Endothelial Cell Viability of Pre-Processed DSEK and DMEK Corneal Donor Transplants

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ABSTRACT

Purpose: To determine the viability of endothelial cells using vital dye staining in processed Descemet's stripping automated endothelial keratoplasty (DSEK) and Descemet's Membrane Endothelial Keratoplasty (DMEK) tissue stored for extended periods of time.

Methods: Trypan blue and alizarin red staining was performed on DSEK donors processed at 1, 2-4, 5-7, and >7 days prior to evaluation and DMEK donors processed at 1, 2-4, and 5-7 days prior to evaluation. Penetrating keratoplasty (PK) donors were used as controls. Grafts were photographed and analyzed to quantify endothelial cell damage by grading software, Adobe Photoshop 7.0.

Results: 46 corneas (17 PK, 18 DSEK, and 11 DMEK donors) were evaluated. DSEK tissue prepared one day prior to surgery had 6.4% endothelial cell damage compared to 8.2% in DSEK donors processed by 2-4 days, 5.2% processed by 5-7 days, 4.5% processed >7 days (single factor analysis of variance, P = 0.6). DMEK tissue prepared one day prior to surgery had 2.0% damage compared to 3.8% in donors processed by 2-4 days, and 5.3% processed by 5-7 days (single factor analysis of variance, P = 0.4). There was no significant effect of preservation time (<7 vs 7-14 days) on endothelial cell viability between control PK and DSEK/DMEK donors (PK 3.1% vs 5.5%, P = 0.7; DSEK 7.2% vs 5.1%, P = 0.3; DMEK 4.4% vs 1.6%, P =0.1).

Conclusion: Extended storage of processed DSEK and DMEK tissue has no significant effect on endothelial cell viability up to seven days after tissue preparation, supporting the use of corneas prepared greater than one day prior to surgery.

Keywords: Descemet's Membrane Endothelial Keratoplasty, Descemet's stripping automated endothelial keratoplasty, corneal endothelial cell

Descemet's stripping endothelial keratoplasty (DSEK) and Descemet membrane endothelial keratoplasty (DMEK) have increased in success and popularity in the recent past and have become the treatment of choice for endothelial cell dysfunction. The Eye Bank Association of America reports that rates of endothelial keratoplasty have increased by 6% in 2015,[1] and notes improvements in tissue preparation and storage aid in the proliferation of these procedures. Aiming to optimize the utilization of each donor graft will offset future pressures such as loss of donors from increased screening of new infectious agents and an increased demand for tissue due to an aging population.[2] The Cornea Preservation Time Study has shown that most surgeons request tissue that has been stored less than seven days for implantation for DSEK procedures and corneal tissue grafts are typically used within a few days after the grafts are harvested and.[2] The FDA permits 14 day storage of corneal tissue in Optisol GS[3] and DSEK grafts have been shown to be viable in culture for the full two weeks when properly stored.[4] Processed corneal tissue can be stored in organ culture media and placed in room temperature incubators, as in Europe, and cold storage media and placed at 4°C as in the United States;both methods have been shown to preserve endothelial cell function in the grafts for at least a four day period.[5,6] Endothelial keratoplasty (EK) grafts stored in 4°C media and implanted at a mean of 4 days were clear for at least six months after surgery.[6] However, the effects of prolonged storage beyond four days after endothelial keratoplasty preparation has been minimally investigated. Extending the time between preparation and use of EK grafts promises to extend the available pool of tissues for transplant while reducing processing time burdens on eye banks.

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Technical difficulties while implanting EK grafts, along with postoperative complications, can lead to increased pressure on the available cornea donor pool. DMEK in particular has been increasing in popularity among U.S. corneal surgeons over the past few years[1] due to its advantages over DSEK, including rapid visual improvement, greater likelihood of achieving improved vision (20/25 or better), and decreased rates of tissue rejection.[7,8,9] Eye banks offer pre-processed DSEK and DMEK tissues for transplantation to increase preoperative assessment of transplant tissue and operating room efficiency.[10] Although this practice decreases the need for surgeons to prepare their own grafts, the DMEK preparation technique requires more skill than that of DSEK processed tissue. Substantial donor cornea wastage can occur due to a preparation failure rate of up to 13.2 %.[4,11] Recent studies have found no difference in DMEK grafts prepared by surgeons versus those that are pre-processed,[4,11] thus opening the door for increased demand of eye bank prepared grafts in the future. Furthermore, recent trends have leaned towards eye banks preloading DMEK tissue for surgeons to reduce operating room time, increase the quality assurance of the prepared tissue, and reduce costs to surgery centers. These partnerships can also reduce the chance of failed graft preparation in the operating room, ensuring quality tissue for transplant for a patient's benefit, as demonstrated in recent studies by Khao et al and Parekh et al.[12,13] Recent data shows that pre-prepared DMEK tissue that is pre-loaded in an IOL cartridge can be stored up to four days with low amounts of endothelial damage.[13] Other limitations of DMEK surgery include difficulty of surgical technique and greater number of early complications in comparison to DSEK.[14] These considerations emphasize the importance of increasing available donor tissue.

