

# A morphometric study of endothelial cells of human corneas stored in MK media and warmed at 37°C

DAVID S ROOTMAN, SYED M HASANY, AND PRASANTA K BASU

*From the Department of Ophthalmology, University of Toronto, Canada*

**SUMMARY** No study has yet been done to investigate the changes in endothelial cell size, perimeter, and density that may result from the warming of corneas in MK (McCarey-Kaufman) medium for specular microscopy. In the present investigation eye bank eyes were stored in MK medium at 4°C and rewarmed daily for six days at 37°C before specular photography of the endothelium was performed. These photographs were compared with wet mount preparations stained with trypan blue and alizarin red made from the same corneas and those stored without rewarming for six days. In addition all corneas were qualitatively analysed with the scanning electron microscope (SEM). The data from serial specular photography were insufficient to allow significant conclusions to be drawn about day to day changes in cell morphology. However, analysis of wet mount preparations revealed that cell density and perimeter varied significantly between those corneas rewarmed daily and those held in cold storage for six days. SEM studies showed an intact cell monolayer with cell loss along the folds of corneal endothelium. We therefore concluded that repeated rewarming at 37°C of corneas stored in MK medium at 4° has a deleterious effect on cell morphology and that folds induced by swelling of corneal tissue result in endothelial cell damage with some loss.

The morphological study of the corneal endothelial monolayer has been greatly facilitated by the advent of specular microscopy.<sup>1</sup> This has allowed for viewing of the living endothelium both *in vivo*<sup>2</sup> and *in vitro*.<sup>3</sup> Initial studies looked at cells in terms of density,<sup>4</sup> that is, cells per unit area. These density calculations did not always provide an accurate prediction of cell function.<sup>5</sup> Consequently investigators have been looking at other parameters to quantitate cell size variability (polymegethism) and cell shape variability (pleomorphism).<sup>6,7</sup>

We wanted to answer a question that often worries eye bank personnel: Do the characteristics of endothelial cells of corneas stored in the MK (McCarey-Kaufman) medium at 4°C change when corneas are briefly warmed at 37°C for specular microscopic examination?

For the purpose of studying the variables of corneal endothelial cell density, mean cell area, and cell perimeter, chronological specular photographs

were compared with photographs taken of an endothelial wet mount preparation from the same cornea at the end of the storage period.

## Materials and methods

Five pairs of human donor corneas were put in MK medium within 12 hours post mortem. One of each corneal pair was placed in MK medium in a corneal viewing chamber (Product Research Organization (PRO) Inc., Tustin, California). This cornea (experimental cornea) was stored at 4°C, removed daily, and warmed at 37°C for one hour for six days. The endothelium was then photographed with the specular microscope (PROEB-I). Afterwards the cornea in the storage chamber was returned to cold storage. The other cornea of the pair acted as a control and was put in a vial of MK medium at 4°C continuously for six days. After six days of storage each cornea was removed and bisected. One half of the cornea was processed for scanning electron microscopy by the method described previously.<sup>8</sup> The other half was stained with trypan blue and

Correspondence to Dr P K Basu, Department of Ophthalmology, University of Toronto, 1 Spadina Crescent, Toronto, Ontario, Canada M5S 2J5.

alizerin red<sup>9,10</sup> with the endothelium being photographed as a wet mount preparation.

To count the cell density (cells per square mm), individual cell area, and cell perimeter all the photographs of the wet mount photographs and calibration grid (0.01 mm) were magnified to 428 times. The cell boundaries in each photograph were enhanced manually with a felt tip marker. A quantitative morphometric analysis of the cells was done with an image analyser (Omnicon 3000 Bausch and Lomb, attached to a computer: Nova IV Data General), located at the Department of Mechanical Engineering, University of Toronto.

## Results

### SPECULAR MICROSCOPY

Good quality photographs were obtained when the cornea and its MK medium was warmed at 37°C for 1 hour. Since the quality of specular photomicrographs varied daily at different sites of the same cornea, it was not possible to have data from all corneas suitable for statistical analysis. Table 1 shows the number of photographs available for each cornea on each day of observation. It was possible, however, by comparing the photographs on a daily basis to detect changes in endothelial cell shape and size. Figs. 1 and

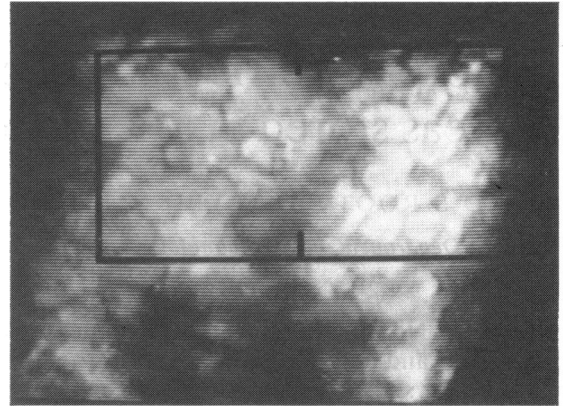


Fig. 1 Specular photomicrograph of human corneal endothelium after one day of storage in MK medium at 4°C. Cornea was warmed at 37°C for one hour. Note photograph shows some guttae.

