Histological study of corneas preserved in two new media

KOICHI TAMAKI, TATSUO YAMAGUCHI, EMILY D VARNELL,
AND HERBERT E KAUFMAN

From the Lions Eye Research Laboratories, LSU Eye Center, Louisiana State University Medical Center School of Medicine, New Orleans, LA, USA

SUMMARY A new corneal preserving medium (K-Sol), developed by Kaufman and others, contains purified chondroitin sulphate, TC 199, HEPES buffer, and gentamicin. Another new medium (JM) containing bicarbonate-free glucose-phosphate Ringer solution and dextran 70 has been developed in Japan. New Zealand white rabbit corneas with scleral rims were stored in each medium at 4°C for one or two weeks. The condition of the endothelium was evaluated histologically. Corneas preserved in both media were in good condition at the end of one week. Corneas preserved in K-Sol for two weeks showed fewer endothelial changes than similar tissue stored in JM for two weeks. Corneal swelling was also less in corneas preserved in K-Sol, than in corneas preserved in JM.

Many eye banks now use M-K medium, which was developed in the United States by McCarey and Kaufman in 1974 to preserve donor corneas until surgery. M-K medium maintained viability (as measured by temperature reversal) of rabbit corneal endothelium and generally preserved rabbit corneas for up to 14 days.¹ However, clinical results with human corneas suggested that storage time in M-K medium should be limited to three or four days.²

Recently Kaufman and others developed a new corneal preservation medium (K-Sol). Corneas stored in this new medium have been tested in animal studies and used in human corneal transplants. Corneas stored in K-Sol have been successfully transplanted following storage for up to two weeks.^{3,4}

Mizukawa and associates⁵ in 1968 developed a new liquid preserving medium, consisting of a mixture of TC-199, NaHCO₃, 1.5% chondroitin sulphate, inosine, adenine, adenosine, streptomycin, and penicillin. This medium was used as a corneal preserving medium in Japan. Mayes *et al.*⁶ reported that rabbit corneas stored in medium with a low bicarbonate concentration functioned better than corneas stored in medium with high bicarbonate concentration. Based on these findings a new corneal preservation medium (JM) was developed, and Manabe *et al.*⁷ have reported that this new medium is

an excellent and safe corneal storage medium for clinical usage.

In this study, corneas with scleral rims were stored in JM and in K-Sol, and the condition of the endothelium was evaluated histologically.

Materials and methods

PRESERVING MEDIUM

The Japanese medium (JM) (Kaken Pharmaceutical Company, Hongo, Tokyo) named EP-II is based on bicarbonate-free glucose-phosphate Ringer's solution and contains dextran 70, a few electrolytes, and a mixture of streptomycin and penicillin. The medium has an osmolarity of 305 mosm and pH of 7.40 (Table 1).

Table 1 Japanese Medium (JM)

Component	Concentration
Glucose phosphate Ringer (HCO ₃ ⁻ free)	
No CaCl ₂	
No MgCl ₂ ·6H ₂ O	
Dextran 70	3.5%
Na-citrate·2H ₂ O	900 µg/ml
MgSO ₄ ·7H ₂ O	218 µg/ml
Streptomycin sulphate	1000 µg/ml
Potassium penicillin G	200 units/ml
pH	7.40
Osmolality	305 mosm

Correspondence to Herbert E Kaufman, MD, LSU Eye Center, 2020 Gravier Street, Suite B, New Orleans, LA 70112, USA.

Table 2 K-Sol

Component	Concentration
TC 199	
HEPES buffer	0.25 M
Chondroitin sulphate, purified MW>10 000	2.5%
Gentamicin sulphate	100 µg/ml
pH	7.40
Osmolality	310 mosm

K-Sol contains 2.5% purified chondroitin sulphate (molecular weight >10 000) in TC 199, 0.25 M HEPES buffer, and gentamicin sulphate (Cilco, Sandford, NC). The medium has an osmolality of 310 mosm, and a pH of 7.40 (Table 2).

TISSUE

New Zealand white rabbits were killed with an overdose of sodium pentobarbital and both eyes were immediately enucleated. Corneas with scleral rims

were stored in either JM or K-Sol at 4°C. Eight pairs of corneas were used: the right corneas were preserved in JM and the mate corneas were preserved in K-Sol. Four pairs of corneas were stored for one week, and another four pairs were stored for two weeks at 4°C.

MICROSCOPY

At the end of the period of observation the specimens were divided into two pieces. One part was fixed immediately in 2.5% glutaraldehyde and 3% formalin with 1/15 M potassium sodium phosphate buffer (pH 7.35) for 12 hours, washed in the same buffer, postfixed in buffered 1% osmium tetroxide for 90 minutes, washed again in buffer, dehydrated in graded alcohols from 50% to 100% and propylene oxide, and embedded in Epon. Embedded specimens were sectioned with a microtome and stained with toluidine blue for light microscopy or uranyl acetate and lead citrate for transmission electron microscopy.

