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Influence of Pre-Descemet'S Layer in Precut Endothelial Grafts Digital Measurements and Scrolling Characteristics

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Abstract

Purpose: This study aims to evaluate the impact of the pre-Descemet layer on central graft width (CGW) and scrolling scores (SS) in endothelial keratoplasty. **Methods**: This experimental study included 47 corneoscleral buttons unsuitable for transplantation dissected by intrastromal air injection into pre-Descemet's endothelial keratoplasty (PDEK) or Descemet's membrane endothelial keratoplasty (DMEK) grafts. The central graft width (CGW) was calculated using FIJI-ImageJ software, SS characteristics was determined. The Student's "t-test" analyzed paired samples. Pearson's correlation coefficients were calculated for CGW and age, and statistical significance was set at $P < 0.05$. Using confocal microscopy a representative PDEK graft had endothelial cell pool analyzed with vital dies. **Results**: The average age was 53.8±14.5 years and 18 were male (66.6%). Twenty-four PDEK grafts (51%) and 17 DMEK grafts (36.2%) were dissected. Six dissections (12.8%) failed, three (50%) were diabetic donors ($P<0.001$). Mean CGW of 2.35 mm for PDEK grafts, had higher values than DMEK with mean of 1.95 mm ($P<0.05$). The R^2 correlation of age and CGW was 0.2834 and 0.004 for DMEK and PDEK, respectively. The PDEK grafts were SS-2 (50%) and the DMEK grafts were SS-3 (70.5%). TB had no significant effect on CGW or SS. **Conclusion**: PDEK had a significantly higher CGW than DMEK ($P<0.05$), and there was an inverse relationship between CGW and SS. Donor age and CGW had a weak correlation with DMEK, and no correlation with PDEK grafts.

Keywords: endothelial keratoplasty; eye bank; trypan blue; fuchs endothelial dystrophy

1. Introduction

Precut corneas processed by the eye bank enable the distribution of dissected grafts, which decreases the surgical complexity of posterior lamellar corneal transplantation [1,2]. A submerged endothelial graft spontaneously assumes a roll shape with the endothelial face outwards [3], Descemet's membrane (DM) presents high elastin content in the anterior fetal portion that explains the tissue tendency to scroll [4]. Progressively with age, the posterior portion of the DM increases in thickness due to constant endothelial cells secretions; Thus, surgeons prefer donor age over 50 years old [5] for endothelial keratoplasty with Descemet's membrane (DMEK) due to the lower tissue scrolling tendency.

In 2013, Dua et al. redefined the cornea anatomy by describing the pre-Descemet layer (PDL), which is on average 10 μm thick with a regular distribution of elastin [6], thus, pre-Descemet endothelial keratoplasty (PDEK) [7] grafts are thicker than DMEK with less scrolling tendency, which results in looser grafts. Furthermore, the air impermeability of PDL allows the dissection of endothelial grafts in donors of all ages[8]. This increase endothelial grafts donor pool and obtain tissue with high endothelial cell density (ECD).

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The eye bank team and surgeons usually stain endothelial grafts with trypan blue (TB) to improve visualization and prevent primary failure due to incorrect tissue orientation within the anterior chamber. The dye applied for a few minutes on the tissue had low cellular toxicity and remained detectable even after storage time [9], the literature described endothelial cell loss of 16 to 18% of preloaded and pre-stained tissues shipped internationally for three days[10].

Two variables that characterize the endothelial graft roll were previously described: the central graft width (CGW) [11] represents the measurement of the parchment diameter, and the Scrolling Score (SS) [12] which classifies the morphology of the rolled graft into four stages. To assemble the endothelial tissue into injector cartridge, the standard technique uses fluid for aspiration and subsequent injection; however, there are few discussions about cartridge tip size and corneal incision size for both endothelial keratoplasty modalities. One study demonstrated an increase in tissue stiffness after TB staining using an atomic force microscope [13]; however, there are no studies on the effect of the dye on graft measurements and morphology.

There is a general consensus among ophthalmologists regarding differences in graft size between pre-Descemet endothelial keratoplasty (PDEK) and Descemet membrane endothelial keratoplasty (DMEK); however, these differences have not yet been formally quantified. This study aims to evaluate the grafts morphological characteristics, and in addition, a representative PDEK graft underwent cellular viability analysis using confocal microscopy with vital dyes.