A better understanding of the effects of extended cold-storage on the endothelium of pre-processed DSEK and DMEK tissue could allow for lengthened storage time and, thus, increase the pool of available tissue. It would also allow greater flexibility for banks to schedule preparation in advance of surgery. The ability to store these tissues for a longer period of time could increase placement of backup tissue returned by surgeons learning DMEK and provide greater flexibility in international placement.

MATERIALS AND METHODS

Seventeen human donor corneas processed for DMEK, 18 donor human corneas processed for DSEK, and 11 control PK human donor corneas were obtained from eleven eyebanks of VisionShare (Chicago, Illinois). Corneal tissues prepared for penetrating keratoplasty were used for control. Tissue was shipped to our laboratory in Baltimore, MD overnight. The tissues were then stored at 4°C in Optisol GS (Bausch and Lomb, Rochester, NY) for 1 day, 2-4 days, 5-7 days, and 14 days. These tissues were then stained with 0.4% trypan blue (MP Biomedicals, LLC, Solon, OH) and 0.2% alizarin red (GFS Chemicals, Inc., Columbus, OH) after each storage time point.

At each time point or interval, all stained corneal graft tissue was removed from the donor cap and placed on viscoelastic with the endothelial side up on a glass slide, and photographed under a light microscope. Images were analyzed to quantify endothelial cell damage by a masked grading system using Adobe Photoshop 7.0 software.[15] The grading system was masked as to which type of tissue was presented for grading. Any tissue stored beyond 14 days was excluded.

Statistical analysis was then performed using one-way analysis of variance (ANOVA) to compare the four groups at each time point of 1 day, 2-4 days, 5-7 days, and greater than 7 days of storage time.

The Institutional Review Board granted approval for this study at the Greater Baltimore Medical Center in Baltimore, MD, and the study was conducted in adherence to tenets of the Declaration of Helsinki.

RESULTS

44 corneas were obtained and evaluated. These included 11 penetrating keratoplasty (PK) donors, 18 processed DSEK donors, and seventeen processed DMEK donors. Table 1 details tissue demographics for the DSEK and DMEK tissue studied. 5 DSEK tissues, 1 PKP tissue, and 11 DMEK tissues were not cooled. One DSEK donor did not have endothelial cell counts measured. No significant changes were noted between DSEK and DMEK tissues from 1 to 7 days and between DSEK and PKP tissue from 7 to 14 days.

All tissues were processed within seven days of donor death. DSAEK tissue prepared one day prior to surgery had 6.4% endothelial cell damage (standard deviation 0.05) compared to 8.2% in DSEK donors processed by 2-4 days (standard deviation 0.06), 5.2% processed by 5-7 days (standard deviation 0.04), 4.5% processed >7 days (standard deviation 0.02; single factor analysis of variance, P = 0.6) as shown in Figure 1 and Table 2.

DMEK tissue prepared one day prior to surgery had 2.0% damage (standard deviation 0.04) compared to 3.8% in

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Table 1: DMEK, DSEK, and PKP Tissue Demographics
Tissues donor age, average endothelial cell count, average death to preservation time, and average death to cooling time in donor DSAEK, DMEK, and PKP corneas.