The other part of each cornea was fixed in 2.5%

Fig. 1A JM preservation for one week. Scleral rim. Scanning electron micrograph of the corneal endothelium. Some of the endothelial cells are swollen and protruding (arrow). Normal mosaic-like appearance can be seen. Bar=5 µm. Inset: Light micrograph. Corneal thickness is 0.56 mm. Corneal swelling is prominent. The epithelial cells and stromal cells are well preserved. Toluidine blue.

1B

2A

2B

glutaraldehyde and 3% formalin with potassium-sodium buffer (pH 7.4) for 12 hours, dehydrated in alcohol, dried in a critical-point dryer, placed on aluminum stubs, coated with gold, and photographed by means of scanning electron microscopy. Corneal thickness was measured on light micrographs, which were enlarged 30 times.

Results

The appearance of the preserved corneas was similar in all specimens.

PRESERVATION FOR ONE WEEK

On light micrographs the average thickness of the corneas preserved in JM was 0.54 mm and stromal swelling was prominent (Fig. 1A, inset). The control corneal thickness was 0.31 mm. The corneas preserved in K-Sol (Fig. 2A, inset) measured 0.39 mm in thickness, and no stromal swelling was seen. The epithelial cells were intact and the stromal cells were preserved in both corneas.

Both corneas appeared similar by scanning electron microscopy. A few swollen endothelial cells were seen protruding into the anterior chamber.

Fig. 1B JM preservation for one week. Scleral rim. Transmission electron micrograph of same cornea as in Fig. 1A. Cleft (*) and small vacuolations (V) can be seen on the apical side. The cytoplasm is slightly less dense anterior to the nucleus. The cytoplasmic organelles are almost intact. DM=Descemet's membrane; N=nucleus; AC=anterior chamber; M=mitochondria. Bar=0.7 μ m.

Fig. 2A K-Sol preservation for one week. Scleral rim. Scanning electron micrograph of the corneal endothelium. A few endothelial cells (arrow) are swollen. Normal mosaic-like appearance can be seen. Bar=5 μ m. Inset: Light micrograph. Corneal thickness is 0.40 mm. The stroma is not swollen. The epithelial cells and stromal cells are well preserved. Toluidine blue.

Fig. 2B K-Sol preservation for one week. Scleral rim. Transmission electron micrograph of the same corneas as in Fig. 2A. Cleft (*) and a few vacuolations (V) can be seen. Cytoplasmic organelles are almost intact. DM=Descemet's membrane; N=nucleus; M=mitochondria; AC=anterior chamber. Bar=0.7 μ m.

3A

3B

4A

Intercellular cytoplasmic membranes were distinct. The mosaic-like arrangement of the hexagonal endothelial cells was nearly normal (Figs. 1A, 2A).

By transmission electron microscopy the corneas also looked similar. An endothelial cleft and small vacuolations were seen. The cytoplasm seemed less dense anterior to the nucleus. The mitochondria were somewhat swollen, but the cristae were not disrupted (Figs. 1B, 2B). The endothelial sheets were preserved in good condition.

PRESERVATION FOR TWO WEEKS

On light micrographs the average thickness of the

corneas preserved in JM measured 0.53 mm, and stromal swelling was prominent (Fig. 3A, inset). The thickness of the cornea preserved in K-Sol was 0.35 mm, which is almost normal (Fig. 4A, inset). The epithelial cells and stromal cells were well preserved in both corneas.

By scanning electron microscopy corneas preserved in JM showed some swollen endothelial cells protruding into the anterior chamber, and cellular pleomorphism was prominent. Several pits were seen in the intercellular junctions (Fig. 3A). The corneas preserved in K-Sol showed a few swollen endothelial cells and small pits in the cytoplasmic membrane.

Fig. 3A JM preservation for two weeks. Scleral rim. Scanning electron micrograph of the corneal endothelium. About 20% of the endothelial cells are swollen and cellular pleomorphism is prominent. Pits (arrow) can be seen on the surface of the endothelium. Mosaic-like appearance can still be seen. Bar=5 μ m. Inset: Light micrograph. The epithelial cells and stromal cells are well preserved. Corneal thickness is 0.54 mm. Corneal swelling is prominent. Toluidine blue.

Fig. 3B JM preservation for two weeks. Scleral rim. Transmission electron micrograph of the cornea seen in Fig. 3A. Large clefts (*) and small vacuolations (V) can be seen on the apical side of the endothelium. The cytoplasmic organelles are intact. DM=Descemet's membrane; N=nucleus; M=mitochondria; AC=anterior chamber. Bar=0.7 μ m.