2. Materials and Methods

Study design: The Institutional Ethics Committee of Londrina University Hospital, Brazil approved the study protocol; the informed consent was obtained during organ donation. A single experienced surgeon (Oguido APM) prepared the donor tissues between September 2018 and August 2020. The eye bank provided forty-nine corneoscleral buttons intended for disposal, the inclusion criterion was endothelial cell density (ECD) above 2000 cells/mm^2 by specular microscopy (Konam, Nishinomiya, Japan). Patients excluded had history of glaucoma, intraocular surgery, or uveitis. According to the type of big bubble (BB) formed during air injection, two groups made up the study: PDEK group, type-1 and type-3 BB, and DMEK group, type-2 BB. Dissection failure as well as risk factors were quantified and analyzed. Each graft dissection video had four frames selected and using the FIJI-ImageJ software obtained digital measurements. To set the scale, a three millimeters (mm) caliper appeared in the plane of the cornea, and the average of three measurements was calculated: CGW, posterior white-to-white distance (PWTWD), BB diameter (BBD), graft length (GL), graft area (GA), and graft margin circularity (GMC). SS described previously was performed [10]: SS-1, graft edges do not touch; SS-2, graft edges touch; SS-3, single graft roll formed; and SS-4, more than one-roll or a thin and tight roll formed.

The data obtained regarding the donor's age, sex, donor diabetes mellitus, death-to-preservation time (DPT), total preservation time (TPT), procedure time (PT), and air injected volume (AIV) were documented, statistical analysis was performed using IBM-SPSS Statistics Base, version 20.0.

Graft Preparation: The donor corneas placed over a trepanation block with the endothelial side up and 0.1% TB (Ophthalmos, São Paulo, Brazil) for 30 s. The technique utilized a five-cm^3 syringe filled with air coupled to a 30-gauge needle and injected into Schwalbe's line with the bevel facing upward until it reached the central and posterior corneal stroma. The BB formed with intrastromal air injection had dissection completed with a 15-degree scalpel and scissors for PDEK grafts and an 8.25 mm trephine (Katena, New Jersey, USA) for DMEK grafts. The anterior surface of the cornea received a three-millimeter punch and subsequently the "S" was stamped. The roll of endothelial graft submerged in BSS had digital analysis performed before and after TB staining for three minutes. On a filter paper, the graft had the roll opened with a blunt spatula.

Statistical analysis: Continuous variables were subjected to descriptive statistics and counting data were analyzed using total and percentage frequencies. The Shapiro-Wilk test checked the normality of the residues. The Student's t-test was applied to paired samples of CGW after TB staining. Under PDEK and DMEK conditions, dispersion values and Pearson's linear correlation (R)

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were applied to verify the relationship between donor age and CGW. Unpaired samples were analyzed using the Mann-Whitney U test, and the chi-square association test was used to analyze failed dissections and risk factors. Statistical significance was set at a p value of 0.05.

Confocal microscope analysis: A representative PDEK graft produced using NGENITY® 3D Visualization System (Alcon Laboratories, Inc., Fort Worth, TX, USA) were analyzed with Leica TCS SP8 confocal microscope (Wetzlar, Germany) attached to LAS software (Leica Microsystems). Triple staining procedure were applied using 100 µl solution containing: 60 µl BSS, 30 µl Hoechst 33342 (H) diluted in the proportion of 1:5000, 10 µl Etidium-homodimer (EH-1) and 10 µl of Calcein AM (CAM) [19]. At room temperature, tissue was incubated for 45 minutes followed by washing with BSS drip. By manipulating only the filter paper, graft was positioned with endothelial face on a viscoelastic coating applied into the Petri dish, and tissue was flattened by low pressure from a cover slip. Central graft area was analyzed with 20x magnification objective lens, and a global graft image was produced with 5x magnification objective lens and merge of nine juxtaposed images. Fluorescence emitted by three laser channels was analyzed: H in blue - viable nuclei; EH-1 in red - damaged nuclear membranes; CAM in green – viable cytoplasm cell.

3. Results

Forty-seven corneas from 27 donors were included, 21 were bilateral and seven were unilateral, previous eye surgery excluded two corneas from one donor. Demografics data were demonstrated (**Table 1**).

Table 1. Demographics Data of Cornea Donor Tissue of Eye Bank Records.

Parameters	Value
No of donors	27
Age (y)	53.8±14.5
Range	18-70
Sex	
Male	18 (66.6%)
Female	9 (33.4%)
Diabetes mellitus	4 (14.8%)
No of cornea tissue	47
Endothelial cell density (cells/mm^2)	2.376±244
Death to preservation time (hs)	14.48±8.83
Total preservation time (days)	36.56 ± 25.64
Diabetes mellitus	6 (12.7%)

PDEK group had 24 corneas (51%) with type-1 BB (79.1%) and type-3 BB (20.8%) formation, AIV was 3.5±3.0 cm^3 (range, 0.5-12 cm^3), and PT was 12.5±5.7 minutes. Four PDEK or DMEK graft dissection images were analyzed and variables measurements were calculated. (**Figure 1**). DMEK group had 17 corneas (36.2%) with type-2 BB formation, AIV was 4.6±4.1 cm^3 (range, 0.3-15 cm^3), and PT was 4.7±4.1 minutes. Six corneas (12.7%) from six donors had BB burst, AIV was 4.5±4 cm^3 (range, 0.5-12.2 cm^3), all contralateral corneas were successfully dissected.

