

Development of Novel Corneal Storage Medium: First Report. Examinations of Rabbit Cornea

Atsuko Tachibana and Mitsuru Sawa

Department of Ophthalmology, Nihon University School of Medicine, Tokyo, Japan

Purpose: To develop a new corneal storage medium with a simple formula and evaluate it by histological methods.

Methods: Two corneal storage media containing minimum essential medium and 2.5% chondroitin sulfate (pH 7.33), with osmolarity of 320 mOsm/kg, were compared to Optisol-GS. The differences in the two media were the molecular weight (MW) and the source of chondroitin sulfate. The MW of Medium I was 27,500 and the MW of Medium II was 33,700. Japanese albino rabbits were used in this study. A cornea with scleral rim obtained from a rabbit was stored in either Medium I or Medium II and the fellow cornea was stored in Optisol-GS for 7 or 14 days at 4°C. Histological examination of corneal endothelial cells was performed both by scanning electron microscopy and by transmission electron microscopy.

Results: At day 7, there was no significant difference in histological findings among the rabbit corneas stored in Optisol-GS, Medium I, or Medium II. At day 14, corneas stored in Optisol-GS or Medium I showed similar histological findings. In Medium II, endothelial cells showed marked degeneration.

Conclusions: The results of experiments with rabbit cornea indicated that Optisol-GS and Medium I could preserve endothelial cellular structure better than Medium II. The difference between Medium I and Medium II was only the MW of the chondroitin sulfate used. The MW may be an important factor for determining suitable chondroitin sulfate for use in a corneal storage medium. **Jpn J Ophthalmol 2002;46:377–383**

Key Words: Chondroitin sulfate, corneal endothelial cell, corneal storage medium, Optisol-GS, storage of cornea with scleral rim.

Introduction

There are several factors to consider in corneal storage. One is whether to preserve the whole eye or only the cornea with scleral rim. Another factor is the storage period; short (up to 2–3 days), intermediate (up to 1 month), or longer (more than 1 month) period.¹ The preservation method for a whole eye is limited to short period storage because of the deterioration of corneal endothelial function due to postmortem change in aqueous humor.^{2,3} On the other hand, preservation of the cornea with scleral rim is feasible for an intermediate or longer period of storage, be-

cause corneal endothelial cells are not affected by postmortem change in aqueous humor. Therefore, the latter method is recently widely used in the clinical field in Japan. Because of a relative shortage of donor eyes for patients who required corneal transplantation, keratoplasty was performed shortly after the donation of an eye and intermediate corneal storage was not required. However, an intermediate storage period is now necessary because since 1997 there has been a Japanese government regulation requiring quarantine of donors with infectious diseases. Therefore, sufficient time is now needed for serological examination. Thus, we shifted from a short storage period for the whole eye to an intermediate storage method for the cornea with scleral rim. Until now, there has been no domestic storage medium for intermediate storage, and an imported product such as Optisol-GS^{4,5} is used. Therefore, we

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Correspondence and reprint requests to: Atsuko TACHIBANA, MD, PhD, Department of Ophthalmology, Nihon University School of Medicine, 30-1 Oyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan

studied the development of novel intermediate storage media with simple formulations, and examined their efficacy using rabbit cornea.

Storage Medium

We formulated two storage media, Medium I and Medium II, as shown in Table 1 and compared their efficacy with that of Optisol-GS (Cairon, Irvine, CA, USA). The ingredients for the two storage media are similar except for molecular weight (MW) and sources of chondroitin sulfate, Medium I: MW = 27,500 (average) (Kaken, Tokyo) and Medium II: MW = 33,700 (average) (Maruha, Osaka).

The formulated storage media were sterilized by filtration in clean bench, then stored in sterilized 20-mL glass bottles (180°C for 3 hours) and sealed using a cap. They were autoclaved at 121°C for 20 min, with an additional process of ethanol rinse and ultraviolet irradiation (30 minutes for each side of the bottles).

