

Corneal Temperature Reversal After Storage in Chen Medium Compared With Optisol GS

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Purpose. To compare corneal endothelial cell function by measuring corneal thickness during temperature reversal between corneas stored in two different storage media, Optisol GS and Chen Medium (CM). **Methods.** Twenty paired corneas from 10 human donors were randomly assigned for storage at 4°C in Optisol GS (10 corneas) or CM (10 corneas). The storage media were masked, and measurements were done in a masked fashion. After storage for 48 hours, corneal thickness was measured by ultrasonic pachymetry at 2-hour intervals for 12 hours, during which time the corneas were perfused with BSS (balanced salt solution) Plus at 37°C. Scanning electron microscopy of two pairs of corneas from two donors was performed to assess ultrastructural change after 12 hours of warming. **Results.** Corneal thickness decreased during the first 4 hours of the warming period and then increased during the 6- to 12-hour warming period. These changes in corneal thickness over time were similar for the two storage media ($p = 0.212$). Scanning electron microscopy showed greater amounts of endothelial cell disruption in Optisol GS-stored corneas than those stored in CM after 12 hours of warming and perfusion. **Conclusions.** The endothelial pump of corneas stored in CM appear to be as well-preserved as those stored in Optisol GS, although greater endothelial disruption may be present with Optisol GS by scanning electron microscopy. Further studies are required to compare the clinical effectiveness of these two media.

Key Words: Cornea—Endothelium—Storage media—Optisol—Chen Medium.

Since the introduction of the first 4°C corneal storage medium, McCarey–Kaufman medium, a number of intermediate-term storage media (K-Sol, Dexsol, Optisol [Bausch and Lomb Inc., Irvine, CA, U.S.A.]) have been developed for clinical use.^{1–4} The addition of dextran and chondroitin sulfate have increased the duration of storage and the quality of donor tissue^{5–7}; however, endothelial cell loss continues to occur during cold storage. Corneal storage media (CSM), K-Sol, and Dexsol (no longer commercially avail-

able) were used for some time in eye banks. Optisol is now widely used in U.S. eye banks.

Chen Medium (CM; Chen Laboratories, Baltimore, MD, U.S.A.) is a new corneal storage medium that differs from Optisol GS in some components (Table 1), one of which is the addition of β -hydroxybutyrate. Previous studies have shown that the accumulation of metabolic wastes and the inactivation of metabolic enzymes may be responsible for the decreased metabolic activity of corneas stored in commercial eye bank media.^{8–10} Chen et al.^{11,12} demonstrated that corneas stored in media enriched with a non-lactate-generating, high-energy substrate (β -hydroxybutyrate) maintained high levels of adenosine triphosphate with less lactate formation and accumulation. β -hydroxybutyrate is a ketone body, which is the preferred fuel for the brain, kidneys, and muscles when glucose is inadequate.^{13,14}

To further compare the efficacy of CM and Optisol GS, a temperature-reversal experiment was carried out to assess corneal endothelial function after storage in the two media by measuring the change in corneal thickness during a 12-hour warming period.

METHODS

Human donor corneas were obtained from the Ontario division of the Eye Bank of Canada between July and August of 1999. Medical histories were reviewed, and eyes not suitable for corneal transplantation were used in this study. Eyes that were excluded from the study were from donors more than 85 years of age, those who had enucleation done more than 8 hours after death and preserved for more than 36 hours after enucleation, and those with a previous history of intraocular surgeries or intraocular lens implant.

After sterilization for 10 minutes in povidone iodine (0.5%), the corneoscleral rim was excised by means of standard eye bank procedures. The corneas were placed in 20 mL of Optisol GS or 20 mL of CM. For each pair of corneas, one cornea was randomly assigned to be stored in Optisol GS and the other in CM. All measurements were done by a single observer (C.Y.), without knowledge of the storage medium.