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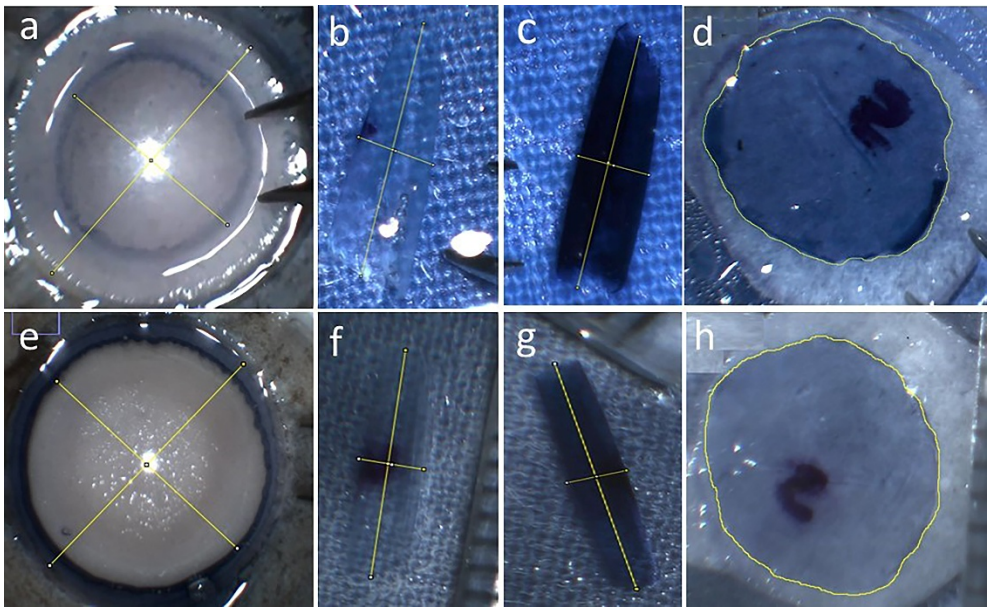

Figure 1. Four selected frames of graft dissection video for Pre-Descemet endothelial keratoplasty (upper boxes) and Descemet membrane endothelial keratoplasty (lower boxes). Digital measurements were obtained using FIJI-ImageJ software: white-to-white distance (PWTWD) (right diagonal; a,e); big bubble diameter (BBD) (left diagonal; a,e); graft length (GL) (vertical line; b,c,f,g); central graft width (CGW) (horizontal line; b,f); CGW after trypan blue (TB-CGW) (horizontal line; c,g); graft area (GA) (d,h); and graft margin circularity (GMC) (d,h).

CGW and SS did not change significantly after TB staining of PDEK and DMEK grafts. The SS frequencies and mean CGW were as follows: PDEK grafts had SS-2 in 50%, SS-3 in 30% with a CGW of 2.34±0.39 mm, DMEK grafts had SS-3 in 70.5%, and SS-4 in 17.6% with a CGW of 1.95±0.41 mm (**Table 2**).

Table 2. Scroll Score (SS) of Pre-Descemet's endothelial keratoplasty and Descemet's membrane endothelial keratoplasty grafts with central graft width (CGW) before and after trypan blue staining (TB-CGW).

Scroll Score	n	CGW	TB-CGW	Age (years)	P
PDEK	24	2.35±0.36	2.39±0.33	50.8±16.2	>0.05
SS-1	4	2.74±0.30	2.81±0.30	41.2±26.9	0.721
SS-2	12	2.41±0.26	2.41±0.27	52.0±13.9	0.946
SS-3	6	2.10±0.38	2.19±0.26	60.0±11.2	0.612
SS-4	2	1.99±0.50	2.11±0.21	35.0+0.0	0.612
DMEK	17	1.95±0.41	1.92 ± 0.40	55.3±12.2	>0.05
SS-2	2	2.47±0.14	2.46 ± 0.06	57.0±0.0	0.986
SS-3	12	1.98±0.34	1.97 ± 0.25	60.9±4.7	0.961
SS-4	3	1.50±0.34	1.34 ± 0.42	32.0±8.6	0.544

PDEK had larger CGW measurements than DMEK grafts (P<0.001), and both had an inverse relationship with SS. The DMEK group showed a positive linear correlation between CGW and age (R=0.53), whereas in the PDEK group, age did not correlate with CGW (R=0.02) **(Figure 2**). PDEK

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grafts that obtained type-3 BB had a higher CGW than those that obtained type-1 BB, without statistical significance (P>0.05).

