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Impact of Donor Age on Corneal Endothelium-Descemet Membrane Layer Scroll Formation

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Abstract

Objectives—To correlate corneal endothelium-Descemet membrane layer (EDM) parameters of scroll tightness with donor age, endothelial cell density, and history of diabetes.

Methods—EDM scrolls were harvested from 26 corneoscleral buttons using the SCUBA technique by a cornea-fellowship trained ophthalmologist masked to donor age. Two independent outcome parameters were used to characterize the scrolling severity of successfully harvested tissue: scroll width and tendency for EDM scroll formation (referred to as scroll rating on a 1 to 4 scale: incomplete scroll formation to tightly-scrolled).

Results—Mean donor age was 59 ± 17 years (15–69). Mean endothelial cell density of EDM scroll was 2451 ± 626 cells/mm² mm (range: 1307 – 3195). Using stepwise linear regression, a significant correlation was found between scroll width and donor age ($R = 0.497$, $P < 0.05$). Additionally, a significant inverse correlation was found between scroll width and endothelial cell density ($R = -0.605$, $P < 0.05$). There was no statistically significant correlation between a donor history of diabetes and the parameters of scrolling tendency.

Conclusions—Our data suggests that using older donors reduces EDM scroll tightness.

Keywords

endothelium; descemet membrane; keratoplasty

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Introduction

For patients with endothelial dysfunction, endothelial keratoplasty (EK) has replaced penetrating keratoplasty (PKP) as the preferred method of corneal transplantation. The main limitations of PKP are long recovery times, unpredictable surface topography changes, suture related complications, compromise of the structural integrity of the globe, and immunologic rejection.^{1,2}

Recent interest in EK began in 1998 when Melles et al. introduced posterior lamellar keratoplasty (PLK).³ In 2000, Terry modified this technique and introduced it in the United States as deep lamellar endothelial keratoplasty (DLEK).⁴ In 2004, Price and Price introduced Descemet's stripping with endothelial keratoplasty (DSEK).⁵ Gorovoy popularized this procedure with Descemet's stripping automated endothelial keratoplasty (DSAEK) by using a microkeratome to prepare the donor tissue.² Studies have shown patients of DSAEK have faster visual recovery, fewer graft failures, and less severe post-op astigmatism compared to PKP.⁶

Descemet's membrane endothelial keratoplasty (DMEK) is the most recent iteration of EK.⁷ In DMEK, only the endothelium-Descemet membrane (EDM) layer is harvested from the donor cornea. Thus, in DMEK, there is no donor stroma/recipient stroma interface. Overall, DMEK offers potential advantages compared to DSAEK. DMEK may offer a more rapid visual recovery, better visual acuity, less induced hyperopia, and a lower immunologic rejection rate, while having a comparable endothelial cell loss.⁸⁻¹² DMEK also has the added benefit of eliminating the need for an automated microkeratome.

There is a tradeoff when working with a thinner layer of tissue: it is more difficult to surgically manipulate than the donor tissue used in DSAEK. The acceptance of DMEK is currently hindered by the difficulty obtaining the donor EDM, inserting it into the patient's anterior chamber, and achieving proper positioning with correct orientation onto the posterior stromal surface.^{13, 14} Anecdotally, surgeons have reported that the stripping of Descemet's membrane from young donor's cornea is more difficult and has a greater tendency for the EDM layer to spontaneously form a tighter scroll compared to one from an older donor.¹⁵ The aim of this study was to correlate donor age, endothelial corneal density, and history of diabetes with EDM 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 (SM) with no previous DMEK experience was masked to the donor age and harvested EDM scrolls within 14 days of donor death.

The corneoscleral buttons were positioned on a trephination block. After centration, an 11.5 mm trephine made a partial cut through the endothelium. 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. 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 placing the entire trephine cut in the periphery. The corneoscleral button was transferred to a viewing chamber filled with Optisol-GS for dissection using the SCUBA technique.⁹ EDM was removed from the underlying stroma using 2 blunt (non-toothed) tying 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 to the block, and an 8 mm trephine (range 7.75 – 8.25 mm) was used to punch through the central Descemet's membrane. Trephine size use was subject to ready availability of the trephines with 79% of trephinations performed with an 8.0 mm trephine. The endothelial surface of the button was re-stained with VisionBlue[®] for 60 seconds. The corneoscleral button was submerged in an Optisol-GS filled viewing chamber and blunt tying forceps were used to completely free the EDM from the underlying stroma in a circumlinear fashion. The EDM scrolls were left submerged in the Optisol-GS filled viewing chamber with an underlying 1×1 mm grid to measure the scroll dimensions (Figure 1).

Three different methods were used to characterize scrolling severity of successfully harvested EDM tissue: scroll width and the 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 touched, 3 when the EDM formed one complete scroll, and 4 when more than one complete scroll formed (Figure 2).

Donor diabetic history was ascertained by the eye bank by review of available medical records and interviews with the next of kin and/or knowledgeable historians. Between these two sources, the type of diabetes (NIDDM vs IDDM) was usually known. Hemoglobin A1c levels were not obtained.

Statistical analysis was performed including calculations of Pearson's coefficient (R) to assess the relationship between age and other parameters with scroll width and scroll rating. P values were calculated using a two-tailed probability and adjusted for multiple comparisons using stepwise linear regressions.