Materials and Methods

Six Japanese albino rabbits, weighing from 2.5 to 3.0 kg, were used. The rabbits were sacrificed using an overdose of sodium pentobarbiturate (Nembutal); then the eyeballs were enucleated. After the cornea with scleral rim was removed from the eyeballs of each rabbit in clean bench, one cornea with rim was stored in Optisol-GS solution and the cornea with rim of the fellow eye was stored either in Medium I or in Medium II solution, $n = 3$ for each, and stored at 4°C. On day 7 or 14 of storage, 9 corneas for each stage were fixed using 2.5% glutaraldehyde solution and cut into two halves. One of the two halves was dehydrated using increasing gradients of alcohol solution, then processed by critical

point desiccation (HCP-2 Critical Port Dryer; Hitachi, Tokyo) and gold particle evaporation coating (Quick Auto Coater; Sanyu Electronics, Tokyo), and observed by a scanning electron microscope (SEM) (S-650; Hitachi, Tokyo). The other half was double-stained using 1% tetra osmium solution and dehydrated using increasing gradients of alcohol solution. Then specimens were embedded in Epon. An ultrathin section of each specimen was double-stained using uranyl acetate and Pb citrate and observed by transmission electron microscope (TEM) (JEOL 1200; Nihon Electronics, Tokyo).

Results

Day 7

In cornea stored in Optisol-GS or Medium I, SEM revealed distinctive corneal endothelial cell boundaries and TEM showed almost normal findings in intracellular organella. Cornea in Medium II showed a paving stone-like bulging of endothelial cell surface but had almost normal intracellular organella (Figure 1).

Day 14

In cornea stored in Optisol-GS or Medium I, cell boundaries became blurred in several places and vacuoles in the cytoplasm were observed. Cornea in Medium II demonstrated irregularly shrunken cell surfaces by SEM examination, and the degenerated cells showed less staining pattern, destroyed cristae in mitochondria and aggregation of intranuclear chromatin by TEM examination (Figure 2).

These findings indicated that corneal endothelial cells were well preserved in their morphological aspects in the three tested media, but cornea stored in Medium II showed greater deterioration than the corneas stored in the other two media.

Table 1. Components of Tested Corneal Storage Media

	Optisol-GS	Medium I	Medium II
Base medium	MEM, TC-199	MEM	MEM
Buffer	HEPES	HEPES	HEPES
Gentamicin (mg/L)	100	100	100
Streptomycin	+	-	-
Chondroitin sulfate (%)	2.5	2.5	2.5
Molecular weight	27,500	27,500	33,700
Dextran (%)	1.0	-	-
ATP	Adenosine, inosine, adenine	-	-
Fe	+	-	-
Vitamins	α -tocopherol etc.	α -tocopherol	α -tocopherol
pH	7.25	7.33	7.33
Osmotic pressure (mOsm/kg)	351	320	320

MEM: minimum essential medium, HEPES: N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid, ATP: adenosine triphosphate.

