In vitro specular microscope perfusion of M-K- and moist chamber-stored human corneas

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Human donor corneas were stored in McCarey-Kaufman (M-K) medium for 3 to 6 days; then they were mounted in the specular microscope, and the endothelial surface perfused with a glutathione-bicarbonate-Ringer solution. During storage, the corneal thickness increased 37 percent above an assumed normal of 0.520 mm. The corneas did not have sufficient active thinning to be accepted as viable when tested by the temperature-reversal phenomenon. Since comparable donor corneas have been used in successful penetrating keratoplasties, there must be a discrepancy between the cell viability as tested by the temperature-reversal phenomenon and clinical application.

Key words: cornea, endothelium, human, eye bank storage, viability, specular microscope.

Eye banks universally store a viable donor eye as an intact globe in a moist enclosed environment at 4°C for a maximum of 24 to 48 hr. A conservative ophthalmic surgeon using donor tissue for a penetrating keratoplasty might prefer to use the tissue within 12 hr. Recently a corneal bathing medium, referred to as McCarey-Kaufman (M-K) medium, has come into widespread use. The M-K medium consists of a tissue culture medium plus dextran as a colloidal osmotic agent. The viability of the rabbit corneal endothelium after M-K storage has been investigated by temperature reversal, electron microscopy, metabolic analysis, and corneal grafting. The evidence indicated that the M-K storage was superior to conventional moist chamber storage in maintaining rabbit endothelial cell viability. The rabbit model is a convenient laboratory starting point, but it differs from the human cornea because the rabbit eye is very young, has short postmortem times, and has excellent endothelial cell regeneration.

In order to expand the laboratory data, the cat was selected as a better animal model. Van Horn et al. used trypan blue...
Fig. 1. After 3 days of storage in M-K medium, the in vitro perfused cornea was able to maintain a reasonably stable corneal thickness but unable to thin to a normal thickness of 0.520 mm. Removal of the epithelium (epi) did not alter the experimental results.

and electron microscopy to evaluate the endothelial cell viability. Their study shows that after 5 days of storage, over 20 percent of the M-K-stored endothelial cells and 100 percent of the moist chamber-stored cells were disrupted. Meyer et al. compared M-K storage to conventional moist-chamber storage of cat corneas via corneal grafting. They found cat corneas stored for 5 days in M-K medium had a higher incidence of postoperative graft clarity and intact ultrastructure than corneas stored in moist chambers.

If a truly pertinent evaluation of endothelial cell viability after M-K storage is desired, then only human cornea data is accurately informative. There is a limited amount of published data evaluating the physiological function of the endothelium after M-K storage. Clinical case reports of corneal grafts performed with M-K-stored donor corneas have been very encouraging.7-10

The purpose of this paper was to directly investigate the human corneal endothelial cell morphology and function with the specular microscope after M-K medium and conventional moist-chamber storage. The endothelial cell pump function, via corneal thickness measurements, and barrier function, via histological appearance of the cells, were assessed during in vitro perfusion studies.

Methods

Human donor eyes from the North Florida Lions Eye Bank were used. The 23 donor eyes were from 17 to 86 years old, with death to enucleation time of 1 to 40 hr., enucleation to storage time of 1 to 14 hr., and death to experimental use time of 62 to 144 hr. The eyes were enucleated and prepared for storage by the eye bank technician who used accepted procedures for M-K- and moist chamber-storage techniques.

The corneas with a 3 to 5 mm, scleral rim were excised from the donor eyes either before or after storage, depending on the storage technique, and mounted in the specular microscope's perfusion apparatus. The isolated cornea was maintained in vitro at 34° C. with 15 mm. Hg intraocular pressure. The epithelium was left intact, except when specified, and covered with 360 Medical Fluid (silicone oil, Dow Corning Corp. Midland, Mich.). The endothelial surface was perfused with a glutathione-bicarbonate-Ringer solution (GBR) of the following composition (in grams per liter): glucose 0.90, NaCl 6.92, KCl 0.35, CaCl 0.37, MgSO4 • 7H2O 0.30,KH2PO4 0.16, NaHCO3 2.10, reduced glutathione 0.00922, and adenosine 0.1536. The GBR was infused at the rate of 60 μl/min. Corneal thickness measurements were recorded along with specular photomicrographs of the endothelium. The photographs were exposed on Kodak Tri-X film with the 6 volt tungsten light source for 1 sec. exposures and developed in Kodak HC-110 (B dilution) for 5½ min. at 23° C. (room temperature).

Experimental group I. Eleven donor corneas after 3 to 6 days of storage in M-K medium were mounted and perfused in vitro as described above. During the course of some experiments, the epithelium was removed in order to get a better view of the endothelium with the specular microscope.

Experimental group II. Six paired donor corneas were stored with one eye in a conventional moist chamber at 4° C. and the paired cornea stored in M-K medium. After 4 days of storage, the corneas were mounted and perfused in the specular microscope as described above.