2 show a qualitative comparison of specular images of similar areas of an experimental donor cornea photographed after one and six days of storage. There is a more compact cellular structure in the corneal endothelium stored for 1 day (Fig. 1) than in the same corneal endothelium photographed after six days of storage (Fig. 2).

Table 1 Number of specular photographs available on each day of observation

Eye Bank number	Day						
	0	1	2	3	4	5	6
19053	3	1	—	—	1	2	2
19059	2	—	2	1	2	3	—
19075	—	2	2	—	—	—	—
19095	1	—	1	—	—	1	—
19103	2	6	—	—	2	—	—

### LIGHT MICROSCOPY OF WET MOUNT PREPARATION

The comparative data of the experimental and the control corneas in terms of changes in cell density, mean cell area, and perimeter are shown in Table 2. By means of the unpaired *t* test it was found that mean cell area and perimeter in the control corneas was significantly smaller in three out of five pairs than in the corresponding experimental corneas (Table 2). When the difference was analysed with the paired *t*

Table 2 Endothelial morphological analysis from wet mount photographs of corneas stored in MK medium at 4°C (controls) compared with corneas in MK medium that were rewarmed daily to 37°C for one hour (experimental)

Donor number	Eye bank no.	Sample	No. of cells	Mean cell area ± SD (μm <sup>2</sup> )	Coefficient of variation of cell area	Cell density (mm <sup>2</sup> )	Cell perimeter ± SD (μm)	Coefficient of variation of perimeter
1	19054	Cont.	(136)	267 ± 122	0.45	3738	64.2 ± 14	0.21
	19053	Expt.	(45)	369 ± 118**	0.32	2710	74.9 ± 12*	0.16
2	19060	Cont.	(198)	201 ± 86	0.43	4955	55.5 ± 11	0.20
	19059	Expt.	(172)	222 ± 108*	0.45	4489	58.7 ± 13*	0.23
3	19076	Cont.	(192)	205 ± 81	0.39	4859	56.4 ± 11	0.20
	19075	Expt.	(131)	222 ± 119	0.53	4504	59 ± 16	0.27
4	19096	Cont.	(190)	212 ± 105	0.49	4714	56.8 ± 13	0.22
	19095	Expt.	(15)	209 ± 100	0.47	4780	56.6 ± 13	0.23
5	19104	Cont.	(221)	180 ± 81	0.44	5527	52 ± 11	0.21
	19103	Expt.	(186)	212 ± 114**	0.53	4714	56.3 ± 16*	0.28

Significant values: \**p*<0.05; \*\**p*<0.01. Cont. = control. Expt. = experimental.

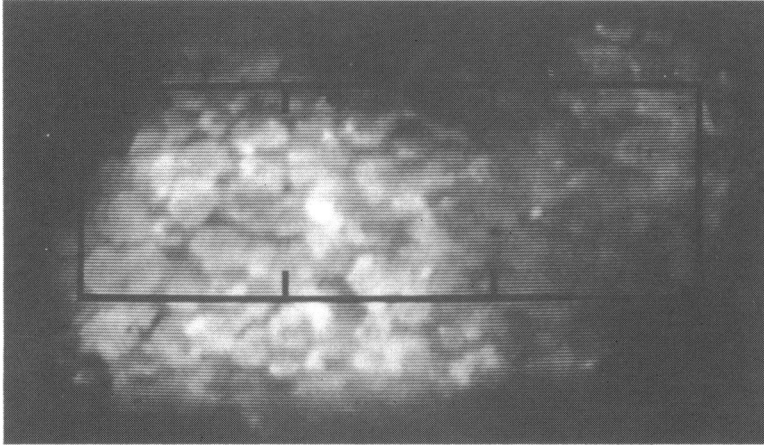


Fig. 2 *Specular photomicrograph of similar area of corneal endothelium as in Fig. 1 after six days of storage. The cornea was rewarmed daily at 37°C for one hour. Note the presence of larger cells and greater guttae than in Fig. 1 (control).*

test, it was found that mean cell area in the total sample did not vary significantly ( $p > 0.05$ ), whereas the cell density and perimeter varied significantly ( $p < 0.05$  and  $p < 0.02$  respectively) (Table 3).

