Utilization of the Novel RE-One Device for Human Eye Procurement Improves the Quality of Transplantation-Grade Corneal Tissue and Research-Grade Posterior Eye

Kayla M. Jones, CEBT,[1,6] Rahul Raghu, MD, MBA,[2,6] Yang Shan, MD, MS,[3] Patrice E. Fort, PhD,[3,4] Kristen E. McCoy, CEBT, CTBS,[1,5] Cheryl Zeleny,[1] Onkar B. Sawant, PhD[1]

ABSTRACT

Purpose: To analyze the effectiveness of the novel RE-One chamber for excising surgical-grade human corneal tissue for transplantation and preserving high-quality posterior eyes for research purposes.

Methods: 56 corneas were excised from 28 research-intent donors using either traditional *in-situ* procurement techniques or RE-One chambers. The corneas were evaluated using standard eye banking practices and stained with Trypan blue to assess the corneal endothelium. An additional 10 whole eyes were procured from 5 donors to analyze the integrity of the retina within the posterior eye after traditional procurement technique or after RE-One chamber utilization.

Results: In the corneal excision study, scleral rim size was significantly more uniform in the RE-One group compared to the traditional method ($P \leq 0.0001$). Corneas excised from pseudophakic donors using RE-One devices demonstrated higher corneal endothelial cell density compared to those excised by the traditional method ($P=0.0405$). None of the posterior eyes procured using the RE-One chamber exhibited any retinal folds, detachments or tears. Adversely, posterior eyes procured using the traditional method resulted in a 60% retinal fold rate, 100% detachment rate and a 40% retinal tear rate.

Conclusion: Our series of investigations validates the beneficial effect of the RE-One chamber in procuring significantly higher-quality post-mortem human posterior eyes for research purposes while simultaneous improving the quality of surgical-grade corneas for transplantation.

Translational Relevance: RE-One not only improves the quality of surgical tissues to treat corneal blindness but also promotes the utilization of human eyes to bridge the translational gap between animal models and clinical studies.

Key Words: Cornea, posterior eye, retina, procurement techniques, eye-bank, RE-One

The first corneal transplant occurred in 1905, leading to the opening of the first eye bank in 1944.[1] Ocular procurement techniques have changed in the rapidly progressing field of corneal transplantation ever since.[1,2] Among the changes is the shift from whole globe enucleation to corneal excision. Corneal excision presents an increased risk of recovery-induced errors as navigating around facial features and increasing demands on short death-to-preservation intervals weigh on the recovery technician.[3] Recovery-induced errors can lead to corneal endothelial cell loss and uneven scleral rims, all affecting the suitability of the cornea intended for transplantation.[3-5]

The scleral rim size is an important factor in corneal preservation. A large rim can lead to irreversible corneal damage by creating folds within the cornea leading to corneal endothelial cell loss if the rim is not trimmed prior to placing in a corneal viewing chamber. For endothelial keratoplasty (EK) procedures, corneal grafts are prepared with a microkeratome. The scleral rim plays an essential role in holding the cornea in place during the microtome pass to ensure a successful, even graft is cut. 6,7

The RE-One chamber (Lugano, Switzerland, see Figure 1) purports to alleviate the challenges working around the donor's facial structure during the excision process (http://www.re-one.ch/about.php). By providing a more ergonomic platform, it allows for more uniform scleral rims

Author Affiliation: [1]Eversight, Cleveland, OH, [2]University Hospitals Cleveland Medical Center, Cleveland, OH, 3University of Michigan Department of Ophthalmology and Visual Sciences, Ann Arbor, MI, [4]University of Michigan Department of Molecular and Integrative Physiology, Ann Arbor, MI, [5]Miracles In Sight, Winston-Salem, NC, [6]Equal contribution first-authors

Financial Disclosures: The authors declare no financial disclosures or material conflicts of interest.

Corresponding author: Onkar B. Sawant, PhD, Eversight, 6700 Euclid Ave. Ste. 101, Cleveland, OH, 44103, Phone: 216-706-4212, E-mail: osawant@eversightvision.org onkarsawant19@gmail.com

 ISSN 2161-5546

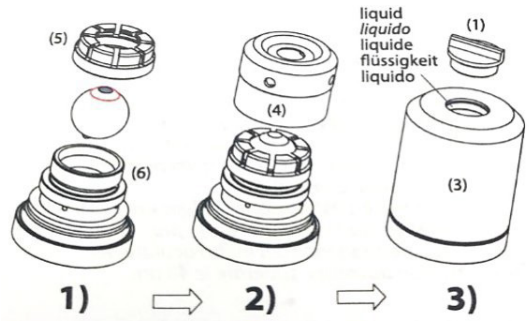

Figure 1. *RE-One diagram for globe transport (http://www.re-one.ch/about.php). 1. Place globe into base (6) and tighten the collet (5) onto the base to hold the globe in position. 2. Place the internal cover (4) onto the base. 3. Place the external cover (3) onto the base. Fill with desired solution and seal container by affixing the screw cap (1) onto the external cover. For more information about the RE-One chamber, including videos, visit: https://medicalinnovationpartners.international/. Permission to use this Figure was granted by Medical Innovation Partners.*

and greater control during the iris separation, presumably leading to reduced corneal damage including less endothelial cell loss. To test this hypothesis, we designed a paired study comparing corneal tissue excised with the RE-One chamber to tissue recovered via traditional *in-situ* corneal excision method.

