelial keratoplasty (DMEK) is an alternative to Descemet's stripping automated endothelial keratoplasty (DSAEK) to surgically replace diseased corneal endothelium. Although graft rejection incidence has been reported to be drastically lower in DMEK, wide adoption may be limited by two factors. Harvesting the endothelium-Descemet membrane layer (EDM) can be difficult, and tight EDM scrolling can hinder unfolding once inserted into the patient's eye. Anecdotally, surgeons have noticed the use of younger donors has exacerbated these factors. We sought to correlate donor age with EDM stripping difficulty and scroll tightness.

Methods: EDM scrolls were harvested by a cornea-fellowship trained ophthalmologist masked to donor age from 26 corneoscleral buttons. An 11.5 mm partial trephination was used instead of blunt dissection for a consistent and even outer cut. 7.0 to 8.25 mm EDM scrolls were prepared using the SCUBA technique in Optisol GS. VisionBlue® (.06 % trypan blue) staining was used to harvest and assess EDMs. The surgeon subjectively rated stripping difficulty on a 1 to 5 scale (easiest to unable to strip) based upon DM adherence to underlying stroma and radial tear formation. Three different methods were used to characterize scrolling severity: scroll width, normalized scroll surface area (scroll width × scroll length/surface area of EDM), and tendency for EDM scroll formation (referred to as scroll rating). A scroll rating of 1 corresponded to opposite ends of the EDM not touching, 2 when EDM ends touch, 3 when the EDM forms one complete scroll and 4 when more than one scroll formed.

Results: Mean donor age was 59 ± 14 years (15–69). Mean diameter of EDM scroll was 7.9 ± .23 mm (7.0 – 8.25). Stripping difficulty was shown to be inversely correlated with donor age ($p < .05$). The three methods to determine scrolling severity had different results. Inverse relations between donor age was significant for scroll rating ($p < .05$), nearly significant for normalized surface area ($p = .0508$), and not significant for scroll width ($p < .10$).

Conclusions: Our data supports previous observations that harvesting of EDM scrolls may be easier in older donor corneas. There may be a decreased scrolling tendency based on an inverse correlation between age and scroll rating ($p < 0.05$) and normalized surface area ($p = 0.0508$). Use of older donor corneas should reduce surgical difficulty of DMEK procedures.

INTRODUCTION

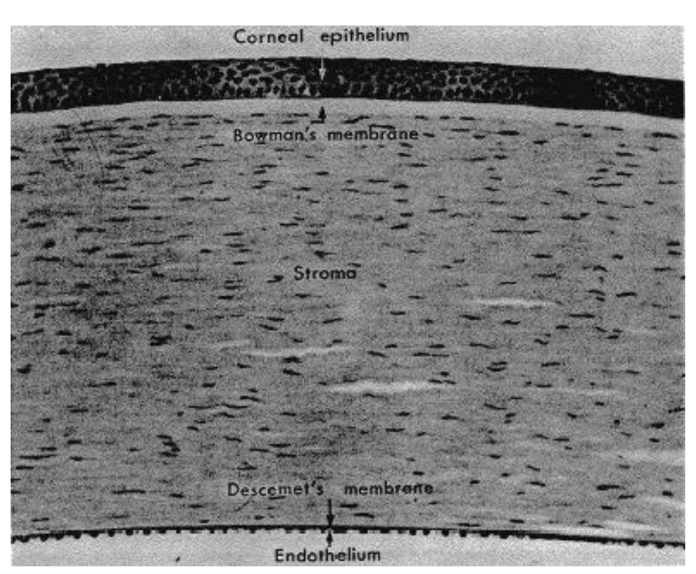

Figure 1: Cross section of human cornea (160 X; SEM)[1]

Corneal transplantation has undergone multiple changes in the last fifteen years. Penetrating keratoplasty (PKP), a technique that replaces the full thickness of a patient's cornea with that of a donor, was used for many decades but required long recovery times, unpredictable surface topography changes, suture related complications, and immunologic rejection.[2]

The cornea has five layers: the epithelium, Bowman's membrane, stroma, Descemet's membrane, and the endothelium (see Figure 1). The endothelium is responsible for keeping the cornea in a relatively dehydrated state called deturgescence that maintains corneal clarity. The corneal endothelium creates an osmolarity gradient using ion pumps, causing water to also flow out of the cornea and into the aqueous membrane. In addition, endothelial cells are tightly adhered to one another, preventing water influx from the aqueous membrane. Descemet's membrane is the basement membrane of the endothelium and thickens with age. When the structure of the endothelium is compromised, such as in Fuch's Dystrophy, the cornea loses its transparency and transplantation is needed to restore proper function.