Fig. 4A K-Sol preservation for two weeks. Scanning electron micrograph of the corneal endothelium. The intercellular cytoplasmic membranes are distinct, and the hexagonal endothelial cells demonstrate a normal mosaic-like appearance. A few swollen endothelial cells and pits can be seen. Bar=5 μ m. Inset: Light micrograph. Corneal thickness is 0.35 mm. No stromal swelling is seen. The epithelial cells and stromal cells are well preserved. Toluidine blue.

Fig. 4B K-Sol preservation for two weeks. Scleral rim. Transmission electron micrograph of the same cornea seen in Fig. 4A. A few small vacuolations can be seen. Cytoplasmic organelles are intact. DM=Descemet's membrane; N=nucleus; M=mitochondria; AC=anterior chamber. Bar=0.7 μ m.

The intercellular cytoplasmic membranes were well preserved, and the hexagonal endothelial cells demonstrated a normal mosaic-like appearance. The endothelial sheets were almost intact (Fig. 4A). By transmission electron microscopy the corneas preserved in JM showed large clefts and small vacuolations. The cytoplasmic organelles were almost normal (Fig. 3B). In the corneas preserved in K-Sol the endothelial cells were almost intact except for small vacuolations (Fig. 4B).

Discussion

JM, which contains no bicarbonate, was developed by modifying glutathione bicarbonate Ringer's solution (GBR).⁸ Endothelial function is almost stopped by cooling to 4°C. Mayes *et al.*⁶ reported that a preserving medium with low bicarbonate concentration intensifies this effect. Therefore, nutritional materials are not included in this medium except for glucose. In contrast, K-Sol contains tissue culture medium for corneal nutrition, though K-Sol preser-

vation also reduces corneal endothelial function by cooling to 4°C.

Because cooling of the cornea to 4°C stops the function of the corneal endothelium, it also triggers corneal swelling. Dextran 70 and chondroitin sulphate are both high molecular weight materials that prevent stromal swelling.⁹⁻¹¹ JM contains dextran 70 (MW 70 000) and K-Sol contains purified chondroitin sulphate (MW >10 000). For penetrating keratoplasty it is desirable to use grafts of normal thickness. In our experiments corneas with scleral rims preserved in K-Sol maintained normal thickness for up to two weeks. No stromal swelling was seen (Fig. 4A). However, corneas with scleral rims preserved in JM were thicker than those in K-Sol and stromal swelling was prominent (Fig. 3A); thus K-Sol preservation appears to be superior in preventing stromal swelling. It is believed that purified chondroitin sulphate prevents corneal swelling, as first reported by Kida.⁹

Characteristic changes seen by transmission electron microscopy in the endothelium were clefts,

cytoplasmic vacuolations, and decreased cytoplasmic densities. The clefts were parallel to the cytoplasmic membrane and crescentic in shape. Small vacuolations were located on the apical side. Similar changes have been reported in the past with 4°C preservation.^{11,12} Sakimoto *et al.*¹² reported that the clefts disappeared after penetrating keratoplasty. This change may be the result of the loss of endothelial function during 4°C preservation. The density of the cytoplasmic organelles was somewhat decreased in the endothelial cells, but the organelles were almost intact. Overall, the endothelial sheets were in good condition in both corneas preserved for two weeks, though nearly all the endothelial cell nuclei were swollen.

By scanning electron microscopy the changes seen in endothelial cells preserved in both media for one week were negligible. Endothelial cell size was normal and the cytoplasmic membranes were almost intact (Figs. 1B, 2B). Corneas preserved for two weeks showed no endothelial cell loss. The hexagonal cells were well preserved. However, a few endothelial cells were enlarged, and endothelial pleomorphism was prominent. Endothelial swelling and pits in the cytoplasmic membrane were also visible, though the cytoplasmic pits were not confirmed by transmission electron microscopy. These endothelial changes (pleomorphism and pits) were more prominent in the corneas preserved in JM. Mizukawa *et al.*⁵ reported that even at 4°C the function of the cornea cannot be stopped completely; therefore a preserving medium should contain some nutritional additives. It is conceivable that lack of nutrition results in more changes in corneas preserved in JM, and that chondroitin sulphate exerts a beneficial effect on corneal endothelial cells preserved in K-Sol, as reported by Lindstrom and associates.¹³

K-Sol preserved corneas have been used in corneal transplantation in humans with good results.⁴ Human corneal endothelium degenerates much faster than that of rabbits,⁶ and the reproductive power of the endothelium in the rabbit is much greater than in humans. Therefore it seems likely that the results

obtained with rabbit corneas in this study are better than those obtained with human tissue. However, it may also be true that some histological changes that may occur in human tissue during storage may be reversible when the cornea is transplanted into the eye. In this study, although the corneas with scleral rims preserved for two weeks in both K-Sol and JM remained in good condition, somewhat better results were obtained with K-Sol.

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