Subsequently, we looked at the application of the RE-One device in the procurement of posterior eyes for research purposes. Current procurement techniques fall short in terms of retinal quality that is provided by enucleating the whole eye; however, whole eye enucleation for research is often secondary to procuring the cornea for transplantation. The posterior eye cup is what remains after the cornea has been removed from the eye; if the retina remains intact, the posterior eyes can be used for high-caliber research studies[8] According to a recent study, the percentage of ocular tissues distributed for research has gradually declined between 2006 to 2016.[9] Among the factors driving this reduction in available tissues for research purposes is the rapid evolution of endothelial keratoplasty techniques, which created a large influx in the number of corneas procured for transplantation.[10] Indeed, a donor's ocular history of age-related macular degeneration (AMD) or diabetic retinopathy (DR) is not an exclusion for corneal transplantation, clearly impacting the number of whole eyes available for research purposes.[11]

Our goal for this study was to evaluate the effectiveness of the RE-One device for potentially improving both the quality of surgical-grade corneal tissue procured for transplantation and the quality of research-grade posterior eyes . This study demonstrates that use of the RE-One device provides quality corneas intended for transplant and reduces defects of research-grade posterior eyes. Our assessment also revealed that the RE-One chamber improves the quality and retinal integrity of the posterior eyes compared to the traditional technique, specifically by reducing incidences of retinal tears, folds, and/or detachments.

METHODS

This study was performed in compliance with the Declaration of Helsinki, Eye Bank Association of America (EBAA), and Food and Drug Administration (FDA) regulations. Our study was divided into two portions: the cornea study which procured tissues for research purposes only and the posterior eye cup study which procured tissues for transplantation and research. Consent from each donor family was obtained prior to procurement. Table 1 contains the inclusion criteria for each part of the study. Figure 2 outlines each step of the study which was divided into two groups: the cornea study and the posterior eye parts study.

Table 1*. Inclusion Criteria for Donors that Participated in the RE-One Study*

	Inclusion Criteria
Cornea Study	Research intent donors only*
	Age 5-80 years
	Death to preservation interval ≤ 24 hours
	Bilateral donors
	Same lens type bilaterally
	No known communicable diseases
Posterior Eye Parts Study	Must meet screening criteria for surgical grade corneas
	Surgical intent donors with consent for research†
	Age 5 – 75 years
	Death to preservation interval ≤ 10 hours
	Bilateral donors
	Phakic lens type
	No history of retinal diseases or surgeries

* Donors procured for research purposes only.

† Posterior eye cup study included surgical cornea donors, meaning the corneas were procured aseptically with the intention of transplantation. Consent for research was also obtained for each donor to be able to utilize the posterior eye partss for research.

Cornea Study

Traditional In-situ Excision Technique

Using our current traditional *in-situ* excision technique, 28 corneas were excised directly from 28 research donors in northeast Ohio. After a betadine soak and saline rinse, conjunctiva was removed from the eye designated for corneal excision. A trephine was used to score the peripheral sclera without rupturing the vitreous sac. A scalpel was used to make an incision 3mm from the limbus. Castroviejo scissors were used to gently follow the trephine score, resulting in a complete separation of the sclera surrounding the cor-