#### SCANNING ELECTRON MICROSCOPY

It was observed that in both experimental and control corneas a monolayer of cells was present, though it tended to be damaged or absent in areas overlying folds (Fig. 3). In these areas cells had rounded and had lost continuity with their neighbours. This was also true of the cells along the folds in wet mount preparations (Fig. 4). In the wet mount preparations dead cells, indicated by darkly stained nuclei, were observed to lie in aggregates along a fold of corneal

endothelium (Fig. 4). In some areas exposed Descemet's membrane was observed staining red with the alizarin red.

#### Discussion

Specular microscopy of eye bank corneas is a technique being utilised increasingly. Better storage chambers and microscopes have facilitated this increase. Difficulties with the systems still remain. It is not always possible to obtain good quality photomicrographs even immediately on the eyes' arrival in the eye bank. It was found in our laboratory that image quality was poorer with storage greater than 48 hours. This is in agreement with the observations of

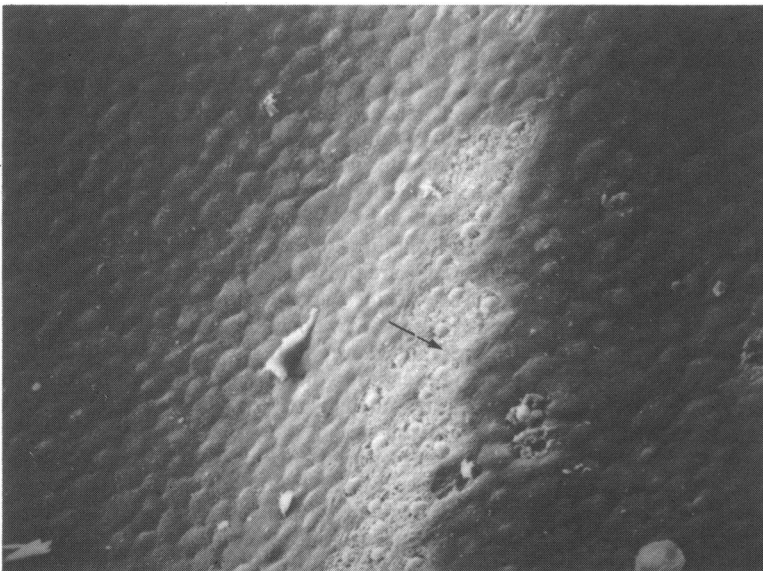
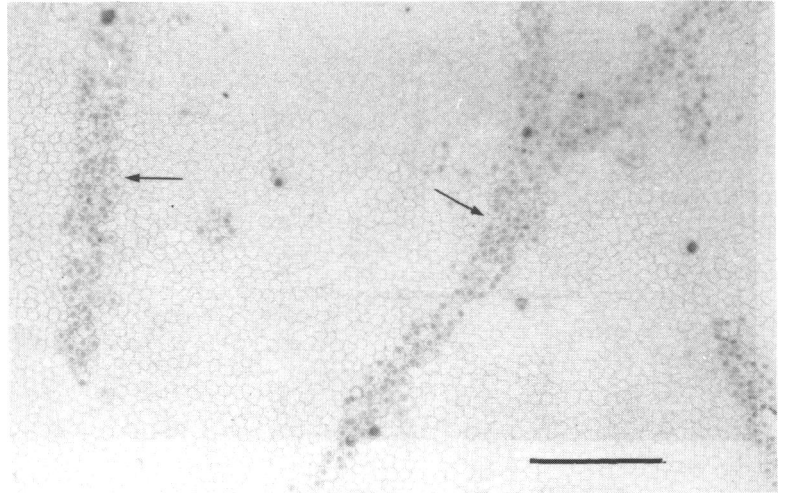


Fig. 3 *Scanning electron micrograph of human corneal endothelium stored in MK medium at 4°C for six days (control). Arrows show dying cells along the fold.*

Fig. 4 Photograph of human corneal endothelium stained with trypan blue and alizarin red after storage in MK medium at 4°C for six days (control). Arrows show the non-viable cells along the fold. Bar=200 µm.



Nesburn *et al.*,<sup>11</sup> who reported similar problems in endothelial examinations by specular microscopy. However, it was observed that an increase of the incubation period at 37°C facilitated observation. In addition, while specular photography may have been quite poor, when corneas were later stained with alizarin red and trypan blue an intact endothelial monolayer was found to be present. Another problem with the specular microscope is that on any given day of observation it may be possible to photograph only 30–40 cells despite a thorough search of the entire cornea. This is due to the optical system of the specular microscope and concavity of the cornea. A low power objective lens may help in scanning the entire endothelial surface and alleviate this difficulty. This makes it difficult to know whether these few cells truly represent the entire monolayer. Moreover it is impossible to identify conclusively the same exact area in sequential observations.