	DSEK	DMEK	PKP
Age			
1 day			
Mean	62.2	62.7	
Range	54-75	54-68	
P value	0.9		
2-4 days			
Mean	70	66.5	
Range	65-72	59-75	
P value	0.3		
5-7 days			
Mean	54	56.5	
Range	20-72	52-61	
P value	0.8		
<7 days			
Mean			59
Range			59
7-14 days			
Mean	70.3		53
Range	69-71		10-65
P value	0.1		
Endothelial Cell Count			
1 day			
Mean	2522	2610	
Range	2463-2597	2237-3106	
P value	0.7		
2-4 days			
Mean	2750.5	2728.3	
Range	1887-3215	2545-2959	
P value	0.9		
5-7 days			
Mean	2671.4	2657.5	
Range	2151-2814	2415-2849	
P value	1		
<7 days			
Mean			2426.5
Range			2193-2660
7-14 days			
Mean	2424.5		2889.4
Range	2045-2814		2315-3636
P value	0.1		
Death to preservation time (hours)			
1 day			
Mean	9.72	10.5	
Range	1.2-18.8	6.7-12.7	
P value	0.8		
2-4 days			
Mean	9.2	17.5	
Range	7.5-12	10.8-23.6	
P value	0.1		
5-7 days			
Mean	7.42	9.9	
Range	3.4-13.1	9.2-10.6	
P value	0.2		
<7 days			
Mean			15.1
Range			15.1
7-14 days			
Mean	10.55		9.7
Range	6.2-20.9		5.6-14.6
P value	0.8		
Death to Cooling time (hours)			
1 day			
Mean	0.8	4	
Range	0-1.8	0-18.1	
P value	0.4		
2-4 days			
Mean	0.5	0.01	
Range	0-1.8	0-0.03	
P value	0.4		
5-7 days			
Mean	2.72	2.1	
Range	1.2-5.2	0-4.1	
P value	0.6		
<7 days			
Mean			12.4
Range			12.4
7-14 days			
Mean	10.55		1.7
Range	6.2-20.9		0-2.8
P value	0.8		

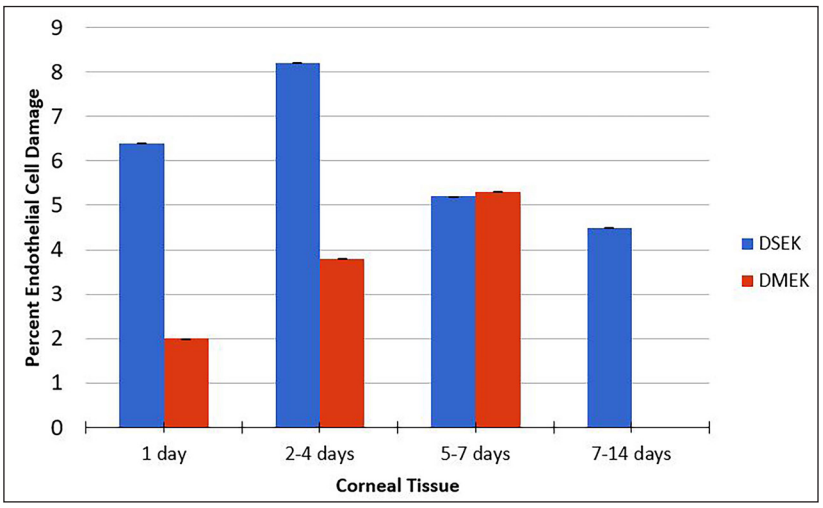

Figure 1: DMEK and DSEK Tissue Endothelial Cell Damage after Cold Storage
Endothelial cell damage determined in DMEK and DSEK corneas placed in cold storage on 1 day, 2-4 days, 5-7 days, and 7-14 days after processing. No significant difference noted at different time points. and PKP corneas.

Table 2: Percentage of Cell Damage in DSEK, DMEK, and PKP Tissue
Percentage endothelial cell damage at various time points in DSEK and DMEK tissue.

	DSEK	DMEK	PKP	
1 day				
Mean	6.4	2		
Range	0.02-0.14	0.0-0.12		
St Dev	0.05	0.04		
2-4 days				
Mean	8.2	3.8		
Range	0.01-0.13	0.0-0.06		
St Dev	0.06	0.03		
5-7 days			**<7 days**	
Mean	5.2	5.3	*Mean*	0.03
Range	0.02-0.1	0.02-0.11	*Range*	0.01-0.04
St Dev	0.04	0.04	*St Dev*	0.01
>7 days			**>7 days**	
Mean	4.5	n/a	*Mean*	0.05
Range	0.02-0.7	n/a	*Range*	0.03-0.09
St Dev	0.02	n/a	*St Dev*	0.02

donors processed by 2-4 days (standard deviation 0.03), and 5.3% processed by 5-7 days (standard deviation 0.04; single factor analysis of variance, P = 0.4). Control PKP tissue was divided into two groups and had 3.0% damage in tissues processed less than seven days (standard deviation 0.01) and 5.0% damage in tissues processed by 7-14 days (standard deviation 0.02).