Introduction

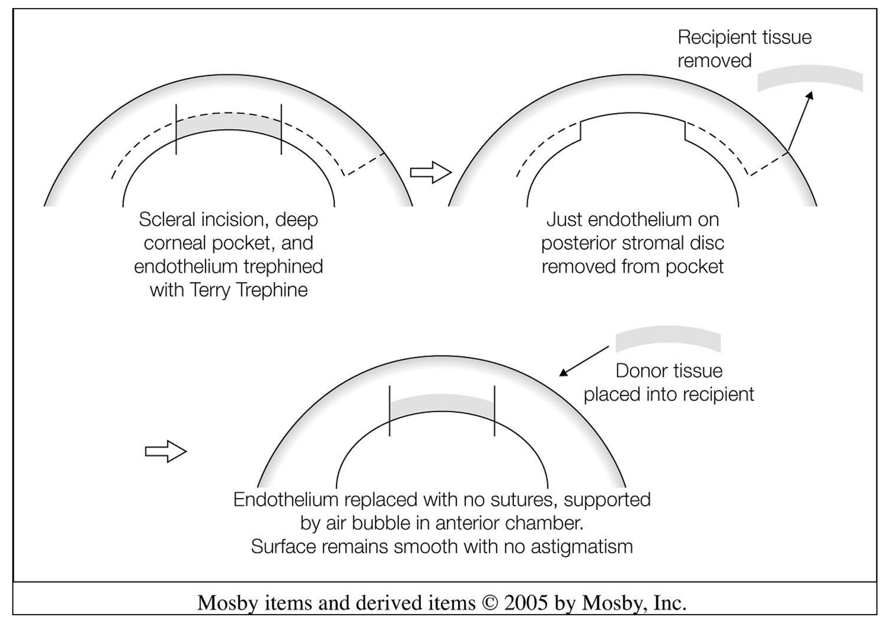

Figure 2: Schematic of PLK/DLEK.[3]

In 1998, Melles et al. introduced posterior lamellar keratoplasty (PLK).[4] This technique introduced the concept of only replacing the diseased posterior layer(s) of the cornea with viable endothelium. This is done in three main steps: donor tissue preparation, excising of recipient tissue, and attachment of donor tissue (see Figure 2). In this case, the surgery involved dissections within the corneal stroma of the donor and the recipient corneas. The donor tissue is folded, inserted into the anterior chamber of the patient, and positioned using air and balanced saline solution. In 2000, Terry introduced this technique in the United States as deep lamellar endothelial keratoplasty (DLEK).[5] They confirmed this method removed the uncertain refractive outcomes of PKP.

In 2004, Price and Price introduced Descemet's stripping with endothelial keratoplasy (DSEK).[6] To perform DSEK, surgeons strip the Descemet's membrane with diseased endothelium off the patient and place the donor tissue directly on the posterior side of the patient's stroma. Gorovoy popularized this procedure by using a microkeratome to prepare the donor tissue.[2] Gorovoy's method, called Descemet's stripping automated endothelial keratoplasty or DSAEK, dramatically cut down surgeon preparation time.[7]

Descemet's membrane endothelial keratoplasty (DMEK) is the most recent iteration of EK.[8] In, DMEK only the endothelium/Descemet's (EDM) layer is harvested from the donor cornea (DSEK also includes a thin portion of stromal layer). Thus, in DMEK, there is no donor stroma/recipient stroma interface. Overall, DMEK offers potential advantages compared to DSEK. DMEK may offer better visual recovery, less induced hyperopia, and a lower immunologic rejection rate, while having a comparable endothelial cell loss.[9-12] Of course, there is a tradeoff when working with a thinner layer of tissue: it more difficult to manipulate than the donor tissue used in DSEK/DSAEK.

The acceptance of DMEK is currently hindered by the difficulty obtaining the donor EDM and challenges of its insertion into the patient's anterior chamber and proper positioning with correct orientation onto the posterior stromal surface.[13,14] Some have advocated that the EDM preparation be handled by eye banks to improve surgical efficiency.[7] Anecdotally, surgeons have reported that the stripping of Descemet's membrane from young donor's cornea is more difficult with a greater tendency for the EDM layer to spontaneously form a scroll compared to one from an older donor. One potential explanation for this finding is that the anterior banded layer of the Descemet's membrane increases with age.[15] The aim of this study was to correlate donor age with EDM stripping difficulty and scroll tightness.

MATERIALS AND METHODS

26 short-term cultured corneoscleral buttons were obtained from the Transplant Services Center at University of Texas Southwestern Medical Center and stored in Optisol-GS (Bausch & Lomb, Irvine, CA) at 4 C. A cornea-fellowship trained ophthalmologist who was masked to the donor age, harvested EDM scrolls within 14 days after each donor's death with the assistance of a medical student.

The corneoscleral buttons were positioned on a suction block. After centration, an 11.5 mm trephine made a partial cut through the endothelium. We used an 11.5 mm trephine blade instead of the traditional method of using a blunt object to score the DM because the trephination gives a smoother edge, allowing an easier separation of the DM and the underlying stroma. VisionBlue® (.06 % trypan blue; Dutch Ophthalmic, USA) staining allowed visualization of the mark. After 60 seconds passed, Weck-Cel® Spears (Beaver-Visitec, Waltham, MA) were used to absorb the blue dye.

MATERIALS AND METHODS

Blunt end forceps were used to separate the cut edge EDM's periphery across the entire circumference of the trephination. If the corneal diameter was small (< 12 mm), eccentric trephination was used to avoid having the entire trephine cut in the periphery.

