

ORIGINAL ARTICLE

Melville M. Martires, MD
 Santiago Antonio B. Sibayan, MD, PhD
 Ma. Elizabeth T. Concepcion, MD
 Ma. Dominga B. Padilla, MD

*Department of Ophthalmology
 Carlos L. Sevilla Eye Center
 Makati Medical Center
 Makati City, Philippines*

Serial endothelial-cell-density and corneal-thickness measurements in corneas preserved in Optisol GS

ABSTRACT

Objective

This study determined the daily rate of change of endothelial-cell density (ECD) and corneal thickness (CT) in donor eyes stored in Optisol GS corneal-storage medium. Correlation between ECD and CT was also determined.

Methods

Twenty-eight corneas from 15 donors (mean age, 38 ± 13.78 years; range, 4 to 78 years) were harvested and preserved in Optisol GS. The corneas were serially examined for ECD and CT using a Konan keratoanalyzer. Readings were performed upon harvest and then daily on the first to fourth postharvest days. Statistical analyses included student's t-test and Pearson's correlation.

Results

There were no statistically significant changes in ECD compared with baseline (all p values > 0.05) up to the fourth postharvest day. There were statistically significant decreases in CT in all readings compared with baseline (all p values < 0.05). There was a weak negative relationship between CT and ECD ($r = -0.15$).

Conclusions

ECD appeared to be stable in donor corneas stored for up to 4 days in Optisol GS. The corneas also became significantly thinner. Definite correlation between CT and ECD could not be established.

Keywords: *Optisol GS, Corneal endothelial-cell density, Corneal thickness*

Correspondence to

Santiago Antonio B. Sibayan, MD, PhD
 Suite 326
 Makati Medical Center
 2 Amorsolo St.
 1229 Makati City, Philippines
 Telephone : +63-2-8151316
 Fax : +63-2-8928686
 E-mail : santiagosibayan@yahoo.com

No financial assistance was received for this study.

The authors have no proprietary or financial interest in any product used or cited in this study.

MILLIONS of people worldwide suffer from opacities and deformities of the cornea, resulting in deficient light transmission and blindness. Penetrating keratoplasty (PKP) is an operation that has enabled cornea-blind individuals to improve or regain their vision. In this procedure, diseased corneal tissue is removed and replaced by cadaveric donor corneal material. These donor corneas are harvested from the eyes of recently deceased individuals and transferred to nutrient solution until transplanted to a recipient.¹

Endothelial cells line the inner surface of the cornea and are responsible for corneal nutrition and clarity. These cells do not divide and have a finite population. As a general rule, the lower the endothelial-cell density (ECD), the less viable is the cornea. ECD is, therefore, used as an indicator of corneal viability.^{2,3}

Although there is no established relationship between corneal thickness (CT) and graft survival, minimization of corneal swelling following preservation is ideal. This allows for better immediate postoperative visual results. Optisol GS (Bausch & Lomb, Irvine, CA, USA) is a corneal storage medium supplemented with dehydrating agents (dextran and chondroitin sulfate) that lessen swelling and maintain corneal clarity.^{4,5}

Corneas remain viable in a storage medium for a finite period due to endothelial-cell loss over time. A recent literature search showed no available studies on the daily rate of ECD loss of donor corneas. Such data would be helpful in prognosticating the outcomes of those who underwent PKP.^{5,6} Thus, this study determined the daily ECD change in Filipino donor corneas stored in Optisol GS. The change in CT was also evaluated.

METHODS

This is a prospective cohort study. A total of 28 donor corneas from 15 donors (mean age, 38 ± 13.78 years; range, 4 to 78 years) were evaluated at the Santa Lucia International Eye Bank of Manila. All donor tissues met the inclusion and exclusion criteria for human corneal transplantation (Table 1). After obtaining consent from the donor's family, corneal tissue was retrieved by trained eye-bank personnel either by whole-globe enucleation or by *in situ* removal. Corneoscleral rims were then transferred to Optisol GS.

Specular microscopy and pachymetry of the central corneal endothelium was performed daily in triplicate from preservation time to day 4 using a Konan Kerato-analyzer (Konan Medical, Hyogo, Japan). These readings were then averaged and subjected to statistical analysis. All measurements were performed by a single examiner (MMM). As corneas are generally shipped for use by the fifth day, no further readings were performed.

Statistical analysis of ECD and CT was performed using

student's t-test. *P* values ≤ 0.05 were considered statistically significant. The relationship between CT and ECD was determined using Pearson's correlation coefficient (*r*).