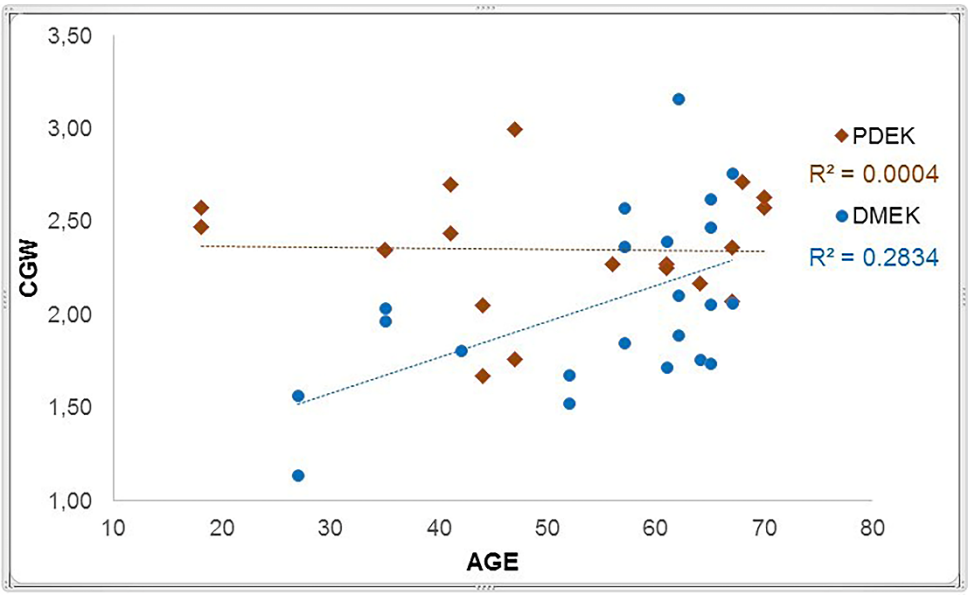

Figure 2. Dispersion values, determination coefficient, and Pearson linear correlation of central graft width (CGW) and age for Pre-Descemet's endothelial keratoplasty (PDEK) and Descemet's membrane endothelial keratoplasty (DMEK) grafts.

There were significant differences in the BBD, GL, and GA between the PDEK and DMEK groups; only PWTWD showed similar results (**Table 3**). The incidence of diabetes in the failure group was three out of six individuals (50%), while dissection success was three of 41 individuals (7.3%). The chi-square association test for failed dissection and diabetes mellitus was statistically significant (P<0.001).

Table 3. Pre-Descemet's endothelial keratoplasty (PDEK) and Descemet's membrane endothelial keratoplasty (DMEK) grafts mean values, standard deviation (SD) and P value of posterior white to white distance (PWTWD), big bubble diameter (BBD), graft length (GL), graft area (GA), and graft margin circularity (GMC).

	PDEK group	**Range**	**DMEK group**	**Range**	*P*
	n=24		n=17		
PWTWD	11.66 ±0.38	(11.07-12.41)	11.75±0.44	(11.07-12.56)	0.496
BBD	7.40 ± 0.44	(6.48-8.37)	10.40±0.46	(9.11-10.81)	**<0.001**
GL	7.91 ± 0.41	(7.10-8.69)	8.71±0.51	(7.71-9.72)	**<0.001**
GA	50.76 ± 7.12	(33.20-66.19)	60.99±9.2	(47.86-78.18)	**<0.001**
GMC	0.81 ± 0.06	(0.69-0.91)	0.89±0.02	(0.82-0.92)	**<0.001**

Corneal endothelial cells exhibited active nuclei and cytoplasm when visualized via confocal microscopy using vital dyes. The density of viable cells, stained with H, was 2407 cells/mm^2, while the density of non-viable cells, stained with EH-1, was 37 cells/mm^2 (**Figure 3**). Furthermore, the green fluorescence of the cytoplasm in live cells (CAM) showed high colocalization with H (**Figure 4**). Global viability assessment of a representative PDEK graft was performed using a digital montage of nine juxtaposed images. Green fluorescence identifies the cytoplasm of viable cells, while dark areas indicate cellular dropout (**Figure 5**).

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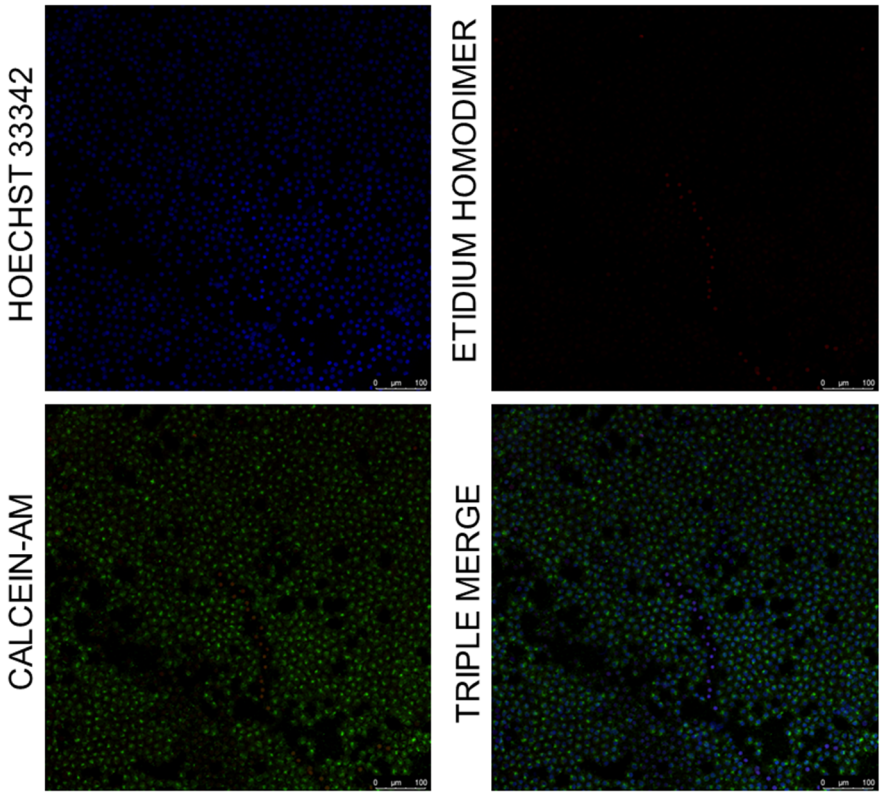