After storage for 48 hours, the corneas were removed from their media and mounted in a multicorneal perfusion chamber (Medical Engineering, University of Toronto, Ontario, Canada). The epithelium was left intact and covered with Balanced Salt Solution Plus (BSS Plus; Alcon, Fort Worth, TX, U.S.A.) throughout the experiment to prevent evaporation. The temperature of the perfusion medium was maintained at 37°C by means of a constant-

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TABLE 1. Components of Optisol GS and CM

Components	Optisol GS	CM
Base medium	Hybrid of TC-199 and MEM	Modified medium 199
Dextran	Yes	Yes
Chondroitin sulfate	Yes	No
β -hydroxybutyrate	No	Yes
HEPES buffer	Yes	Yes
Gentamicin	Yes	Yes
Streptomycin sulfate	Yes	Yes
Sodium carbonate	Yes	No
Nonessential amino acids	Yes	No
Vitamins	Yes	Yes

MEM indicates minimal essential media.

temperature water bath, and the pressure was set at 15 mm Hg. A perfusate of BSS Plus was delivered at 30 μ L/min by a constant-rate infusion pump. Thickness measurements were obtained at 2-hour intervals during a 12-hour period, starting immediately after mounting.

In spite of other studies of a shorter perfusion time,¹⁵⁻¹⁷ the decision for this study was based on a previous study that compared corneal thickness during perfusion with BSS of Optisol-stored and H-sol-stored corneas carried out at the University of Toronto in 1996 (Wong AM, et al. unpublished data), which showed continual thickening for the entire 6 hours of the study. It was hypothesized that a longer rewarming time would be required to observe deswelling after the initial swelling period. Thus, a 12-hour rewarming time was chosen for this experiment.

Corneal thickness was measured by ultrasonic pachymetry (Teknar Ultrasonic A-Scan/Pachymeter; Teknar, St. Louis, MO, U.S.A.). The mean corneal thickness was determined from three central readings at each time point. After 48 hours of storage and 12 hours of rewarming, ultrastructural changes in the endothelium of the two randomly selected pairs of corneas from two donors were assessed by fixation in 2% glutaraldehyde and 1% paraformaldehyde for examination by scanning electron microscopy.

RESULTS

Twenty paired corneas from 10 donors were studied. The mean donor age was 76.3 years (SD = 5.1 years). The mean time from death to enucleation was 2.92 hours (SD = 2.3 hours), and the mean time from enucleation to preservation in medium was 14.3 hours (SD = 2.4 hours).

Figure 1 graphically illustrates the changes in corneal thickness over time according to the type of medium used for storage. The corresponding pachymetry values and their associated standard errors are given in Table 2. The trend was similar for corneas stored in CM and in Optisol GS; corneal thickness decreased during the first 4 hours of the warming period and then increased during the 6- to 12-hour warming period. Because corneal thickness was measured repeatedly, the effect of the two media on corneal thickness needed to be compared across all levels of time. This was accomplished with a two-way repeated measures analysis of variance, which can be thought of as comparing the two curves in Figure 1. The difference in the mean values of thickness among the corneas stored in Optisol GS (652.1 μ m) and CM (644.2 μ m) was 7.9 ± 11.0 (CI = -13.7 to 29.5). This difference was not statistically significant ($p = 0.212$). Thus, there was no difference

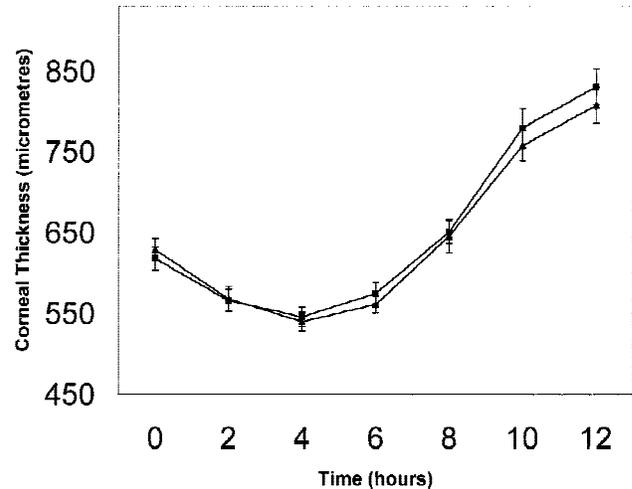


FIG. 1. Change in corneal thickness at 2-hour intervals during a warming period of 12 hours, with standard error bars.

between the two storage media with respect to changes in corneal thickness during the rewarming period.

Scanning electron microscopy of two pairs of corneas from two donors, at completion of the 12-hour warming period, showed greater amounts of endothelial disruption for Optisol GS-stored corneas than corneas stored in CM (Fig. 2).