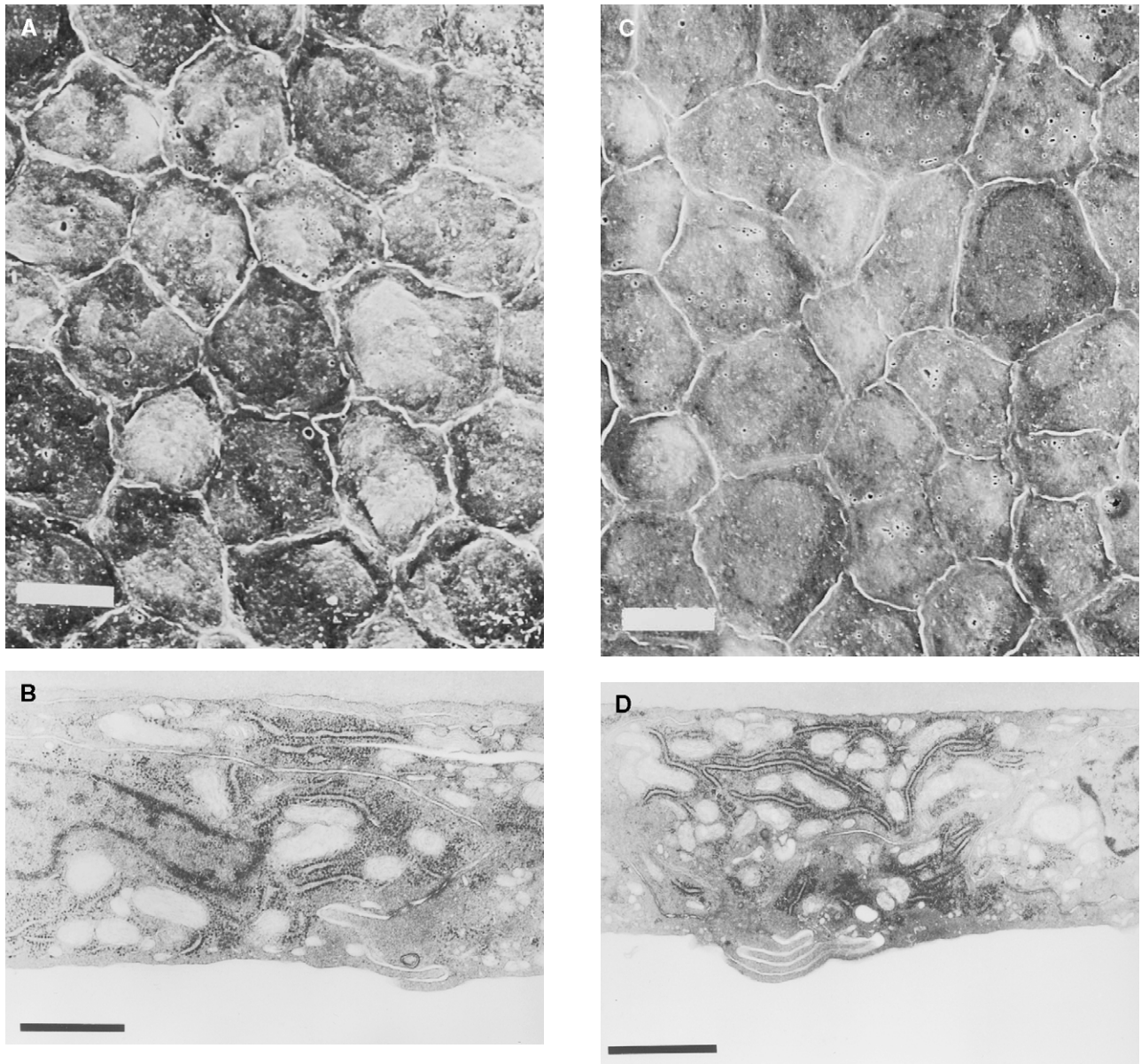


Figure 1. Electron microphotographs of rabbit corneal endothelium stored for 7 days. (A,C,E) Scanning electron microscope. Bar = 10 μ m. (B,D,F) Transmission electron microscope. Bar = 1 μ m. There is no significant difference between (A) Optisol-GS and (C) Medium I, but E: Medium II shows bulging of cell surface. Features of cell organella are maintained in good condition in (B) Optisol-GS. (D) Medium I, and (F) Medium II.

Discussion

Cornea has been stored by two methods, preserved either as the whole eye or as the cornea with scleral rim. Recently, the latter method is predominantly used. So far, several corneal storage media have been developed. For the cornea with scleral rim preservation solution, M-K medium was developed by McCarey in which the basic component was tissue culture medium, TC-199.⁶ The medium also con-

tained dextran, which was effective in suppressing corneal stromal swelling. Thus, it made it possible to maintain corneal endothelial functions for 8 to 14 days and M-K medium was used widely in the clinical field.⁷⁻¹¹ Then, K-sol,¹²⁻¹⁹ CSM,²⁰⁻²³ and Dexsol^{24,25} comprising either dextran or chondroitin sulfate were developed. In 1991, Optisol was developed based on K-sol and Dexsol.²⁶⁻²⁸ Currently Optisol-GS, in which gentamycin and streptomycin are