There was no significant effect of preservation time (<7 vs 7-14 days) on endothelial cell viability between control PK and DSEK/DMEK donors (PK 3.1% vs 5.5%, P = 0.7; DSEK 7.2% vs 5.1%, P = 0.3; DMEK 4.4% vs 1.6%, P =0.1). Figure 2A and B showcase tissues prepared with Alizarin Red and Trypan Blue and photographed with slit lamp microscopy.

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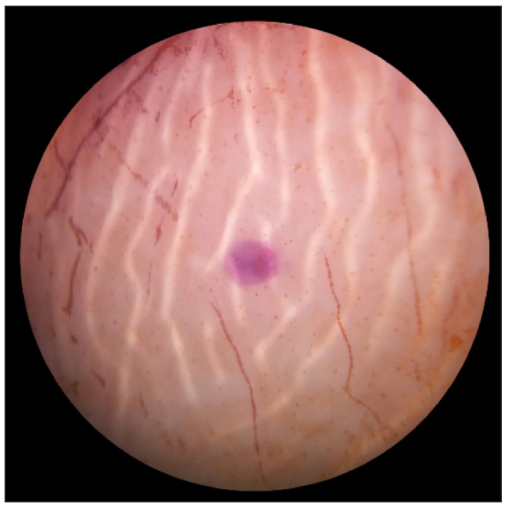

Figure 2A: DMEK Tissue Stained with Alizarin Red and Trypan blue

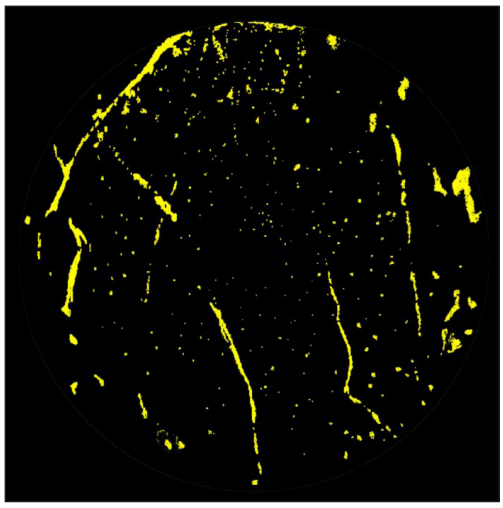

Figure 2B: DMEK Tissue Stained with Trypan Blue and Imaged with Slit Lamp Microscopy

DISCUSSION

This study suggests there is deterioration of endothelial cell viability by vital dye staining in extended storage of EK tissue up to seven days after preparation by an eye bank. This lays the groundwork for a possible paradigm shift encouraging surgeons to routinely use EK grafts up to one-week post-preparation. Such a shift in surgical planning should allow more flexibility for eye banks to schedule tissue preparation.

Most surgeons currently prefer to use the prepared EK tissue within one day of processing and unused tissue runs the risk of being discarded. The Cornea Preservation Time Study is currently underway to determine outcomes of DSEK tissue used between 1 to 7 days post-preparation compared to DSEK tissue used between 8 to 14 days. While this study examines transplant tissue after it has been prepared, it does not examine the effect of time after tissue has been prepared and then used for transplant.[2] Other studies support the resiliency of endothelial cells after extended storage. Feng and colleagues[16] determined that endothelial cell loss at 3 months after DMEK did not differ between recipient eyes that received processed tissue stored in a refrigerator for 0, 1, or 2 days, however, the effects of a longer preparation-to-use time were not explored. Similarly, Heindl et al[17] observed that an increased storage time of split donor DMEK tissue in organ culture or cold-storage media was not associated with an increase in endothelial cell loss. In a study by Kobayashi et al[18] comparing endothelial cell damage by trypan blue staining between processed DMEK and DSEK tissue which was sent overseas by airplane, overall endothelial cell loss was low in both types of tissue (0.3% and 0.1%, respectively), but the time between preparation and staining was not specified. In contrast, our study found no significant difference when pre-prepared EK tissue was examined at specific time points up to seven days after cold storage.

One limitation of this study is that the donor grafts did not undergo the additional manipulation expected with performing surgery. We do not know if extended storage makes tissue more prone to damage during intraocular manipulation. Another limitation was that the study was limited to vital dye staining and did not explore metabolic analysis looking at mitochondrial respiration or glycolysis activity. The lack of a large number of tissue is another limitation, due to tissue availability and the cost of donor tissue. Follow-up studies should focus on clinical outcomes in vivo in a larger cohort of patients randomized to receive EK grafts stored for longer periods after preparation.