The corneoscleral button was transferred to a viewing chamber filled with Optisol-GS for dissection to follow the SCUBA technique.[9] EDM was removed from the underlying stroma using 2 forceps following a technique described by Kruse.[16] The forceps pulled the EDM edge toward the center of the graft. If a radial tear occurred, the dissection was attempted at another location. About 50 % of the EDM was separated from the stroma before being placed flat again.

The corneoscleral button was transferred back to the suction block, and an 8 mm trephine (range 7.0 – 8.25 mm) cut the central portion of the sample. 60 second staining is repeated with VisionBlue®.

The corneoscleral button was transferred back to an Optisol-GS filled viewing chamber. One pair of 0.12 forceps is used to stabilize the donor cornea, while two pairs of non-toothed forceps are used to peel back and remove the rest of the EDM in circumlinear fashion.

The surgeon subjectively rated stripping difficulty on a 1 to 5 scale (from easiest to unable to strip) based upon DM adherence to underlying stroma and radial tear formation. Three different methods were used to characterize scrolling severity: scroll width, normalized scroll surface area (scroll width × scroll length/surface area of EDM), and tendency for EDM scroll formation (referred to as scroll rating). A scroll rating of 1 corresponded to opposite ends of the EDM not touching, 2 when EDM ends touch, 3 when the EDM forms one complete scroll and 4 when more than one scroll formed (see Figure 4).

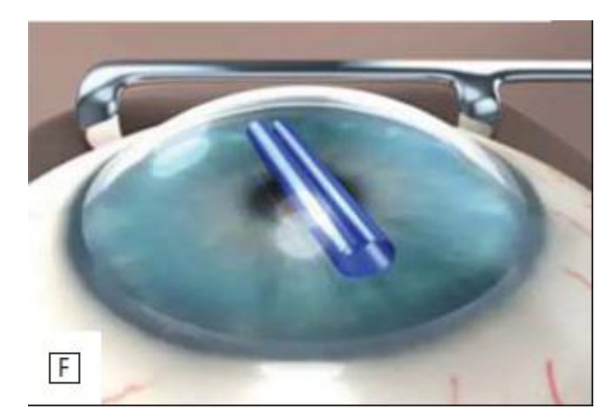

Figure 3: A model of an EDM stained with VisionBlue® and placed in Optisol-GS for measurement.[11]

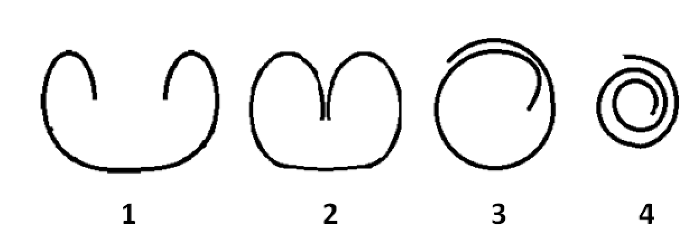

Figure 4: A schematic of EDM cross-section to assess the scroll rating on the 1 to 4 scale. Note: the endothelium is the outer layer of the scroll.

RESULTS

Table 1: Demographics for Study

Parameter	Mean ± Std. Dev. or Percentage
Age	59 ± 14 years old
Time between death and dissection	9.4 ± 3.1 days
Male	62%
Female	38%

RESULTS

Table 2: Statistical Results for Correlation between Age and Parameters of Interest

Parameter	N	R	P-value
Stripping Difficulty (1-5)	26	-.438	.0254
Scroll Width (mm)	18	.407	.0936
Scroll Length (mm)	18	-.0127	.960
Scroll Area/Surface Area	19	.454	.0508
Scroll Rating (1-5)	18	-.484	.0420

Statistical analysis was performed including calculations of Pearson's coefficient (R) to assess the relationship between age and stripping difficulty, scroll width, scroll length, scroll area/surface area, and scroll rating. Mean donor age was 59 ± 14 years (15–69 range). Mean diameter of EDM scroll was 7.9 ± .23 mm (7.0–8.25 range). Stripping difficulty ($R = -.438$; $p = .0254$) and scroll rating ($R = -.484$; $p = .0420$) were found to have a significant inverse correlation with age (Table 2). The scroll area/surface area ratio had a nearly significant correlation ($R = .454$; $p = .0508$). Scroll width ($R = .407$; $p = .0936$) and scroll length ($R = -.0127$; $p = .960$) did not have a significant correlation with age.

CONCLUSION

Our data supports that the use of older corneas should reduce the surgical difficulty of DMEK procedures. The study showed a significant decrease in the subjective category of stripping difficulty with the use of older donors. In addition, there may be a decreased scrolling tendency based on an inverse correlation between age and scroll rating ($p < 0.05$) and a positive correlation between age and normalized scroll surface area ($p = 0.0508$).

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Funding provided by:

Research to Prevent Blindness

National Eye Institute EY020799

UT Southwestern Undergraduate Medical Research Fellows Program (UT-SUMR)