Figure 3. Pre-Descemet endothelial keratoplasty graft with 20x objective lens of central area and confocal microscope fluorescence analysis with triple staining. Viable endothelial cell nuclei stained with Hoechst 33342; unviable endothelial cell stained with Ethidium-homodimer; green fluorescence of live cell cytoplasm stained with Calcein AM; three laser channels overlapping images.

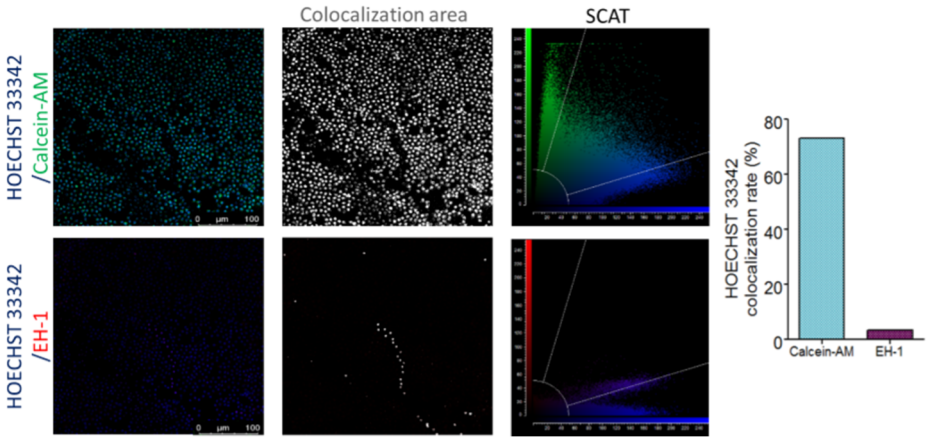

Figure 4. Pre-Descemet endothelial keratoplasty graft and confocal microscope fluorescence analysis. FIJI-ImageJ processed images with percentage bar graph of Hoechst 33342 stained cells and colocalization rate with Calcein-AM or ethidium-homodimer.

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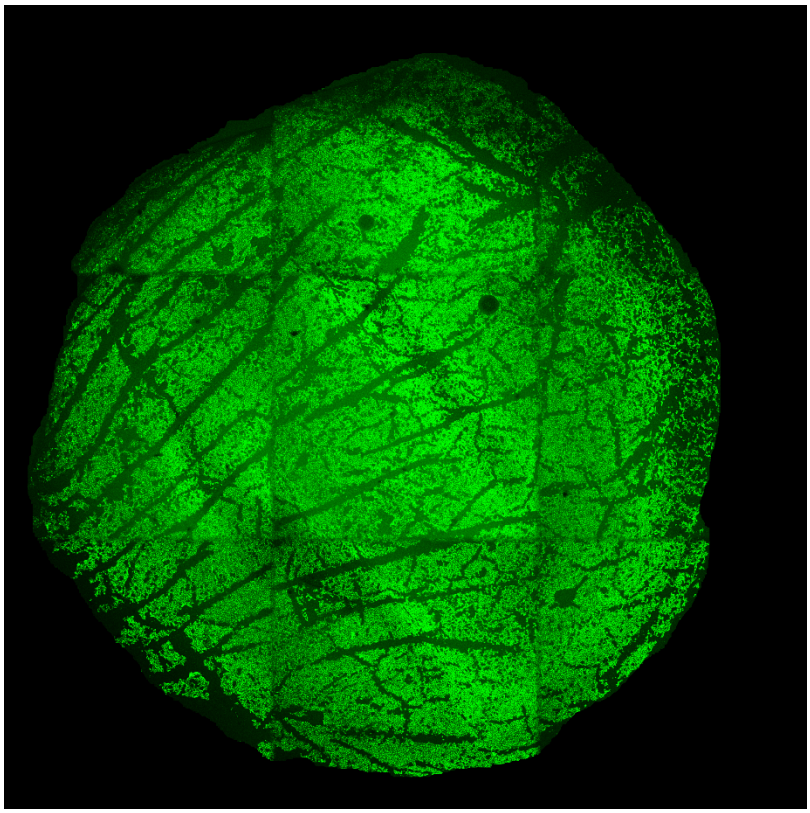

Figure 5. Global graft viability assessment of a representative pre-Descemet endothelial keratoplasty graft digitally mounted with nine juxtaposed images. Green fluorescence represents viable cells cytoplasm and dark areas are indicative of cellular dropout.