 ISSN 2161-5546

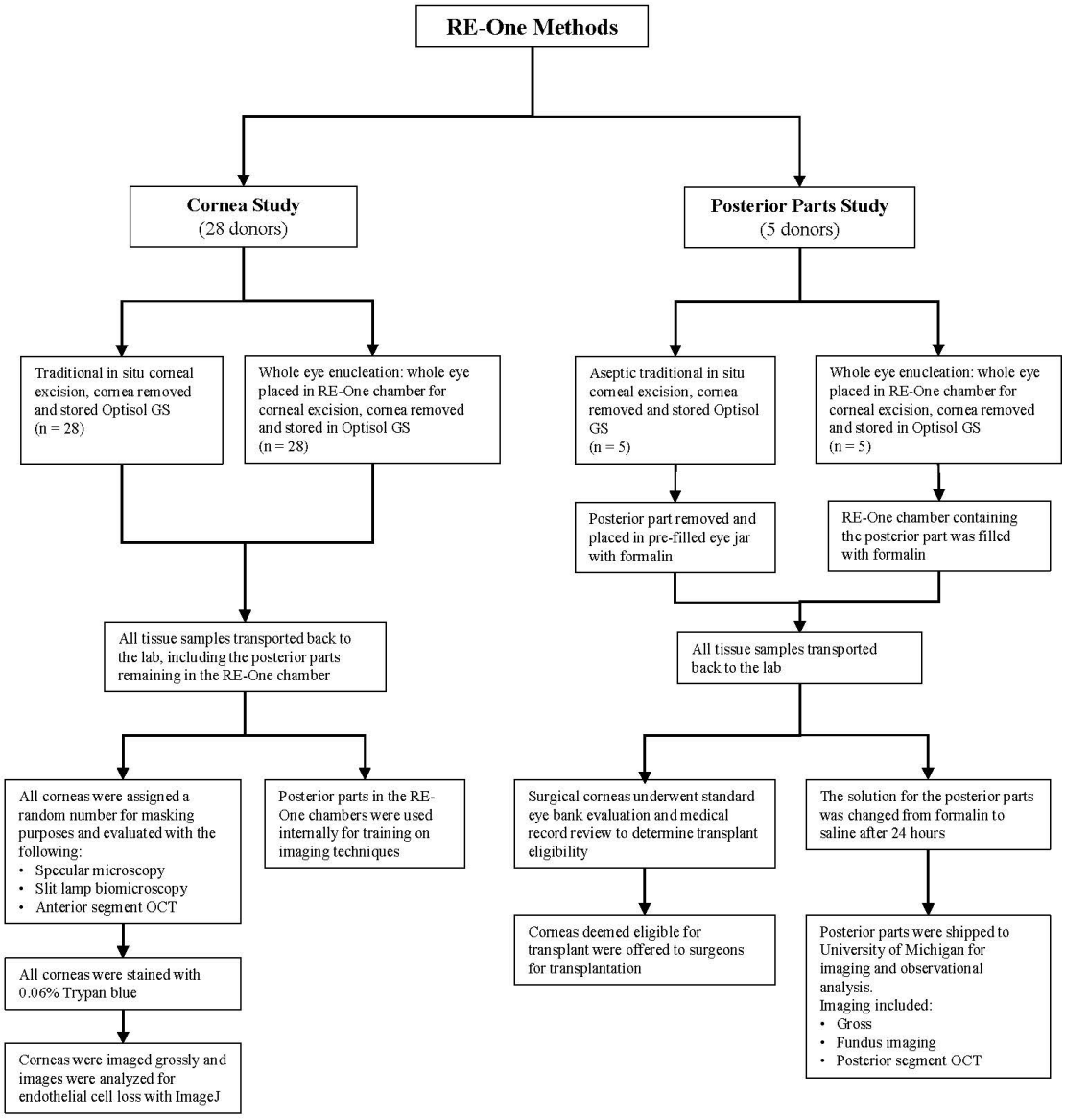

***Figure 2**. Paired Study Design. This figure outlines the steps taken in each study.*

nea. Next, two pairs of toothed forceps were used to gently separate the iris from the cornea. The cornea was placed into a corneal viewing chamber (Bausch & Lomb, St. Louis, MO) with Optisol GS (Bausch & Lomb, St. Louis, MO) for storage and transportation back to the lab.

RE-One Procurement Technique

The 28 contralateral eyes were enucleated prior to corneal excision in the RE-One chamber (Figure 1). After a betadine soak and saline rinse, the conjunctiva was removed from the eye. The extraocular muscles followed by the optic nerve were clipped. The whole eye was removed and positioned in the RE-One. Once the collet was tightened over the enucleated eye, the corneal excision was completed following the aforementioned steps above starting with the trephine score. Once the cornea was stored in the viewing chamber, the RE-One chamber containing the posterior eye cup was assembled for transport.

Tissue Transport

All of the tissues, two corneas and one posterior eye cup, were packed on wet ice for transportation. The tissue was transported from the recovery site to the Eversight lab for the remainder of the cornea study.

Corneal Evaluations

Post procurement, the corneas were logged into a tracking database and stored in the refrigerator (2-8°C) until evaluations could be completed, typically within 12 hours. The evaluations were performed by a single certified eye bank

 ISSN 2161-5546

technician. Each cornea was assigned a random number for masking purposes. After the corneas were allowed to warm in an incubator (36°C) for 60 to 90 minutes, they were analyzed for endothelial cell density (ECD) using the CellChek D+ (Konan Medical, Irvine, CA) specular microscopy.

The corneas were examined via slit lamp biomicroscopy (Haag-Streit, Köniz, Switzerland). A slit beam was used to measure the smallest and largest sections of the scleral rim and assess endothelial cell damage. Endothelial cell damage caused by the procurement was assessed in terms of stress lines, a linear pattern of cell loss illuminated by the slit lamp. The stress line quantity and location were recorded.