Results

Demographic information of the donor corneas is presented in Table 1. Mean donor age was 59 ± 17 years (15–69 range). Cause of death of donors included: cardiac illness, myocardial infarction, trauma, pulmonary embolus, chronic obstructive pulmonary disease, respiratory illness, subarachnoid hemorrhage, and cerebrovascular accident. The surgeon was able to successfully harvest the EDM scroll from 19 out of 26 donor corneas. Mean diameter of EDM scroll was $7.97 \pm .11$ mm (7.75–8.25 range).

Using stepwise linear regression (with scroll width and scroll rating as independent variables), a significant correlation was found between scroll width and donor age ($R = 0.497$, $P < 0.05$) (Table 2). Scroll rating added no additional predictive value. There was a

significant inverse correlation between scroll width and scroll rating ($R = -0.712$, $P < 0.005$). Using stepwise linear regression (with scroll width and scroll rating as independent variables), a significant inverse correlation was found between scroll width and endothelial cell density ($R = -0.605$, $P < 0.05$). Again, scroll rating did not add additional predictive value. There was no statistically significant correlation between a donor history of diabetes mellitus and the parameters of scroll rating or scroll width (Table 2).

Discussion

This masked study of the endothelium-Descemet membrane (EDM) layer's biomechanical characteristics demonstrated significant differences based upon donor age. We found an inverse correlation ($R < 0$) between age and scroll rating and a positive correlation ($R > 0$) between age and scroll width. Each of those findings shows that using older donors reduces EDM scroll tightness. Since younger donors typically have higher endothelial cell density, it was not surprising that donors with higher ECD had a more robust scrolling tendency. Finally, we found no correlation of scrolling tendency to a donor history of diabetes.

One potential explanation for changes in biomechanical characteristics of the EDM is the growth of the DM's posterior non-banded layer. Endothelial cells produce secretions that form this relatively homogenous layer after birth.¹⁷ The posterior non-banded layer width has a significant direct correlation with age, increasing from 2 to 10 μm on average between the ages of 10 and 80 years old.¹⁷ The increase in thickness could provide increased support for the EDM layer, reducing scroll tightness. Endothelial cell density may also impact on scrolling tendencies of the EDM and requires further study. A recently reported thin a cellular pre-Descemet's layer, could also potentially impact the harvesting and characteristics of the EDM, but its independent existence and ultra-structure require further validation and study.¹⁸ Perhaps, residual attached stromal fibrils to the EDM may also impact its biomechanical traits.

The EDM was harvested using the previously published SCUBA technique with the refinements described by Kruse et al.¹⁶ However, we found that use of an 11.5 mm trephine blade instead of the traditional method of using a blunt object or needle to score the DM gave a smoother edge, allowing an easier separation of the DM and the underlying stroma.

The scope of our study was limited by the harvesting surgeon's previous inexperience with DMEK tissue harvesting. Further studies with an experienced DMEK surgeon or eye bank technician performing the procedure are required to study the impact of donor age and diabetes on the stripping difficulty and rate of successfully harvesting the EDM scroll.

Recently, Heinzlmann et al. also showed that using older donors for DMEK results in wider EDM scrolls.¹⁹ They also demonstrated that older donors had a decreased intra-operative unfolding time for the scrolls. However, they found that donors with a lower ECD had a tendency to form a tighter scroll, which is the opposite of our findings. One possible explanation for the discrepancy between our findings is the difference in the endothelial cell counts studied. Our range was 1307 – 3195 cells/ mm^2 with an average of 2451 and standard

deviation of 626. Their study had a range of 2117 – 2920 cells/mm² with an average of 2397 and standard deviation of 223. Thus, we might have observed an overall trend that reflects donor age while they found a different trend in that narrower range.

Our study demonstrated that use of older donors resulted in less tight EDM scrolls. This provides support for observations, such as those made by Price and Price, that use of older donor corneas may facilitate DMEK.¹⁵

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Figure 1. Photographs of VisionBlue[®] stained endothelium-Descemet membrane (EDM) layer in Optisol solution. The left panel (a) shows an EDM scroll in solution. The right panel (b) shows a scroll placed in a viewing chamber with a 1×1 mm grid to measure the scroll.

Figure 2.

Upper panel (a) is a schematic of endothelium-Descemet membrane (EDM) layer cross-section to assess the scroll rating on the 1 to 4 scale. The rating of 4 was given to scrolls that had multiple turns (left) or when the EDM formed two tight scrolls (right). Red dots indicate the outer layer of the scroll, which is the endothelium. The lower panel (b) includes representative images of stained EDM (arrow) representative of each scroll rating. The ages of the donors were 75, 55, 21, and 21 from lowest to highest scroll rating.

Table 1

Demographics of Donor Corneas

Parameter	N	Mean ± Std. Dev. or Percentage
Age	19	59 ± 17 years old
Death to Preservation Time	18	11.9 ± 4.7 hours
Death to Dissection Time	17	9.0 ± 3.1 days
Endothelial Cell Density	11	2451 ± 626 cells/mm ²
Male	12	63%
Female	7	37%
History of Diabetes	5	28%

Table 2

Statistical Results for Correlation between the Parameters of Interest and Age, Endothelial Cell Density (ECD), and Diabetic Status.

Scrolling Parameter	Age			Endothelial Cell Density			Diabetic		Non-Diabetic		Diabetic Status	
	N	R	P-value	N	R	P-value	N	Median	N	Median	P-value	
Scroll Width (mm)	18	.497	<0.05	11	-.605	<0.05	5	2.0	12	2.0	.45	
Scroll Rating (1–4)	18	-.484	<0.05	10	.251	.49	4	3.5	13	3.0	.34	