Endothelial Cell Viability of Donor Corneas Preserved in Eusol-C Corneal Storage Medium

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Abstract

Objectives: To evaluate the efficacy of Eusol-C as a corneal storage medium on the survival of donor endothelium.

Materials and Methods: Twenty-seven corneas not suitable for transplant were included in this study. All donor corneas were stored in Eusol-C at 4°C. Daily donor corneal endothelial cell counting was performed with an eye bank specular microscope. All corneas were discarded after the study process.

Results: Mean donor age was 51.3 ± 18.8 years (range, 25-94 y). The mean duration between death and corneal excision was 9.5 ± 6.7 hours (range, 3-23 h). Mean endothelial cell density was 2195 ± 383 cells/mm² at the beginning of the preservation (range, 1361-2899 cells/mm²). Donor endothelial cell density was between 1500 to 2000 cells/mm² in 9 corneas, 2000 to 2500 in 11 corneas, 2500 to 3000 in 5, and higher than 3000 in 2 corneas at baseline. Mean endothelial cell density was found 1658 cells/mm² on the eighth day of storage, with a mean endothelial cell loss rate of 24.5%. Corneas stored 9 to 24 days in Eusol-C had a rate of endothelial cell damage of 3.1% per day.

Conclusions: Although our results revealed a higher endothelial cell loss than previous reports, overall performance of Eusol-C in preserving the donor endothelium may be satisfactory for clinical use.

Key words: Eusol-C, Corneal storage medium, Endothelial cell density

Introduction

The period of preservation has been classified as short, intermediate, long, and very long-term storage in terms of the duration of storage of the donor cornea. Donor corneas can be stored at 4°C for short-intermediate term and at 30.5°C to 37°C for organ culture in the long-term. To prolong donor corneal survival and improve postoperative outcome, cold storage at 4°C and organ culture at 32°C are the most common methods. Because of the inadequacy of the moist chamber and the M-K medium methods, intermediate-term corneal storage medium has been a good alternative for a better maintenance of the donor cornea for longer periods. Storage of donor corneas for a longer time allows flexibility for surgical planning and provides time to evaluate the donor material, blood testing, and transportation. The addition of chondroitin sulfate has been a key development in the genesis of intermediate-term corneal storage media.

This study was conducted to evaluate the effect of Eusol-C as a corneal storage medium on the survival of the donor endothelium, not suitable for transplant were used for study purposes.

Materials and Methods

Twenty-seven corneas (7 unilateral, 10 bilateral excision) recovered from 17 cadavers at Izmir Bozyaka Education and Research Hospital Eye Bank between 2011 and 2013 were included in this study. The duration between death and corneal excision was noted. This study was performed on the donor corneas not suitable for transplant. So that an ethical committee approval was not taken. However, the study was conducted under the tenets of Helsinki Deceleration and donor anonymity was secured.

Donor corneas were stored in Eusol-C (Corneal Chamber, Alchimia, Ponte San Nicolò, Italy) at 4°C. Eusol-C includes dextran as osmotic agent, sodium pyruvate and glucose as energetic sources, amino acids, mineral salts and vitamins as
nutrients, gentamicin as antibiotic, Hepes and bicarbonate as buffers, and phenol red as pH indicator. Hydrogen ionic activity (pH) is 7.40 (range, 7.20-7.60) and osmolality (mOsm/kg) is 300 (range, 255-345 mOsm/kg). Daily endothelial cell counting of the donor corneas were performed by a trained eye bank technician with an eye bank specular microscope (Konan Eye bank Keratoanalyzer EKA-04, Konan Medical Inc., Hyogo, Japan). Donor corneas were left in room temperature at least 1 hour before cell counting, and turned upside down just before the tissue evaluation under specular microscope. Cell counting was repeated until the corneal haziness rendered it impossible to view the details of the donor endothelium. All the corneas were destroyed after the completion of study process. Reasons to eliminate these corneas were seropositivity for hepatitis B virus in 12 corneas, epithelial defect in 5 corneas, human immunodeficiency virus in 2 corneas, sepsis in 6 corneas, low endothelial cell density in 1, and stromal opacity in 1 cornea.

Statistical analyses
Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 21.0, IBM Corporation, Armonk, NY, USA). Repeated measures analysis of variance tests and paired *t* tests were used for statistical analyses and a *P* value < .05 was considered as statistically significant.

Results
The mean donor age was 51.3 ± 18.8 years (range, 25-94 y). The cause of death was cardiac arrest in 11, and intracranial hemorrhage in 4. The mean duration between death and corneal excision was 9.5 ± 6.7 hours (range, 3-23 h). Mean endothelial cell density (ECD) was 2195 ± 383 cells/mm² (range, 1361-2899 cells/mm²) immediately after excision. Donor ECD was between 1500 to 2000 cells/mm² in 9 corneas, 2000 to 2500 in 11 corneas, 2500 to 3000 in 5, and higher than 3000 in 2 corneas at the beginning of preservation.