Table 1. Inclusion and exclusion criteria for obtaining donor corneal tissues.

| Inclusion Criteria |
|---|
| Corneas of deceased individuals of any age with a known cause of death. Proper consent from the donor and/or relatives must have been obtained. Corneas must have been harvested within 12 hours from time of death. Corneas must be undamaged or minimally damaged. Eyes with no known ocular surgery. |
| Exclusion Criteria |
| CMV infection Congenital rubella Conjunctivitis Creutzfeld-Jacob disease Death from CNS disease of unknown etiology Death of unknown cause Dementia Encephalitis Hepatitis (active) High risk for blood-borne diseases HIV infection Hodgkin's disease Leukemia Lymphosarcoma Multifocal leukoencephalopathy Pneumonia Post-refractive surgery (e.g., LASIK, EPILASIK) and/or intraocular surgery Pulmonary TB (active) Rabies Reye's syndrome Septicemia Subacute sclerosing panencephalitis Syphilis (active) |

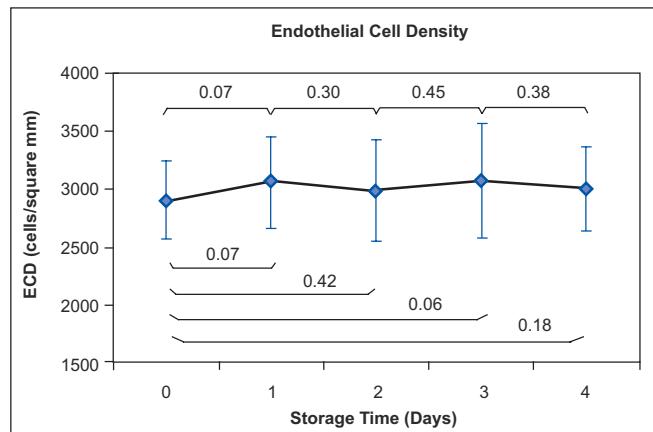


Figure 1. Line graph representing endothelial cell density (ECD) at various periods following corneal harvest. There were no statistically significant changes in ECD compared to baseline (*p* values indicated below line) or to the previous day's readings (*p* values indicated above line). Error bars = 1 standard error of the mean.

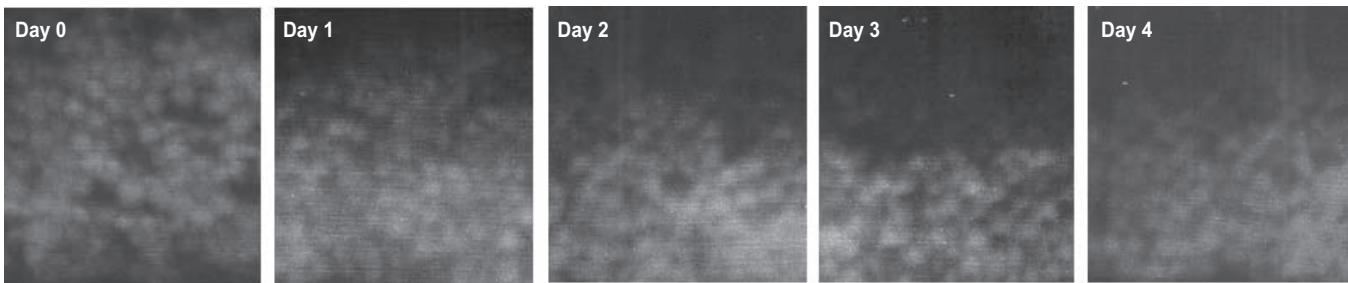


Figure 2. Serial photographs showing the absence of endothelial-cell-density changes over time.

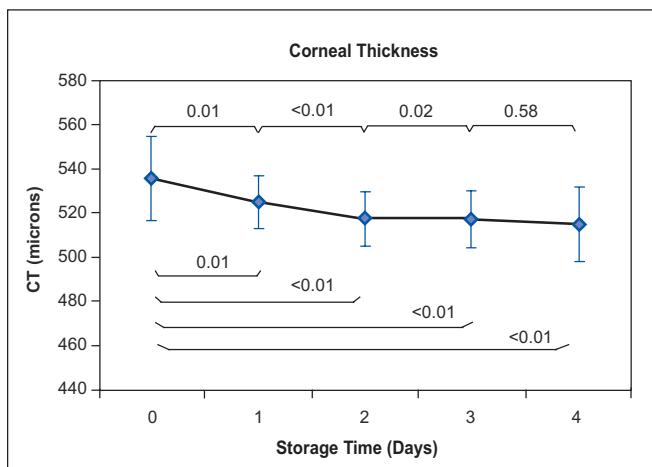


Figure 3. Line graph representing corneal thickness (CT) at various periods following corneal harvest. There were statistically significant decreases in CT compared to baseline on days 1 to 4 (p values indicated below line). When compared to the previous day's readings, there were statistically significant decreases in CT on days 1 and 2. No further changes were observed on days 3 and 4 (p values indicated above line). Error bars = 1 standard error of the mean.

RESULTS

Endothelial-Cell Density and Storage Time

There were no statistically significant changes in ECD compared with baseline or the previous day's readings (all p values > 0.05) (Figure 1). Serial photographs illustrate the absence of ECD changes over time (Figure 2).