Results

Experimental group I. The 11 corneas after 3 to 6 days of storage in M-K medium had corneal thicknesses (epithelium included) ranging from 0.615 to 0.821 mm. (0.714 ± 0.019, mean ± S.E.M.). If 0.520 mm. is assumed as normal thickness, then this group of corneas increased in thickness by 37 percent during storage. The corneas were grouped with 3 to 4 days of storage and 5 to 6 days of storage. There were five
corneas stored 3 days in M-K media and one cornea was stored 4 days. In the second group, three corneas were stored 5 days and two were stored 6 days.

The corneal thickness profile of an in vitro perfused 3-day M-K-stored donor cornea is illustrated in Fig. 1. After 4 hr. of a slight swelling of 0.005 mm./hr., the epithelium was gently removed to reduce the light scatter and improve the specular photomicrographs. The corneal swelling continued at a rate of 0.004 mm./hr. During the perfusion the endothelial cells could be seen to undergo cell surface change (Fig. 2). Immediately after the cornea was mounted, normal endothelial cells could be seen adjacent to cells which have swollen in a blisterlike fashion. Within 15 min. at 34° C., the swollen cells returned to their normal appearance. Thus it is presumed that the blisters are a reversible cold storage-induced change. The cells retained their normal morphology for the remainder of the 6.5 hr. in vitro perfusion. This was consistent with the ability of the endothelium to maintain a relatively constant corneal thickness. A composite graph of the corneal thickness measurements during the in vitro perfusions of the M-K corneas stored for 3 to 4 days is illustrated in Fig. 3, with one cornea having an unusual and unexplained increase in corneal
thickness. A regression analysis of the data reveals a 0.004 mm./hr. swelling rate (note that the cornea with unusual swelling was excluded).

After 5 days of storage in M-K medium, endothelial cell function was still present, as demonstrated by a thinning rate of 0.006 mm./hr. (Fig. 4). The accompanying specular photomicrographs (Fig. 5) reveal normal endothelial cells at the start of the perfusion, i.e., immediately after storage. Some cells became slightly swollen after 5 hr. of the in vitro perfusion. Fig. 6 is illustrative of a 5-day M-K-stored cornea which has marginal endothelial pump function. A slow corneal swelling (0.023 mm./hr.) was present for the first 1½ hr., which was followed by a stabilization in thickness and finally a loss of thickness control. The changes in endothelial cell appearance as seen in the specular photomicrographs (Fig. 7) correspond to the corneal thickness profile. At first, numerous swollen endothelial cells can be seen. Just prior to the stabilization in corneal thickness, the cell resumed a normal appearance. Finally, an increasing number of cells became swollen, which was expressed by the increasing corneal thickness. Thus there appeared to be a correlation between the blisteredlike, swollen endothelial cells and their lack of "pump" function.

The cornea which was evaluated in Fig. 8 was stored in M-K medium for 6 days. The epithelium rapidly became edematous and had to be removed. Afterward, the cornea had a steady decrease in thickness (0.013 mm./hr.). The endothelial cells appeared normal throughout the experimental perfusion (Fig. 9).

A composite graph of the corneal thickness measurements reveals the variability of endothelial cell function after 5 to 6 days of storage in M-K medium (Fig. 10). A regression line was calculated to have a thinning rate of 0.003 mm./hr.

**Experimental group II.** In each of the six pairs of cornea tested in this experimental group, the moist chamber—stored cornea was thicker than the M-K-stored cornea after 4 days of storage. The average corneal thickness (epithelium included) were 0.728 ± 0.030 mm. (mean ± S.E.M.) for the M-K corneas and 0.990 ± 0.061 mm. for the moist-chamber corneas. Thus the M-K-stored corneas had a corneal thickness increase of 40 percent above an assumed normal of 0.520 mm., whereas the moist-chamber corneas increased 80 percent in thickness.

Fig. 11 is a representative experimental graph of the corneal thickness measurements taken during an in vitro perfusion of an eye stored for 4 days in a moist-chamber.
Fig. 5. Specular photomicrographs of the human endothelial cell taken during the experiment in Fig. 4, which was a 5-day-stored cornea. Endothelial cell swelling, observed as a dark blister covering the central zone of the cell, appeared after 3 to 5 hr. of the in vitro perfusion period.

and a cornea stored for 4 days in M-K medium. During the perfusion period, the M-K-stored endothelial cells appeared normal, as described in more detail in the previous section. It was at first impossible to see the endothelium of the edematous moist chamber-stored corneas because of stromal light scatter. After 2 to 3 hr., the edema reduced sufficiently for the presence of normal endothelial cells to be observed, but the presence of many posterior stromal folds limited the cell observations to the fold crests. The abnormal cell changes, i.e., blister formations which have already been described, could be found with either type of storage.