Five of the corneas became uncountable at the ninth day, 5 of them at tenth day, 5 at thirteenth day, 2 at fourteenth day, 2 at fifteenth day, 2 at sixteenth day, 2 at eighteenth day, and 2 at the twentieth day. Nineteen corneas became uncountable within 15 days had an ECD under 2500 cells/mm² at the beginning. Two corneas with an ECD over 3000 cells/mm² at the beginning stayed clear enough for cell counting until the 24th day of preservation. Corneas stored 9 to 24 days in Eusol-C at 4°C showed a rate of endothelial cell damage of 3.1% per day. The loss of endothelial viability rate was 24.5% after 8 days of preservation. A 50% loss of endothelial viability occurred after mean 14.2 ± 3.5 days of preservation (range, 8-22 d). Daily endothelial cell countings were compared with the baseline values by using repeated measures analysis of variance test. The decrease in ECDs of the donor corneas were statistically significant starting from the first day of the preservation (*P* < .000). When daily ECDs were compared consecutively; the decrease in ECD was found to be statistically significant, except comparison of the 4th and 5th days (Table 1).

The decrease in mean ECD of all corneas during storage may be seen in the Figure 1. Mean ECD was still over 1500 cells/mm² on the eighth day of the preservation. Daily endothelial loss rate was calculated as 3.1%.

Discussion
The sudden arrest of the aqueous humor formation after death, and the depletion of nutrients and oxygen supply to the eye for variable lengths of time,
especially at room temperature, result in the initial damage of the corneal cells by autolysis. The time during which the cadaver is exposed to the room temperature should, therefore, be as short as possible. For successful corneal transplant, minimum 50% of endothelial cell function is crucial. Donor corneas with a storage time longer than 10 days are usually excluded from transplant. In our study, mean ECD was found approximately 1658 cells/mm² on the eighth day of storage with a mean endothelial cell loss rate of 24.5%. Thus, these corneas may still be considered suitable for transplantation.

Historically, Mizukawa and Manabe reported successful preservation of whole donor globes immersed in a solution of chondroitin sulfate at 4°C for up to 3 days. The exact mechanism by which chondroitin sulfate protects the donor cornea has not yet been established. It probably acts as an antioxidant and free-radical scavenger to protect cell membranes. It also may act as a cation-exchange resin regulating cation fluxes across cell membranes through the formation of chelation complexes. Several corneal storage media containing chondroitin sulfate have been developed for clinical use in the United States and Europe. They are K-Sol (Cilco, Huntington, West Virginia), Chondroitin Sulfate Storage Medium, Dexsol, Optisol (Chiron Ophthalmics Inc. Irvine, California), and Likorol (Opsia Pharma, France). However, K-Sol and Chondroitin Sulfate Storage Medium are no longer commercially available. It has been demonstrated that Optisol as compared with Dexsol, K-Sol, and MK medium, can preserve the corneal endothelium better than yielding a thinner cornea up to 2 weeks. A thinner cornea permits a better evaluation and easier manipulation of the donor tissue at the time of the surgical procedure and also helps an earlier visual rehabilitation. Nelson and associates compared Chen medium with Optisol-GS in their study. Although corneas stored in Chen medium were found thicker during storage than those stored in Optisol-GS medium, they reported no difference between 2 media regarding endothelial cell loss or keratocyte density during storage.

Although initially successful, using glycerol as a corneal preservative was largely supplanted by the introduction and availability of intermediate-term storage media (eg, Optisol GS and Eusol-C) for tissues destined for optical keratoplasties. These storage media have the clear advantage of supporting viable cells, including endothelium, epithelium and kerocytes. Eusol-C is a widely used European media for cold corneal storage. There are few studies concerning efficacy of Eusol-C as a corneal storage medium. In their study comparing Optisol-GS and Eusol-C, Chaibpracob and associates reported similar corneal ECD and corneal thickness results with Optisol-preserved and Eusol-preserved corneas after transplant. In a recent study, Kanavi and associates also found no significant difference in the endothelial loss rate between Optisol-GS and Eusol-C during 7 days of preservation. In our study, Eusol-C maintained donor corneal viability for at least 8 days. However, the loss of clarity on specular microscopy was encountered in some of the corneas after 8 days of storage. We found that the donor corneas with higher ECD keep clear throughout the preservation period, those corneas preserve viable ECD of 2000 cells/mm² up to 9 days, and they are still clear enough for cell counting until the 24th day. This finding indicates that the donor corneas with higher ECD at the beginning stay suitable for transplant for longer periods in Eusol-C. Means and associates reported a 50% loss of endothelial viability in moist chamber-stored corneas after 2 days and by 35 days in corneas stored in Optisol-GS. In our study, the loss of endothelial viability was 24.5% after 8 days of preservation. Fifty percent loss of endothelial viability occurred after mean of 14.2 days. This rate seems higher than their report. However, they used different methods to evaluate the donor endothelium including endothelial staining and electron microscopy. Thus, it is not reasonable to directly compare the results. We used an eye bank specular microscope allowing endothelial cell counting through the bottom of the glass vial of Eusol-C. This method is easy to use and requires less tissue manipulation. In our study, corneas stored 9 to 24 days in Eusol-C had a rate of endothelial cell damage of 3.1% per day. This rate is also higher than previous reports of approximate 0.6% daily loss rate with Optisol-GS. Eusol-C does not contain chondroitin sulfate. This may be the cause of the higher endothelial loss rates in our study. Tissue evaluation methods employed in different studies may be another explanation.

Our results suggest that, Eusol-C keeps endothelium viable for at least 8 days which may be considered sufficient for clinical use; however, it
would be preferable to avoid longer term preservation before surgery. Comparative studies with Optisol-GS and other medias may provide more information about endothelial cell survival in this relatively new corneal storage medium.

References