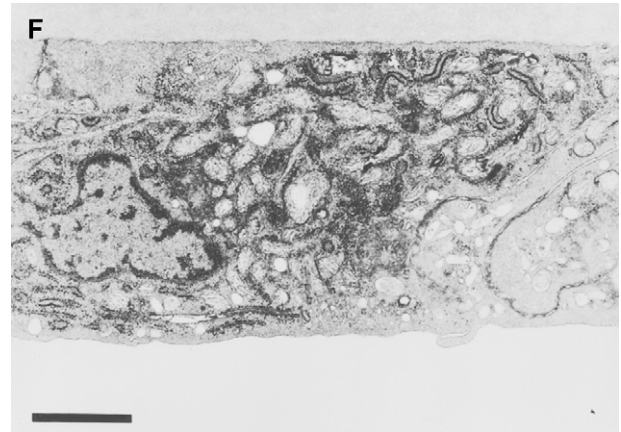
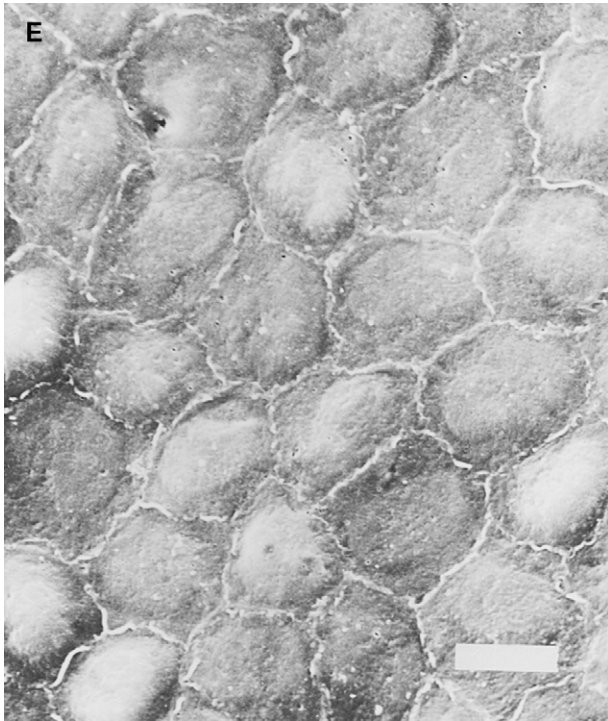


Figure 1. *Continued*

added to Optisol, is mainly used as the corneal storage medium.^{4,5} In Japan, several media were developed for storing the whole eye. These include K-solution,²⁹ in which the ingredients are bicarbonate buffer

solution and 0.1% ascorbate or 1% chondroitin sulfate; EP-solution,^{30,31} in which the ingredients are tissue culture medium, TC-199, inosin, adenin, and chondroitin sulfate; and EP-II in which the ingredi-

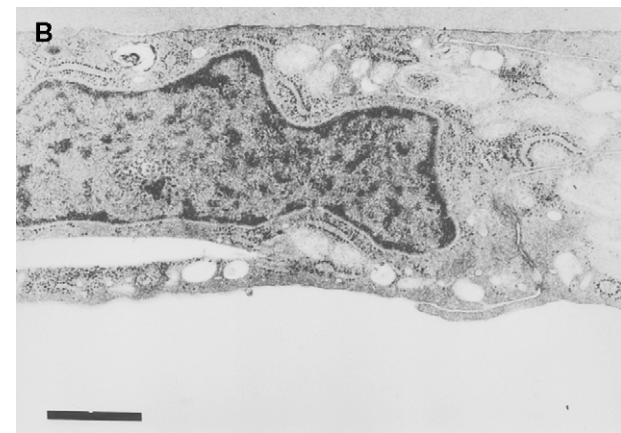


Figure 2. Electron microphotographs of rabbit corneal endothelium stored for 14 days. (A,C,E) Scanning electron microscope. Bar = 10 μ m. (B,D,F) Transmission electron microscope. Bar = 1 μ m. There is no significant difference between (A) Optisol-GS and (C) Medium I, but (E) Medium II shows presence of degenerated cells. Features of cell organelles are maintained in good condition in (B) Optisol-GS and (D) Medium I, but (F) Medium II shows degeneration of cytoplasm such as aggregation of chromatin in nucleus and deterioration of cristae in mitochondria.

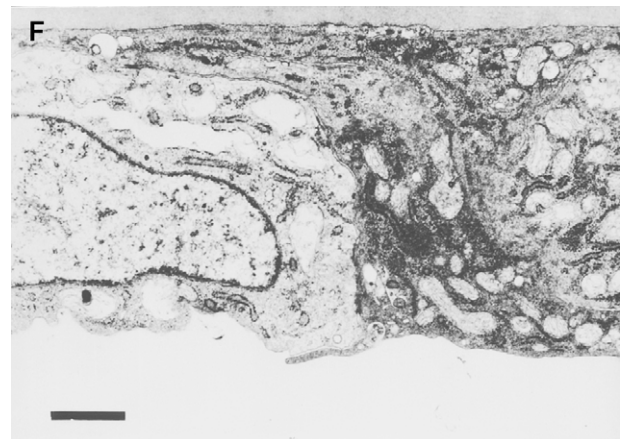
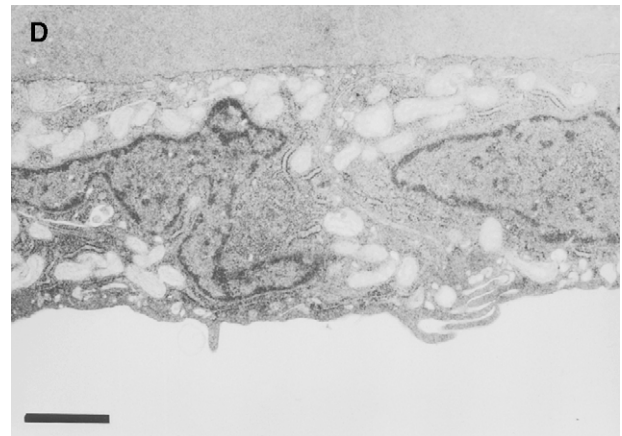