The swollen moist chamber-stored cornea showed a reduction in corneal thickness during the 5 hr. in vitro perfusion, to approximately the thickness of its paired cornea after M-K storage. Some of the moist-chamber corneas continued to dehydrate, but others began to rehydrate. Data collection after 5 or 6 hr. from an in vitro perfusion is unreliable, especially on a less than fresh cornea. Thus it was difficult to know whether the moist chamber-stored cornea would continue to decrease in thickness toward a normal value of 0.320 mm.

Fig. 6. After 5 days of storage, this human cornea had marginal endothelial pump function, as illustrated by the unstable corneal thickness during the in vitro perfusion.

Discussion

Corneal transplants have been successfully performed with donor corneas stored in the M-K storage medium for 5 days and in a few cases after 6 and 7 days of storage. The laboratory data have not been conclusive about the viability of the endothelial cell function.

The in vitro perfusion of a human cornea stored for 3 days in M-K medium (Fig. 1) indicates the presence of endothelial cell function, but apparently not at full capacity because the cornea was unable to reduce
AT SET UP

1 HR

2.5 HRS

Fig. 7. Human endothelial cells photographed during the in vitro perfusion illustrated in Fig. 6. During the early minutes of the perfusion the endothelial cells had dark blister formations. After 1 hr. the blistered cells reversed to normal, with a period of stable corneal thickness. Then as the thickness increased, dark cells once again became prominent.

Fig. 8. Human cornea was perfused in vitro after 6 days of M-K storage. Following the removal of the edematous epithelium (epi), the stromal thinning rate of 0.013 mm./hr. was observed.

its slightly edematous state. Yet the endothelial cells of that cornea (Fig. 2) remained normal in appearance throughout the 6 hr. perfusion. The seemingly marginal endothelial cell function was illustrated more dramatically in Figs. 6 and 7 of a 5-day-stored cornea. The corneal thickness at first increased, then stabilized, and finally showed a continuous increase. These changes correlated with the presence or absence of abnormal endothelial cells. Thus cell morphology reflected cell function. Some of the experimental perfusions did show active thinning of the corneal
thickness after storage in M-K medium for 5 days (Fig. 4) and for 6 days (Fig. 8).

A direct comparison of the moist-chamber storage to M-K storage showed that after 4 days both storage methods resulted in the corneas having similar endothelial cell appearance. The corneal thickness profiles (Fig. 11) seemed to indicate the M-K corneal endothelium to have less pump activity than the moist-chamber corneas.

This statement must be considered carefully before accepting it. Recently, Hull et al. reported that dextran from the M-K medium enters the cornea during storage. This could explain the lack of corneal thinning relative to the moist chamber-stored corneas without assuming a loss of endothelial cell viability in the M-K corneas. During the in vitro perfusion, the dextran within the cornea could be osmotically taking up water from the GBR maintenance medium. This could result in a new pump-to-leak ratio and thus stabilization at a corneal thickness value other than normal, i.e., 0.520 mm. The fact that the endothelial cell appearance during the perfusion study is approximately the same in the moist-chamber and M-K corneas is supportive of an osmotic water shift explanation instead of cell viability.

The effect of intraocular pressure on corneal thickness during the in vitro perfusion must also be considered. Ytteborg and Dohlman have shown that at the normal intraocular pressure of 15 mm. Hg the corneal thickness should stabilize at 0.68 mm. because of the tissue-pressure relationships. Thus it is possible, but has
Fig. 11. Corneal thickness of paired human corneas stored for 4 days, with one in M-K medium and the other in a moist chamber. Of the six corneal pairs tested, each of the M-K-stored corneas stabilized at a corneal thickness greater than 0.520 mm. (the assumed normal thickness). The corneal thicknesses of the moist chamber-stored corneas at the end of the 5 hr. experiments were approximately the same as that of the M-K-stored corneas.

It has not been confirmed, that the very edematous moist-chamber corneas were thinning purely by the physical effects of the intraocular pressure. In support of this premise, stromal water was found to be continually passed into the silicone oil covering the outer stromal surface during the perfusion. This did not happen during the M-K cornea perfusions.

In summary, temperature reversals are normally considered to be a sensitive test of endothelial cell function, i.e., viability. The perfusions reported in this paper can be considered to be temperature-reversal perfusions, since the perfusions were being performed with corneas of greater than normal thickness. The data show that the stored corneas are unable to actively thin to normal corneal thickness. Does this mean that the stored corneas are less than 100 percent viable? Which corneal storage method results in less viability? These questions can not be answered with the data in this paper. Viability testing via temperature reversals is informative but not precise within the given experiments. The moist-chamber corneas were excessively swollen (approximately 1 mm.), and thinning was probably reflecting a physical squeezing out of the stromal water by the intraocular pressure. The M-K corneas did not show a dramatic thinning from the approximate 0.7 mm. storage thickness. This is not too surprising, since it appears that the dextran in the M-K medium can slowly equilibrate across the endothelium and into the stroma. Thus the corneal thickness would no longer reflect only endothelial function. From the data in this paper, it can be concluded that both the moist-chamber and M-K corneas are viable after 4 days of storage, but it is difficult to conclude which storage method results in better tissue viability.

REFERENCES