Corneal Thickness and Storage Time

There were statistically significant decreases in CT compared with baseline on days 1 to 4 (all p values < 0.05).

Compared to the previous day's readings, there were statistically significant decreases in CT on days 1 and 2 (all p values ≤ 0.01). No further changes were observed on days 3 and 4 (all p values > 0.05) (Figure 3).

Corneal Thickness and Endothelial Cell Density

Pearson's correlation coefficient (r) measured -0.15 , indicating a weak negative relationship between CT and ECD (Figure 4).

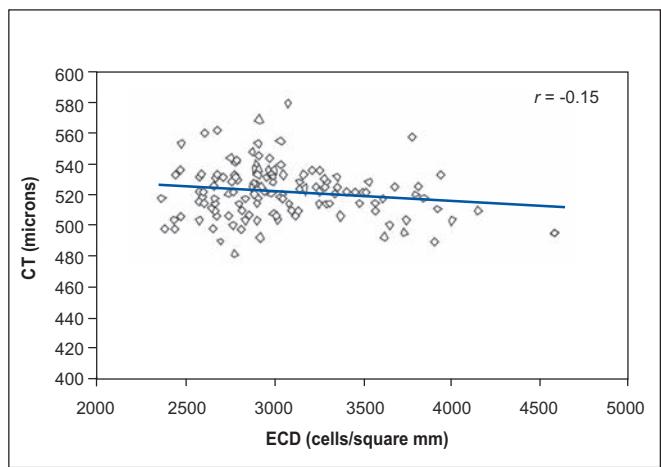


Figure 4. Scatter plot correlating CT with ECD.

DISCUSSION

For donor corneas to remain viable after harvest, they must be preserved in a nutrient medium such as Optisol GS. ECD determination using specular microscopic analysis serves as a measurement of the functional status of the endothelial cells and can serve as a measure of corneal button viability. A critical cell density is necessary to maintain corneal qualities necessary for successful transplantation.⁷⁻⁸

Our study showed that ECD remains unchanged following preservation in Optisol GS at least up to 4 days following harvest. This suggests that the corneal endothelium is adequately preserved in Optisol GS and that the graft may remain suitable for transplantation for at least this length of time.

CT was noted to decrease significantly over time. This may be attributed to dehydrating agents (dextran and chondroitin sulfate) present in the medium. While there is no established relationship between CT and graft survival, minimization of corneal swelling following preservation is ideal, as this allows better postoperative visual results.⁹⁻¹¹

Based on the correlation analysis, no definite relationship

could be established between ECD and CT. This implies that ECD and CT are independent of each other.

In conclusion, over a period of 4 days of storage in Optisol GS, ECD remained stable and CT decreased. These findings suggest that corneas preserved in Optisol GS remain viable and do not deteriorate for at least this length of time.

References

1. Thuret G, Chiquet C, Bernal F, et al. Prospective, randomized clinical and endothelial evaluation of 2 storage times for corneal donor tissue in organ culture at 31 degrees centigrade. *Arch Ophthalmol* 2003; 121: 442-450.
2. Amman J, Holley GP, Lee SB, et al. Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. *Am J Ophthalmol* 2003; 135: 584-590.
3. Ing JJ, Ing HH, Nelson LR, et al. Ten year prospective results of penetrating keratoplasty. *Ophthalmology* 1998; 105: 1855-1865.
4. Sperling S. Endothelial cell density in donor corneas. *Arch Ophthalmol* 1980; 58: 278-282.
5. Bourne WM, Nelson LR, Maguire LJ, et al. Comparison of Chen medium and Optisol GS for human corneal preservation at 4 degrees centigrade. *Cornea* 2001; 20: 683-686.
6. Mannis MJ, Holland EJ, Beck RW, et al. Clinical profile and early surgical complications in the cornea donor study. *Cornea* 2006; 25: 164-170.
7. Naor J, Slomovic AR, Chipman M, et al. A randomized, double masked clinical trial of Optisol GS vs. Chen Medium for human corneal storage. *Arch Ophthalmol* 2002; 120: 1280-1285.
8. Benetz BA, Gal RL, Ruedy KJ, et al. Specular microscopy ancillary study methods for donor endothelial cell density determination of cornea donor study images. *Curr Eye Res* 2006; 31: 319-327.
9. Lass JH, Gal RL, Ruedy KJ, et al. An evaluation of image quality and accuracy of eye bank measurement of donor cornea endothelial cell density in the specular microscopy ancillary study. *Ophthalmology* 2005; 112: 431-440.
10. Amann J, Holley GP, Lee SB, et al. Increased endothelial cell density in the paracentral and peripheral lesions of the human cornea. *Am J Ophthalmol* 2003; 135: 584-590.
11. Saggau DD, Bourne WM. A comparison of two preservation media (CSM and K-Sol) by scanning electron microscopy of preserved corneal endothelium. *Arch Ophthalmol* 1989; 107: 429-432.