After the slit lamp evaluation was completed, a suitability grade was assigned adhering to surgical corneal parameters. A suitability grading scale of 0 to 10 was used to numerically grade tissue quality, a score of 10 representing the highest quality and a score of 0 representing the lowest quality, not suitable for transplant. The scores are based on different quality indicators such as endothelial cell density, scleral rim size, endothelial cell damage and other slit lamp examination findings. This scoring system allows the eye bank to categorize tissues for different types of transplants.

Vital Staining and Imaging

The corneas were removed from the viewing chamber and stained with 0.06% Trypan Blue (Dutch Ophthalmic Research Center, Zuidland, Netherlands) for two minutes. The corneas were rinsed using saline and returned to the original viewing chamber containing Optisol GS. Gross imaging was performed on a light pad (Artograph, Delano, MN) with a ruler for scaling. Images were analyzed for the percentage of endothelial cell loss with ImageJ's Weka Trainable Segmentation software following previously described protocol (Figure 3 C, D).[12]

Circularity of the rim was measured with trainable segmentation software but instead of differentiating between cells, we differentiated the scleral rim from the cornea and the background (Figure 4 C, D). After the result was created, the magic wand was used to select the border of the rim. The circularity of the scleral rim was then measured. Results for cell loss and circularity were recorded.

Posterior Eye Cup Study

In-situ vs RE-One

The extraocular muscles of five donor eyes were cut prior to undergoing traditional aseptic *in-situ* corneal excision technique as described above. After the cornea was excised from the donor, the optic nerve was cut. Two pairs of

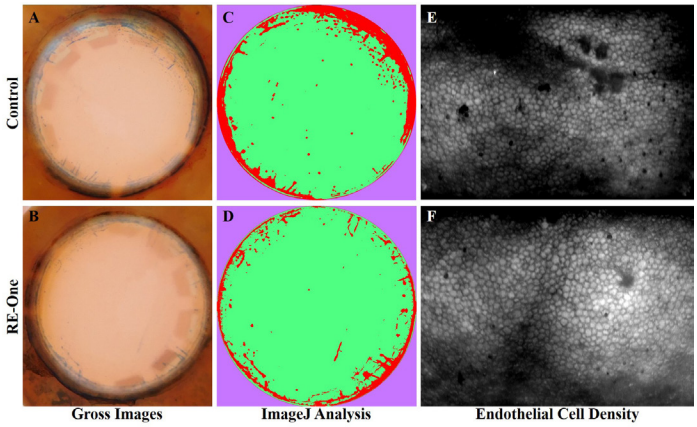

Figure 3. *Corneal endothelial cell staining with 0.06% Trypan Blue (A, B) and accompanied ImageJ analysis (C, D) to determine endothelial cell loss. The representative control cornea was estimated to have 14% endothelial cell loss (red area in panel C) while the RE-One was estimated to have 9% endothelial cell loss (red area in panel D). Representative images depicting corneal endothelial cell densities (ECD) in corneas from control (E) and RE-One (F) groups. This representative images had ECD of 2,786 cells/mm² (E) and 3,115 cells/mm2 (F), respectively.*

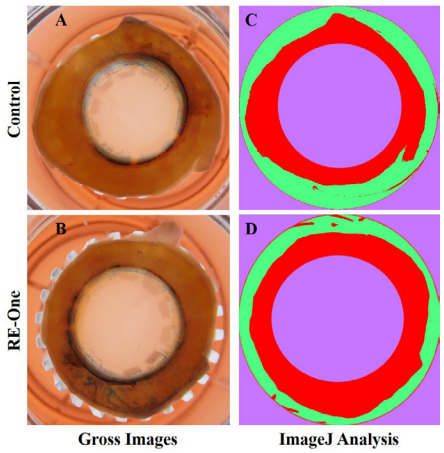

Figure 4. *Representative gross images (A, B) and ImageJ software processed images (C, D) collected as part of the scleral rim and circularity evaluation process for the cornea study. (C, D) The area in red depicts the area inside scleral rims which was used to measure circularity of the rim.*

toothed forceps were used to lift the posterior eye cup from the donor's eye socket and place it into the pre-filled eye jar containing formalin. These posterior eyes were classified by the storage container referred to as "Eye Jar" in the remaining sections. The contralateral eyes of the five donors were procured as previously described in the RE-One chamber. Formalin was poured into the RE-One chamber until the posterior eye cup was fully submerged.

 ISSN 2161-5546

Tissue Transport
All of the tissues, two surgical intent corneas and two posterior eyes, were packed on wet ice for transportation. The tissue was transported from the recovery site to the Eversight lab for surgical evaluation of the corneas and for solution transfers of the posterior eyes.

Solution Transfer
The posterior eyes were stored at room temperature for 24 hours. After 24 hours, the formalin in both the eye jars and the RE-One chambers wasreplaced with saline. The eye jars and RE-One chambers were sealed and stored in a refrigerator (2-8°C) and later shipped to the Kellogg Eye Center (Ann Arbor, MI) for subsequent analysis.