4. Discussion

Endothelial grafts represent the ideal lamellar thickness for posterior replacement surgery, and eye-bank tissue processing plays an important role in this scenario [14,15]. This article highlights CGW and SS as two variables that describe tissue roll characteristics, and for the first time, it was performed a standardized approach using FIJI-ImageJ software to characterize PDEK and DMEK grafts. These two variables describe the resultant forces acting on the tissue roll and improve the eye bank information to the transplant team, thus predicting the surgery complexity and PT. Tight grafts increase intraocular manipulation, it was described high endothelial damage in long-term surgeries [16] CGW less than 1.5 mm or SS-4 describe these kind of tissue. Loose grafts with CGW greater than 2.5 mm or SS-1 could present easy intraocular opening; however, the preloaded graft size could increase the cartridge wall contacts. This study obtained ECD only before the procedure, which represents a limitation of the study that did not analyze the impact of eye bank manipulation and endothelial cells damage.

PDEK grafts cut by the eye bank represent a promising technique for posterior lamellar transplantation, mainly because it allows the dissection of corneas from young donors with high ECD. This study demonstrated a significant difference of CGW and SS between the PDEK and DMEK techniques. An endothelial graft submerged in BSS naturally rolls with the endothelial cell layer outwards, which occurs with less intensity in PDEK tissues [7], and the present study showed a mean CGW 20% higher in PDEK grafts than in DMEK. PDL increases posterior lamella thickness, which reduces the scrolling force induced by the anterior (fetal) band of DM, with a high concentration of elastin [17]. SS-2 was predominant (50%) in PDEK grafts and that described loose grafts in a double-roll format. The constant secretion of endothelial cells throughout life is responsible for increasing the thickness of the DM posterior band, which reduces the scrolling tendency in DMEK tissue of elderly donors [5].

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A previous study using atomic force microscopy demonstrated increased stiffness of DM fragments after TB [10]. CGW and TB-CGW were no significantly different ($p>0.05$). Therefore, it was not confirmed the hypothesis that staining could alter graft roll characteristics. The explanation for TB increasing tissue stiffness is a phenomenon similar to the crosslinking induced by light energy. However, in this study, the amount of light energy exposure from the surgical microscope and the operating room lamps was not controlled. It was applied the S-stamp to the anterior surface of the posterior lamella to prevent an inverted intraocular opening, which is an important cause of primary failure in endothelial transplantation. The stamp in DMEK grafts applied to the anterior face of the DM, which is anatomically close to the endothelium; a study reported the loss of endothelial cells adjacent to the marking [18]. In the PDEK graft, the PDL anterior surface was marked, and there are no studies describing cell viability. There are no reports in the literature on cell viability in PDEK grafts; the result expected is less endothelial damage because the labeling performed on the PDL anterior surface.

Young donors with a high ECD are desirable for endothelial keratoplasty, although, the greater scrolling tendency of these grafts makes intraocular opening and adherence to the recipient bed difficult. This study obtained PDEK and DMEK grafts from donors aged less than 50 years using air dissection, however, these grafts had CGW of 1.1 mm and SS-4, which describe extremely tight grafts that the eye bank should not distribute. The air impermeability present in the PDL constitutes an important reference for PDEK graft manufacture; small peripheral fenestrations in this layer are responsible for type 2 BB formation during air injection [19]. To prevent air escape in the periphery, a rounded clamp was develop to guarantee type-1 BB formation, and has become an essential tool for eye banks in the PDEK graft preparation. In this study, we expected to produce all types of BB to include comparative groups of DMEK and PDEK.

The presence of the pre-Descemet's layer stabilizes the scrolling tendency; however, the higher CGW values in PDEK grafts draw attention to the size of the injector cartridge tip and the corneal incision. This article raises the discussion about the measurement of the injector cartridge orifice; surgeons often adapt intraocular lenses cartridges with 2.2 mm to 3.0 mm. There is no standard practices in this important moment of endothelial transplant surgery, the exit extremity of cartridge is often cut to increase the diameter to prevent endothelial damage since the submerged rolled graft overcome this obstacle twice, first when aspirated into the device, and second when injected into the anterior chamber. Eye bank process could easily include the CGW and SS measurements for transplant team with a single photo, this article suggest the adoption of this practice mainly with PDEK grafts, which allows planning the cartridge dimensions. In our sample, the average CGW was 2.4 mm, however, SS-1 grafts resulted in a CGW of 2.8 mm in younger donors.