DISCUSSION

It has been shown that the ability of tissue to generate ATP and clear metabolic wastes after donor death is markedly reduced.^{11,18} Physiologic processes that are energy-dependent (such as corneal fluid pump activity) may be subsequently compromised,¹⁹⁻²³ resulting in endothelial swelling and degeneration. Studies by Chen et al.¹¹ found that β -hydroxybutyrate stimulates corneal endothelial cell proliferation, increases adenosine triphosphate levels, and decreases lactate production in human and rabbit corneas. Using a rabbit cornea transplantation model, corneas stored in β -hydroxybutyrate-enriched isotonic solution at 4°C for 6, 7, and 11 days deturgescd at higher rates than those stored in Optisol under the same conditions.¹²

In that study, temperature reversal was performed on 20 corneas from 10 human donors in vitro over a 12-hour rewarming period

TABLE 2. Corneal thickness values: CM and Optisol GS

Time (h)	CM average thickness, μ m (SE)	Optisol GS average thickness, μ m (SE)
0	628.7 (13.7)	617.6 (13.7)
2	567.6 (15.4)	565.5 (14.0)
4	539.6 (11.5)	546.0 (12.0)
6	561.2 (9.9)	574.6 (13.5)
8	645.8 (20.6)	650.5 (14.2)
10	757.9 (19.4)	779.9 (24.1)
12	808.4 (22.4)	830.7 (21.9)
Average	644.2 (40.0)	652.1 (42.1)
Mean difference (μ m)		7.9 (11.0)
CI for mean difference (μ m)		-13.7 to 29.5
Minimal detectable difference (μ m)		
($\alpha = 0.05$, power = 80%)		35