Evaluations & Observations
Once the team at the University of Michigan received the posterior eyes, one technician unpacked the box and set up the field for dissection. To remain masked to the container type, a second technician performed the subsequent qualitative assessment. Observations were recorded for the general appearance of the posterior eyes at time of receipt.

Imaging & Analysis
The iris and lens were carefully removed from each posterior eye cup. The posterior cups were imaged grossly with a Nikon SMZ1000 stereo microscope using the Nikon Plan Apo 0.5X widefield objectives and the Nikon Digital Sights DS-Fi1 camera and Nikon NIS-elements software (Minato City, Tokyo, Japan). The posterior eyes were then scanned using the Bioptigen Envisu Preclinical Spectral Domain Optical Coherence Imaging System with a Biotigen 12mm telecentric lens (Durham, NC) (Figure 5). After the images and scans were all collected, the images were reviewed for general observations that included the presence of retinal detachments, folds and tears.

Statistics
Means and standard deviations were calculated. Paired t-tests for approximately normal variables or nonparametric Wilcoxon signed-rank tests were used as appropriate to compare variables measured on paired corneas from the same donor for the corneal excision study. One cornea was assigned to RE-One and the cornea from the contralateral eye of the same donor was assigned to the traditional procurement technique (control). P-values were 2-tailed and, owing to the multiple comparisons, variables with P<0.01 were considered statistically different between RE-One and the traditional procurement technique. Data preparation and statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used for the posterior eye cup study due to the small sample size.

RESULTS

Corneal Study

Donor Demographics and Tissue Characteristics
Age of the donors for the cornea cohort was between 9-80, with an average age of 67.7 and majority of the 28 donors were male (n=21, 75%) (Supplemental Table 1). Of the 28 donors, 13 had undergone bilateral cataract surgery and were pseudophakic.

Across the primary endpoint measures of corneal suitability (P=0.19), circularity of the scleral rim (P=0.65), percent of endothelial cell loss (P=0.79), endothelial cell density (P=0.30) and recovery-induced cell loss categorized by stress line quantity (P=1.00), there were no statistically significant differences between the RE-One corneas and *in-situ* corneas (Table 2). The difference between the largest and smallest sections of scleral rim was statistically significant (P<0.0001), indicative of RE-One corneas demonstrating greater uniformity of the scleral rim as compared to the traditional *in-situ* excisions (Table 2, Figure 4).

Table 2. *Cornea Study End Measures for all Donors*

End Measure[1]	Control (n = 28)	RE-One (n = 28)	*P*-value
Suitability[2]	4.54 (± 3.76)	5.18 (± 3.87)	0.19
ECD (cells/mm²)	2,393 (± 592)	2,468 (± 434)	0.30
Stress Line Quantity	2.82 (± 0.72)	2.79 (± 0.74)	1.0
Stress Line Location	3.14 (± 1.01)	2.93 (± 1.02)	0.37
Endothelial Cell Loss %	10.1 (± 6.86%)	10.9% (± 7.8%)	0.79
Corneal Diameter (mm)	11.3 (± 0.08)	11.3 (± 0.37)	0.77
Smallest Rim Size (mm)	2.25 (± 0.63)	2.63 (± 0.57)	0.0078*
Largest Rim Size (mm)	4.25 (± 0.70)	3.70 (± 0.66)	0.0001*
Rim Size Difference (mm)	2.0 (± 0.73)	1.07 (± 0.65)	<0.0001*
Circularity[3] (mm)	0.74 (± 0.12)	0.76 (± 0.07)	0.65

1. Descriptive statistics are provided as Mean (+ Standard deviation).
2. The higher the suitability, the better the tissue quality is.
* Statistically different between RE-One and the traditional procurement technique (P < 0.01).
3. For circularity 1.0 is a perfect circle. The closer the number is to 1, the more circular the scleral rim.

Over half of the donors (n=16, 57%) had the same suitability rating bilaterally (Supplemental Table 1). The RE-One had a better suitability score in 8 donors versus 4 donors for the control method. Of the 8 donors with a better suitability score with the RE-One, 6 (75%) were pseudophakic (Supplemental Table 1), which prompted us to conduct a sub-analysis based on this criteria. In a 13-paired comparison for pseudophakic donors, RE-One procured eyes exhibited significantly higher (P=0.0405) ECD (2,243 +367) than eyes procured using traditional *in-situ* technique (1,980 +442). As mentioned earlier, overall, there was a statistically insignificant (P=0.30) but an increasing trend for ECD to be higher in corneas procured with the RE-One compared to the traditional *in-situ* method regardless of lens type (Table 2, Figure 3 E, F). Taken together, these