Because of these problems it was difficult to obtain sufficient data to analyse changes occurring on a day-to-day basis and to compare specular microscopy

values with the wet mount preparations values. However, there is an indication from the specular photography in this study that cell area and perimeter of corneal endothelial cells increase with storage time (Figs. 1, 2).

From the wet mount preparations it was also seen that, when all pairs of corneas were compared, there was no significant increase in mean cell area as a result of rewarming and storage. Cell density and perimeter did, however, vary at levels of significance of 5% and 2% respectively. Intraindividual variation amongst paired corneas varied significantly for mean cell area and perimeter in three out of five corneas.

These results (Tables 2 and 3) suggest that rewarming at 37°C of the experimental corneas (kept in MK medium at 4°C) had a deleterious effect on endothelial cell morphology. However, a larger sample is necessary to assess further whether mean cell area is affected, since in our study the mean cell area was not significantly different between the experimental and control groups when the data were pooled.

Scanning electron microscopy served to illustrate that, even when specular microscopy may not image the endothelium of eye bank eyes in MK medium, viable cells are present. It also showed that cell death occurred along folds of corneal endothelium. The folds seen in SEM correlated well with folds seen on the wet mount preparations (Figs. 3 and 4). The folds may be created by imbibition of water by the stored cornea stroma. This suggests that methods of storage which result in greater swelling of stored tissue may induce more damage to endothelial cells, and, conversely, those methods which reduce tissue swelling may be beneficial in reducing endothelial disruption during storage. Moreover surgical techniques that reduce bending and folding of corneal

Table 3 Pooled data from analysis of wet mount photographs from corneas stored in MK medium at 4°C (control) and those rewarmed daily to 37°C for one hour daily (experimental)

	Mean cell area ± SD (µm <sup>2</sup> )	Cell density ± SD (mm <sup>2</sup> )	Cell perimeter ± SD (µm)
Cont. n=5	213 ± 32	4759 ± 289	56.98 ± 4.4
Expt. n=5	246 ± 68	4239 ± 386	61.1 ± 7.8
	t=1.8	t=2.7	t=4.12
4 DF*	p<0.01	p<0.05	p<0.02

\*DF=degrees of freedom.

tissue may result in less endothelial cell loss and a more favourable surgical outcome.<sup>12</sup>

References

- 1 Maurice DM. Cellular membrane activity in the corneal endothelium of the intact eye. *Experientia* 1968; **24**: 1094-5.
- 2 Laing RA, Sandstrom MM, Leibowitz HM. In vivo photomicrography of the corneal endothelium. *Arch Ophthalmol* 1975; **93**: 143-5.
- 3 Bourne WM. Examination and photography of donor corneal endothelium. *Arch Ophthalmol* 1976; **94**: 1799-800.
- 4 Bourne WM, Kaufman HE. Specular microscopy of human corneal endothelium in vivo. *Am J Ophthalmol* 1976; **81**: 319-23.
- 5 Schultz RO, Matsuda M, Yee RN, Edelhauser HF, Schultz KJ. Corneal endothelial changes in type I and type II diabetes mellitus. *Am J Ophthalmol* 1984; **98**: 401-10.
- 6 Rao GN, Aquavella JV, Goldberg SH, Berk SL. Pseudophakic bullous keratopathy. Relationship to corneal endothelial status. *Ophthalmology* 1984; **91**: 1135-40.
- 7 Hartman C, Koditz W. Automated morphometric endothelial analysis combined with video specular microscopy. *Cornea* 1985; **3**: 155-67.
- 8 Basu PK, Hasany SM, Doane FW, Schultes K. Can steroid reduce endothelial damage in stored corneas? Effect on cell viability and ultrastructure. *Can J Ophthalmol* 1978; **13**: 31-8.
- 9 Spence DJ, Peyman GA. A new technique for the vital staining of the corneal endothelium. *Invest Ophthalmol Vis Sci* 1976; **15**: 1000-2.
- 10 Taylor MJ, Hunt CJ. Dual staining of corneal endothelium with trypan blue and alizarin red stain. Importance of pH for the dye-lake reaction. *Br J Ophthalmol* 1981; **65**: 815-9.
- 11 Nesburn AB, Mandelbaum S, Willey DE, Trousdale MD, Maguen E, Ward DE. A specular microscopic viewing system for donor corneas. *Ophthalmology* 1983; **90**: 686-91.
- 12 Grutzmacher RD, Oiland D, McKillop BR, Bunt-Milam AH. Donor corneal endothelial striae. *Am J Ophthalmol* 1986; **102**: 508.

Accepted for publication 1 May 1987.