The PDL tight peripheral adhesion to the corneal stroma limits the size of the type-1 BB; in this study, the cut performed with scissors aim to obtain the greatest size for PDEK graft. Considering the value of 1 as a perfect circle for the GMC variable, the trephined DMEK grafts had a value of 0.89, which was significantly higher than that of the PDEK grafts ($P<0.001$). However, the PDEK grafts had a GMC of 0.81, which represents acceptable roundness values, and this should not influence the clinical results, since GA and ECD represent the variables that could predict the number of transplanted cells and, consequently, the recovery of corneal transparency after transplantation. The first study that introduced CGW for DMEK grafts using FIJI-ImageJ defined the trephination size as equal to GL [9]; this article refuse this statement because the posterior cornea always has a concave shape, and the GL is always higher than trephine measurements. This study demonstrated that an 8.25 mm trephination for DMEK grafts produced a mean GL of 8.71 mm. Similarly, in the convex shape of type-1 BB, produced in PDEK grafts, the GL was greater than BBD because the submerged endothelial grafts straight along the largest diameter and curled perpendicularly.

The main factor responsible for the metabolism of endothelial cells is the aqueous humor, which is in intimate contact with the posterior lamella of the cornea. A strong correlation have been reported between aqueous glucose levels and blood glucose [20], and it is well known that excess glucose in diabetics correlates with a glycosylation process, resulting in strong adhesion between DM and deep

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stroma [21]. Previous DMEK studies described the high occurrence of tears and failure dissection in diabetic patients using stripping techniques [22–24], with the suggestion of excluding the contralateral cornea after unsuccessful DM removal [25]. The present study used air dissection and failed to prepare three corneas from six donors (50%); however, all contralateral corneas were successfully prepared. The interface between the PDL and stroma could be less influence of chronic hyperglycemia due to posterior localization in lamellar anatomy and its impermeability, and future studies may address this issue and investigate whether air dissection has benefits in diabetic donors.

The composite image of the entire graft revealed a pattern of cell loss localized primarily within areas of graft fold formation. Other factors, such as the air injection site and peripheral manipulation, did not contribute significantly to cellular damage. Notably, no damage was observed adjacent to the 'S'-stamp, a feature that typically induces endothelial loss in standard DMEK grafts. While these findings require confirmation by further studies, it is theorized that marking the anterior surface of the Pre-Descemet's Layer (PDL) prevents the endothelial cell damage usually caused by direct contact with stamp ink. In contrast, in standard DMEK grafts, the marking is performed directly on the basement membrane of the corneal endothelium.

5. Conclusions

This article describe digital measurements and scrolling characteristics of precut endothelial cornea grafts. PDEK had a significantly higher CGW than DMEK ($P<0.05$), and there was an inverse relationship between CGW and SS. Donor age and CGW had a weak correlation with DMEK, and no correlation with PDEK grafts. CGW did not change significantly after TB in either modality.

Author Contributions: Substantial contribution to conception and design: Prado, R.B.; Casella, A.M.; Oguido, A.P; Acquisition of data: Prado, R.B.; Oguido, A.P; Analysis and interpretation of data: Prado, R.B.; Casella, A.M.; Oguido, A.P.; Borghi; S.M; Drafting of the manuscript: Prado, R.B; Critical revision of the manuscript for important intellectual content: Casella, A.M.; Oguido, A.P.; Borghi; S.M.; Verri, W.A; Have given final approval of the submitted manuscript: Prado, R.B.; Casella, A.M.; Oguido, A.P.; Borghi; S.M.; Verri, W.A; Statistical analysis: Prado, R.B; Administrative, technical, or material support supervision: Casella, A.M.; Research group leadership: Casella, A.M.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Universidade Estadual de Londrina.

Data Availability Statement: The datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations were used in this manuscript:

EK Endothelial keratoplasty
DMEK Descemet membrane endothelial keratoplasty
PDEK Pre-Descemet endothelial keratoplasty
CGW central graft width
TB trypan blue
TB-CGW central graft width after trypan blue
ECD endothelial cell density
DPT death-to-preservation time
TPT total preservation time