 ISSN 2161-5546

Supplemental Table 1. *Cornea Study Donor Demographics and Corneal Characteristics. A table listing each donor demographics and corneal tissue characteristics for each donor that participated in the cornea study.*

Donor	Age	Gender	Diabetes	Lens Type (OU)	ECD (cells/mm²) (RE-One/Control)	Suitability (RE-One/Control)[1]
1*	78	F	No	Phakic	3436/3425	10/10
2	69	M	Yes	Pseudophakic	2160/1736	9/2
3	68	M	No	Pseudophakic	2421/2336	9/4
4	46	F	Yes	Pseudophakic	2475/2083	1/1
5	75	M	Yes	Pseudophakic	2786/2494	9/4
6	74	M	Yes	Phakic	3021/2924	1/2
7	74	F	No	Pseudophakic	2294/1049	4/2
8	79	M	Yes	Pseudophakic	2128/1876	0/0
9	68	M	Yes	Phakic	3115/2786	5/9
10	66	F	Yes	Phakic	2525/2646	2/9
11	66	M	Yes	Phakic	2874/2915	9/9
12	54	M	No	Phakic	2506/2545	10/10
13	72	M	Yes	Pseudophakic	2016/1712	1 /2
14	72	M	Yes	Phakic	2857/2967	9/9
15	70	M	Yes	Pseudophakic	2155/2273	4/4
16	67	M	No	Pseudophakic	2703/2653	9/9
17	76	M	Yes	Pseudophakic	1577/1709	2/1
18	61	M	No	Phakic	2364/2336	1/1
19	80	M	No	Phakic	2611/3003	10/3
20	75	F	Yes	Pseudophakic	1667/2101	1/1
21	66	M	No	Pseudophakic	2141/1486	4/2
22	70	F	Yes	Phakic	2058/1802	4/2
23	64	M	No	Phakic	2304/2710	10/10
24**	73	M	Yes	Phakic	1931/1919	2/2
25*	9	F	No	Phakic	2874/3436	0/0
26	75	M	Yes	Phakic	2809/2994	9/9
27	74	M	Yes	Pseudophakic	2639/2326	1/1
28	75	M	Yes	Phakic	2646/2857	9/9

1. The higher the suitability score, higher the quality of tissue.

* Black race ** Asian race. All other donors were of Caucasian race.

findings suggest that RE-One device may prevent corneal endothelial cell loss associated with tissue handling in pseudophakic donors and also provide transplantable grade corneas with uniform scleral rim size.

Posterior Eye Cup Study

Donor Demographics

Donor characteristics can be observed in Supplemental Table 2. All donors met surgical criteria at the time of procurement. The average donor age was 57and all were male. All donors had phakic lenses, from which 50% of corneas procured were transplanted. One donor was deemed non-transplantable due to medical history, while another had poor endothelium with severe cell loss and cellular edema bilaterally (Supplemental Table 2). None of the corneas recovered traditionally or in the RE-One chamber sustained technician-induced errors that affected corneal suitability or the ability to be transplanted.

Tissue Observations

In the traditional eye jar group, 100% of cases had retinal detachments, 60% retinal fold rate and 40% retinal tear rate (Table 3). No retinal detachments, tears or folds were observed in the RE-One groups. Iris collapse was

 ISSN 2161-5546

Supplemental Table 2. *Donor Demographics for Posterior Eye Cup Study Including Outcome of Corneas Simultaneously Procured for Transplant. A table describing donor demographics, procurement technique and outcomes of corneal tissue procured as part of the posterior eye cup study.*

Donor	Age	Gender	Diabetes	Device (OS/OD)	Cornea Outcome (OS/OD)
1	57	M	N	RE-One / Eye jar	Research* / Research*
2	60	M	N	RE-One / Eye jar	DMEK / DMEK
3	53	M	N	RE-One / Eye jar	Research** / Research**
4	62	M	N	RE-One / Eye jar	Research*** / PKP
†5	52	M	Y	RE-One / Eye jar	PKP / PKP

* Research: poor endothelial quality. **Research: medical history rule out. ***Research: infiltrate.

† Hispanic race. All other donors were of Caucasian race. **DMEK**: Descemet's membrane endothelial keratoplasty. **PKP**: Penetrating keratoplasty.

observed in each posterior eye cup in the eye jar group, while zero posterior eyes procured in the RE-One groups had iris collapse (Table 3 and Figure 5 A, B). We believe contour support for posterior eye cup provided by RE-One device was the main reason behind preventing retinal folds and detachments (Figure 5 D, F). On contrary, traditional eye-jar method lacks uniform contour support for posterior eye cup which may be resulting in retinal folds and detachments (Figure 5 C, E).