Figure 2. *Continued*

ents are glutathion phosphate ringer solution³² and dextran.^{33,34} In corneal storage, the main objective is to preserve the corneal endothelium. The bicarbonate ion plays an inevitable role in maintaining corneal endothelial function. Thus, bicarbonate ion-free solu-

tion can suppress corneal endothelial metabolism to a minimal level, resulting in a longer storage period for the cornea. EP-II solution was developed as a whole eye preservation medium according to the pathophysiological theory of corneal endothelial function, as de-

scribed above, and it is the medium that has been used mainly in Japan.

In the present study, we developed a newly formulated solution in which the main ingredients are minimum essential medium with chondroitin sulfate, N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES), and gentamycin. Gentamycin is reported to be effective at 4°C. We did not use dextran. Dextran can suppress stromal swelling and facilitate surgical procedures by maintaining corneal transparency. However, it cannot maintain corneal endothelial function.¹³ At its higher concentrations or longer storage periods it may cause damage due to uptake by endothelial cells, resulting in postoperative corneal edema.³⁵ Therefore, we used chondroitin sulfate instead of dextran and its concentration was fixed at 2.5%, the same as that in Optisol-GS, because of the following reports. In terms of optimal concentration, it was reported that 2.5–5.0% of chondroitin sulfate can maintain corneal transparency in a study of concentrations of 2.5–10.0%.¹² Another report indicated that 2.5% was the optimal concentration among 2.0%, 2.25%, and 2.5%.¹⁴ It was also reported that Optisol-GS, with a concentration of chondroitin sulfate at 2.5%, could suppress corneal swelling more effectively than Dexsol, 1.35%.²⁶⁻²⁸

The differences between Medium I and Medium II were the MW and the sources of chondroitin sulfate. Medium I has the same MW and source of chondroitin sulfate as Optisol-GS.

The present study revealed that Medium I could be similarly potent and maintain morphological characteristics as well as Optisol-GS in a 14-day preservation period. However, Medium II showed less potency in a 14-day preservation period than the other two media. The MW of chondroitin sulfate indicates a median value and a certain range of diversity. In the same MW group, it was reported that chondroitin sulfate with a higher viscosity³⁶ and a better purity¹⁶ can be more effective in suppressing corneal swelling. The difference between Medium I and Medium II is the source of chondroitin sulfate, but their quality is the same. The difference between formulations is their MW. Therefore, we think that the difference in potency depends on their choroidal osmolar pressure. Because the main purpose of the present study was to compare our formulated solutions with Optisol-GS, the significant role of choroidal osmolar pressure in corneal storage is our next subject for study.

The osmolar pressure of the present formulated solution is 320 mOsm/kg, which is lower than that of Optisol-GS, at 351 mOsm/kg. The capability of Opti-

sol-GS to maintain corneal transparency and suppress swelling was reported to depend on its higher osmolar pressure, but it had a capability in corneal preservation similar to other media such as Dexsol.²⁸ A tolerable osmolar pressure for corneal endothelial cells ranged from 200–400 mOsm/kg with other essential ions.³⁷ The osmolar pressure of aqueous humor is 303 mOsm/kg³⁸ and DexSol, 309 mOsm/kg. Therefore, osmolar pressure ranging from 309 to 350 mOsm/kg is thought to be optimal.

In terms of pH, the present solution was adjusted to 7.33. It was reported that a tolerable pH for corneal endothelial cells ranged from 6.5 to 8.5.³⁹ A pH of storage medium within this range can be acceptable. In the present study, HEPES buffer solution was added to maintain pH within the above range with phenol red as an indicator.

In the present morphological study, a novel formulated solution with simple ingredients showed a capability for corneal preservation similar to Optisol-GS. The solution can be formulated easily in the laboratory and would be useful for corneal preservation. Further study with Medium I using human cornea is planned.

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