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PT procedure time

AIV air-injected volume

PWTWD posterior white-to-white distance

BBD big bubble diameter

GL graft length

GA graft area

GMC graft margin circularity

H Hoechst 33342

EH-1 Etidium-homodimer

CAM Calcein AM

References

1. Lie JT, Birbal R, Ham L, van der Wess J, Melles GR. Donor tissue preparation for Descemet membrane endothelial keratoplasty. J Cataract Refract Surg 2008;34:1578-1583.
2. Newman LR, DeMill DL, Zeidenweber DA, et al. Preloaded Descemet membrane endothelial keratoplasty donor tissue: surgical technique and early clinical results. Cornea 2018;37:981-986.
3. Melles Gr, Ong TS, Ververs B, van der Wess J. Descemet membrane endothelial keratoplasty (DMEK). Cornea 2006;25:987-990.
4. Mohammed I, Ross AR, Britton JO, Said DG, Dua HS. Elastin content and distribution in endothelial keratoplasty tissue determines direction of scrolling. Am J Ophthalmol 2018;194:16-25.
5. Price MO, Price Jr FW. Descemet's membrane endothelial keratoplasty surgery: update on the evidence and hurdles to acceptance. Curr Opin Ophthalmol 2013;24:329-335.
6. Dua HS, Faraj LA, Said DG, Gray T, Lowe J. Human corneal anatomy redefined: A novel pre-Descemet's layer (Dua's layer). Ophthalmology 2013; 120:1778-85.
7. Agarwal A, Dua HS, Narang P, Kumar DA, Agarwal A, Jacob S, et al. Pre-Descemet's endotelial keratoplasty (PDEK). Br J Ophthalmol 2014;98:1181-1185.
8. Agarwal A, Agarwal A, Narang P, Kumar DA, Jacob S. Pre-Descemet endothelial keratoplasty with infant donor corneas: A prospective analysis. Cornea 2015;34(8):859-65.
9. Majmudar PA, Johnson L. Enhancing DMEK success by identifying optimal levels of trypan blue dye application to donor corneal tissue. Cornea 2017;36(2):217-221.
10. Zeidenweber DA, Tran KD, Sales CS, Wehrer SW, Straiko MD, Terry MA. Prestained and Preloaded DMEK Grafts: An Evaluation of Tissue Quality and Stain Retention. Cornea. 2017;36(11):1402-1407.
11. Heinzelmann S, Huther S, Bohringer D, Eberwein P, Reinhard T, Maier P. Influence of donor characteristics on Descemet membrane endothelial keratoplasty. Cornea 2014;33:664-648.
12. Bennett U, Mahmoud S, Drury D, Cavanagh HD, McCulley JP, Petroll WM, Mootha VV. Impact of donor age on corneal endothelium-Descemet membrane layer scroll formation. Eye Contact Lens 2015;41(4):236–239.
13. John T, Patel A, Vasavada A, Singh M, Nath V, Cheng AM, Sheha H. Effect of Trypan Blue on Descemet Membrane Elasticity. Cornea 2016;35(11):1401-1403.
14. Tran KD, Dye PK, Odell K, et al. Evaluation and quality assessment of prestripped, preloaded Descemet membrane endothelial keratoplasty grafts. Cornea 2017;36:484-490.
15. Parekh M, Ruzza A, Ferrari S, et al. Preloaded tissues for Descemet membrane endothelial keratoplasty. Am J Ophthalmol 2016;166:120-125.
16. Price MO, Lisek M, Kelley M, Feng MT, Price FW Jr. Endothelium-in versus endothelium-out insertion with Descemet's membrane endothelial keratoplasty. Cornea. 2018;37(9):1098-1101.
17. Dua HS, Termote K, Kenawy MB, Said DG, Jayaswal R, Nubile M, Mastropasqua L, Holland S. Scrolling characteristics of pre-Descemet endothelial keratoplasty tissue: An ex vivo study. Am J Ophthalmol 2016;166:84-90.

 doi:10.20944/preprints202602.1413.v1

18. Newman LR, Tran KD, Odell K, Dye PK, Galloway J, Sales CS, Straiko MD, Terry MA. Minimizing Endothelial Cell Loss Caused by Orientation Stamps on Preloaded Descemet Membrane Endothelial Keratoplasty Grafts. Cornea. 2019;38(2):233-237.
19. Dua, H, Said, D. Pre-Descemets endothelial keratoplasty: the PDEK clamp for successful PDEK. Eye 2017;31:1106-1110.
20. Gomel N, Barequet IS, Lipsky L, Bourla N, Einan-Lifshitz A. The effect of the glycemic control on the aqueous humor glucose levels in diabetic patients undergoing elective cataract surgery. Eur J Ophthalmol. 2021;31(2):415-421.
21. Brownlee M, Vlassara H, Cerami A. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. Ann Intern Med. 1984;101(4):527-37.
22. Greiner MA, Rixen JJ, Wagoner MD, Schmidt GA, Stoeger CG, Straiko MD, Zimmerman MB, Kitzmann AS, Goins KM. Diabetes mellitus increases risk of unsuccessful graft preparation in Descemet membrane endothelial keratoplasty: a multicenter study. Cornea 2014;33(11):1129-33.
23. Viana LM, Stoeger CG, Galloway JD, Terry M, Cope L, Belfort JR R, Jun AS. Risk factors for eye bank preparation failure of Descemet membrane endothelial keratoplasty Tissue. Am J Ophthalmol 2015;159(5):829-834.
24. Price, MO, Lisek, M, Feng, MT, Price, FW. Effect of donor and recipient diabetes status on Descemet membrane endothelial keratoplasty adherence and survival. Cornea 2017;36(10):1184-1188.
25. Gorovoy IR, Cui QN, Gorovoy MS. Donor tissue characteristics in preparation of DMEK grafts. Cornea 2014;33(7):683-685.