Additional studies have shown that one donor cornea can be prepared for dual usage to increase tissue efficiency and reduce wastage. The tissue is prepared for use as both a DMEK tissue graft and a DALK graft with no complications associated with the preparation of the grafts and no endothelial cell loss [19, 20]. Extending the time between preparation and surgery can act synergistically with dual usage preparation to increase the donor pool in areas with a shortage of donors, as long as no tissue damage or wastage occurs during tissue preparation. Another limitation is that while our study addresses tissue viability with a vital dye stain, longer storage time could increase stromal edema in DSEK grafts.

Storing pre-prepared EK grafts has minimal effects on corneal endothelium up to one-week post preparation and two weeks post recovery. This can lead to increased tissue utilization, facilitated international distribution, and decreased demands on eye bank staff for tissue preparation.

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REFERENCES:

1. Eye Bank Association of America. 2015 Eye Banking Statistical Report. Washington DC. Eye Bank Association of America. 2016.
2. Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. Cornea 2015; 34:601-8.
3. Terry MA, Shamie N, Straiko MD, et al. Endothelial Keratoplasty: The relationship between donor tissue storage time and donor endothelial survival. Ophthalmology 2011; 118:36-40.
4. Ruzza A, Salvalaio G, Bruni A, et al. Banking of donor tissues for descemet stripping automated endothelial keratoplasty. Cornea. 2013; 32:70-5.
5. Bhogal M, Matter K, Balda MS, et al. Organ culture storage of pre-prepared corneal donor material for Descemet's membrane endothelial keratoplasty. Br J Ophthalmol. 2016; 100:1576-1583.
6. Price MO, Knight OJ, Benetz BA, et al. Randomized, prospective, single-masked clinical trial of endothelial keratoplasty performance with 2 donor cornea 4°C storage solutions and associated chambers. Cornea. 2015;34:253-6.
7. Guerra FP, Anshu A, Price MO, et al. Endothelial keratoplasty: fellow eyes comparison of Descemet stripping automated endothelial keratoplasty and Descemet membrane endothelial keratoplasty. Cornea 2011;30:1382-1386.
8. Tourtas T, Laaser K, Bachmann BO, et al. Descemet membrane endothelial keratoplasty versus Descemet stripping endothelial keratoplasty. Am J Ophthalmol. 2012;153:1082-1090.
9. Price MO, Price FW Jr. Descemet's membrane endothelial keratoplasty surgery: update on the evidence and hurdles to acceptance. Curr Opin Ophthalmol 2013;24:329-35.
10. Boynton GE, Woodward MA. Eye-bank preparation of endothelial tissue. Curr Opin Ophthalmology. 2014; 25: 319-324.
11. Greiner MA, Rixen JJ, Wagoner MD, et al. Diabetes mellitus increases risk of unsuccessful graft preparation in Descemet membrane endothelial keratoplasty: a multicenter study. Cornea. 2014;33:1129-33.
12. 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.
13. Parekh M, Ruzza A, Ferrari S, et al. Preloaded Tissues for Descemet Membrane Endothelial Keratoplasty. Am J Ophthalmol. 2016;166:120-5.
14. Ang M, Wilkins MR, Mehta JS, et al. Descemet membrane endothelial keratoplasty. Br J Ophthalmol. 2016;100:15-21.
15. Saad HA, Terry MA, Shamie N, et al. An easy and inexpensive method for quantitative analysis of endothelial damage by using vital dye staining and Adobe Photoshop software. Cornea. 2008;27:818-824.
16. Feng MT, Burkhart ZN, Price FW Jr, et al. Effect of donor preparation-to-use times on Descemet membrane endothelial keratoplasty outcomes. Cornea. 2013;32:1080-2.
17. Heindl LM, Riss S, Adler W, et al. Split cornea transplantation: relationship between storage time of split donor tissue and outcome. Ophthalmology. 2013;120:899-907.
18. Kobayashi A, Murata N, Yokogawa H, et al. Evaluation of internationally shipped prestripped donor tissue for descemet membrane endothelial keratoplasty by vital dye staining. Cornea. 2015;34:225-7.
19. Menant-Tay CL, Conlon R, Teja S, et al. Dual-purpose corneal tissue for anterior lamellar keratoplasty and Descemet's membrane endothelial keratoplasty. Can J Ophthalmol. 2016;51:408-411.
20. Heindl LM, Riss S, Adler W, et al. Split cornea transplantation: relationship between storage time of split donor tissue and outcome. Ophthalmology. 2013;120:899-907.

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