Table 3*. Posterior Eye Cup Descriptive Statistics for RE-One vs Traditional Procurement*

Variable, N (%)	RE-One (n = 5)	Traditional (n = 5)
Retinal fold	0 (0)	3 (60)
Retinal tear	0 (0)	2 (40)
Retinal detachment	0 (0)	5 (100)
Iris collapse	0 (0)	5 (100)

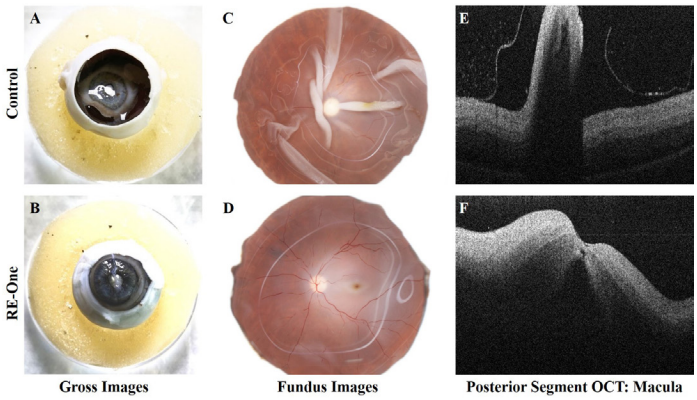

Figure 5*. Representative images of the posterior eyes from a single donor with the Control row being the eye procured in an eye jar, while the contralateral eye was procured with the RE-One. Gross images (A, B) were taken upon receipt of the tissue at the University of Michigan, followed by fundus images (C, D) and posterior segment OCT scans of the macula (E, F). There is significant iris collapse into the posterior chamber in the control (A) compared to the RE-One (B). The control eye had significant retinal folds with retinal detachments observed in the fundus image (C) and posterior segment OCT scan (E), respectively. The fundus image for RE-One has no retinal folds (D) or detachments (F) which is consistent with the posterior segment OCT scan.*

DISCUSSION

The results of the cornea study indicate that excisions using RE-One are very comparable to standard *in-situ* corneal excisions. Although the quality of the corneas procured were the same, the RE-One chamber alleviates the challenges of working around the donor's facial features with its ergonomic design and improves the consistency of the corneal tissue recovered. Once the cornea is excised, the posterior eye cup is left intact, safely supported in the RE-One chamber promoting high-quality retinal tissue for research.

A survey was sent to the technicians that participated in this study after all the corneas and posterior poles were procured. One technician remarked on ease of use, liking the fact that they did not have to work around the forehead while excising the cornea. The technicians consistently remarked on the ease of use for the RE-One but all were apprehensive to incorporate RE-One into use due to the additional time it demands for use. However, if required to recover the posterior eye cup and surgical corneas together, 100% of respondents reported they would rather use the RE-One platform than excising the corneas followed by removing the posterior eyes directly from the donor's eye sockets.

Cornea Study

For the cornea study, there were no statistically significant differences between excision techniques for circularity, ECD, percent endothelial cell loss and suitability. This is consistent with previous studies looking at differences in ocular procurement techniques.[3,4,13]

Circularity and size of scleral rims are important factors when procuring corneal tissue. A uniformed scleral rim is important to ensure proper fit into the corneal viewing chamber to prevent damage to the cornea, for eligibility to undergo advanced processing procedures like DSAEK and to be aesthetically appealing to transplant surgeons. Springs et al. reported a scleral rim size of less than 2mm during DSAEK preparation can cause the tissue to herniate during pressurization of the artificial anterior cham-

 ISSN 2161-5546

ber, resulting in an ovoid lenticule or, in extreme cases, a perforation.7 The size of the scleral rim was statistically significant, in favor of the RE-One chamber, although circularity measurements were similar. We attribute this to the imaging technique and natural horizontally oval shape of the cornea, yet the technique used for measuring circularity cannot be excluded.[14]

Clinically relevant but not statistically significant, the location of stress lines for phakic corneas excised in the RE-One were more peripheral than those removed by traditional *in-situ* techniques. This is clinically significant to eye banks, especially for technicians who routinely procure corneal tissue with diffuse or central stress lines. In general, the central 7.5 - 8mm area of the cornea is transplanted and ECD increases from center to periphery.[15,16] This means that the centermost portion of the cornea needs protection as much as possible from recovery-induced errors that damage the endothelium. We postulate that incorporating RE-One into clinical practice will help to eliminate technician-induced recovery errors resulting in the procurement of high-quality cornea tissue.

Pseudophakic donors had lower ECD's than phakic donors, which is consistent with past studies.[15,17] However, RE-One better preserved ECD for pseudophakic donors than normal *in-situ* corneal excision techniques. Although the beneficial effect of RE-One on ECD was evident only in pseudophakic donors, we found this to be particularly interesting and clinically relevant for the donor pool. Again, these results suggest that corneas excised in RE-One undergo less stress during the excision process compared to those removed with standard *in-situ* excision techniques. Furthermore, it promotes better corneal quality for corneas that have sustained prior trauma, such as wounds from cataract surgery.