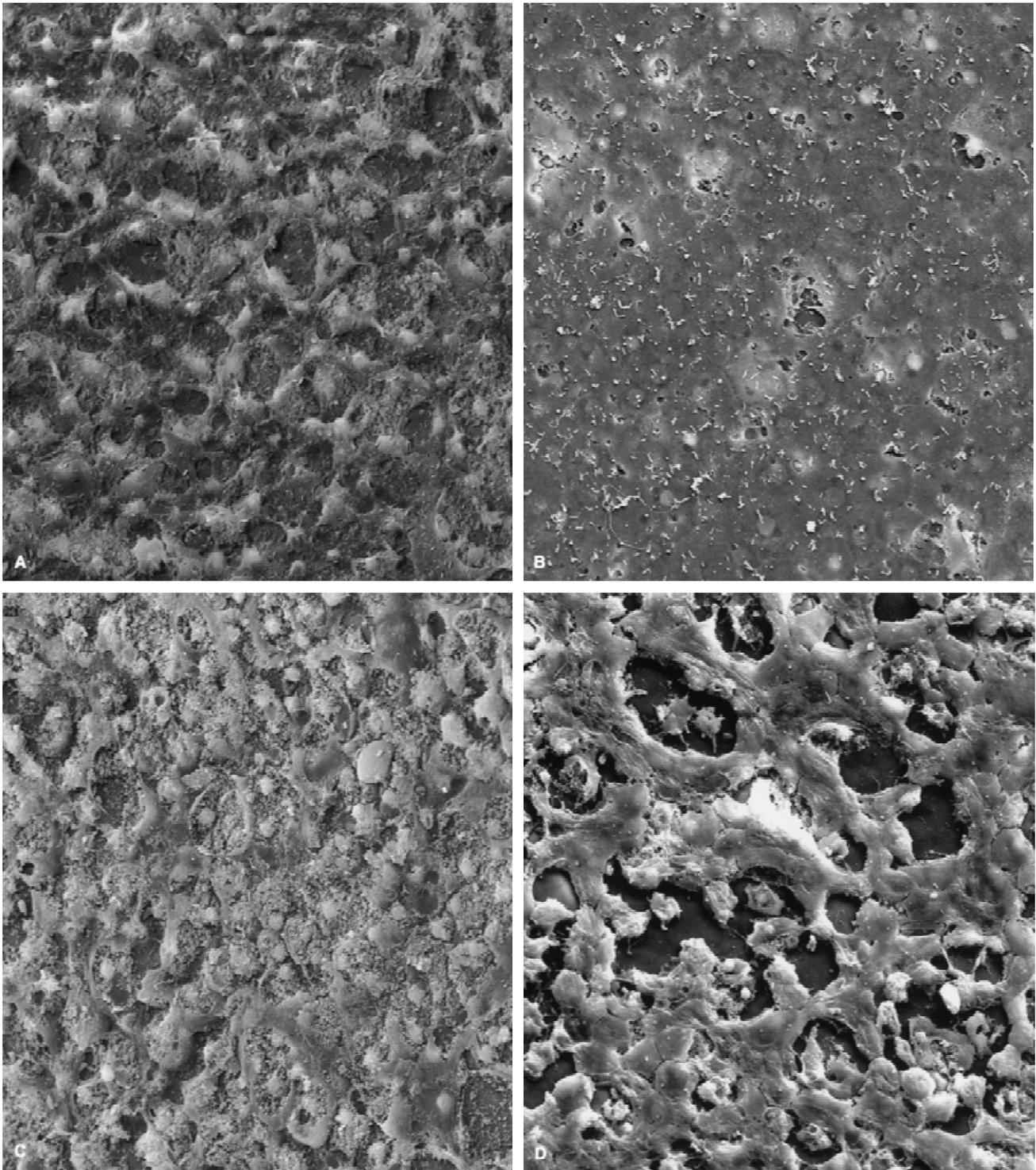


FIG. 2. Scanning electron micrographs under low magnification of corneas after 2 days of storage and warming for 12 hours. **A:** Corneas from donor 1 stored in Optisol GS. **B:** Corneas from donor 1 stored in CM. **C:** Corneas from donor 2 stored in Optisol GS. **D:** Corneas from donor 2 stored in CM. Notice the disruption in cell membrane morphology with vesicular outpouchings seen and loss of cellular contiguity for Optisol GS-stored corneas. For CM-stored corneas there is less pronounced disruption of cell morphology with preservation of intracellular connections and a smooth appearance to the cell membranes and preserved villous projections. In both media, there is loss of cells with exposure of bare Descemet's membrane.

after storage in either CM or Optisol GS at 4°C for 48 hours. Our study found no difference between corneas stored in the two storage media with respect to changes in corneal thickness during the rewarming period. The minimal detectable difference between the mean corneal thickness over the 12-hour period for this study was 35 μm ($\alpha = 0.05$, power = 80%).

The duration of corneal preservation in storage media may have an effect on corneal deturgescence rates. Walkenbach et al.¹⁵ showed that longer corneal storage times resulted in substantial differences in several commercial storage media's abilities to undergo deturgescence during warming. In that study, there were no significant differences in thickness between CM-stored and Optisol GS-stored corneas. All corneas were examined after 48 hours of media preservation. A longer preservation time may be necessary to unmask the differences in deturgescence abilities between corneas stored in the two media.

Other corneal studies using BSS Plus as the perfusate looked at corneal thickness during a shorter period of warming and perfusion (i.e., up to 4 hours).¹⁵⁻¹⁷ In these studies, similar to the first 4 hours of the current study, corneal thickness deswelled immediately for the duration of the warming period. However, in the current study, further observation beyond 4 hours of perfusion in BSS Plus demonstrated an increase in corneal thickness. The loss of epithelial function as a barrier to fluid influx may have contributed to corneal swelling. This can be secondary to poor epithelial preservation in either storage medium, as well as the loss of epithelial integrity from mechanical trauma resulting from handling of the specimen and measuring corneal thickness.

McDermott et al.¹⁶ hypothesized that BSS Plus maintained the endothelial barrier function and Na-K ATPase pump better because it provided the necessary substrates (glucose, glutathione, bicarbonate-buffer) to the endothelial metabolic pump. However, prolonged perfusion in BSS Plus may still cause losses in endothelial cell viability. Kline et al.²⁴ found endothelial cell loss (15.4%) when BSS Plus was used as the irrigant during extracapsular cataract extraction and posterior chamber lens implantation. A cornea to be transplanted would be exposed to BSS Plus for up to 4 hours, which is the time it takes for the aqueous humor to replace the BSS Plus in the anterior chamber. The corneas in the current study were examined during a 12-hour warming period; endothelial cell loss may have occurred as a result of prolonged exposure to BSS Plus.

Scanning electron microscopy showed some disrupted endothelial cells and swelling in corneas stored in both media at the end of the 12-hour warming period. The endothelial disruption was more pronounced for Optisol GS-stored corneas than the corneas stored in CM. A live/dead cell assay would be a future alternative or complementary tool that could be used to assess endothelial cell viability. The swelling of corneas after prolonged temperature reversal suggested impaired endothelial function in both media. However, corneas stored in CM seemed to deturgess at the same rate as those stored in Optisol GS, which suggests preservation of the endothelial pump mechanism is the same in the two different media. Whether this translates to better clinical outcomes is a topic of further investigation.

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