Posterior Eye Cup Study

Our posterior eye cup study demonstrated that using RE-One can provide transplantable corneal tissue as well as high-quality retinal tissue. Implementation of the RE-One into standard eye banking practice could significantly increase the availability of retinal tissues for researchers. This is important as 88% of respondents to an Association for Research in Vision and Ophthalmology (ARVO) survey reported that they would use more human tissue if it was more readily available.[9] Those studying posterior ocular diseases such as age-related macular degeneration and diabetic retinopathy that are not exclusions for corneal transplantation would be particularly impacted. Increasing the availability and improving the quality of human retinal samples for research purposes can lead to scientific breakthroughs in these posterior ocular diseases that are increasingly affecting the world's population.[9,10,18]

In conclusion, RE-One may promote better quality corneal tissue for technicians that struggle with diffuse stress lines or have trouble working around the donor's facial features. RE-One can fully maximize the amount of corneal and retinal tissue available for transplant and research, without compromising quality.

REFERENCES

1. Lambert NG, Chamberlain WD. The structure and evolution of eye banking: a review on eye banks' historical, present, and future contribution to corneal transplantation. *J Biorepository Science for Applied Medicine.* 2017;5:18.
2. Gain P, Jullienne R, He Z, et al. Global Survey of Corneal Transplantation and Eye Banking. *JAMA Ophthalmol.* 2016;134(2):167-173.
3. Jhanji V, Tandon R, Sharma N, Titiyal JS, Satpathy G, Vajpayee RB. Whole globe enucleation versus in situ excision for donor corneal retrieval--a prospective comparative study. *Cornea.* 2008;27(10):1103-1108.
4. Filev F, Bigdon E, Steinhorst NA, et al. Donor Cornea Harvest Techniques: Comparison Between Globe Enucleation and In Situ Corneoscleral Disc Excision. *Cornea.* 2018;37(8):957-963.
5. Lane SS, Mizener MW, Dubbel PA, et al. Whole globe enucleation versus in situ corneal excision: a study of tissue trauma and contamination. *Cornea.* 1994;13(4):305-309.
6. EBAA. 2018 Eye Banking Statistical Report. 2018.
7. Springs CL, Joseph MA, Odom JV, Wiley LA. Predictability of donor lamellar graft diameter and thickness in an artificial anterior chamber system. *Cornea.* 2002;21(7):696-699.
8. Parekh M, Ferrari S, Di Iorio E, et al. A simplified technique for in situ excision of cornea and evisceration of retinal tissue from human ocular globe. *J Vis Exp.* 2012(64):e3765.
9. Stamer WD, Williams AM, Pflugfelder S, Coupland SE. Accessibility to and Quality of Human Eye Tissue for Research: A Cross-Sectional Survey of ARVO Members. *Invest Ophthalmol Vis Sci.* 2018;59(12):4783-4792.
10. Corcoran KP. Accessibility to and Quality of Human Eye Tissue for Research: A Cross-Sectional Survey of ARVO Members - EBAA Commentary. *Invest Ophthalmol Vis Sci.* 2018;59(12):4793-4795.
11. Fields T, Jaber R, James LK, Jones K, McCoy K, Secic M. Suitability of Corneal Tissue Related to Donor Ophthalmic History. *Invest Ophthalmol Vis Sci.* 2019;60(9).
12. Jardine GJ, Holiman JD, Stoeger CG, Chamberlain WD. Imaging and quantification of endothelial cell loss in eye bank prepared DMEK grafts using trainable segmentation software. *Curr Eye Res.* 2014;39(9):894-901.
13. Rootman DB, Wankiewicz E, Sharpen L, Baxter SA. In situ versus whole-globe harvesting of corneal tissue from remote donor sites: effects on initial tissue quality. *Cornea.* 2007;26(3):270-273.
14. Sridhar MS. Anatomy of cornea and ocular surface. *Indian J Ophthalmol.* 2018;66(2):190-194.
15. Al-Mohaimeed MM. Penetrating keratoplasty for keratoconus: visual and graft survival outcomes. *Int J Health Sci (Qassim).* 2013;7(1):67-74.
16. Lee BA, Qureshi S, Lee S, Hou GJ, Bedard P, Hou JH. Peripheral Endothelial Cell Density in Descemet Membrane Endothelial Keratoplasty Grafts. *Cornea.* 2019;38(6):748-753.
17. Singh SG, Satani DR, Patel AP, Doshi DC. Evaluation of quality and utility rate for donor corneal tissue received at tertiary eye care center. *J Clin Ophthalmol Res.* 2017;5(3):4.
18. Luznik Z, Parekh M, Bertolin M, Griffoni C, Ponzin D, Ferrari S. Biobanking of Human Retinas: The Next Big Leap for Eye Banks? *Stem Cells Transl Med.* 2015;4(8):868-872.

 ISSN 2161-5546