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Morphology of graft endothelium and donor age

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SUMMARY The corneal endothelium of 42 clear corneal transplants was studied with a specular microscope. The endothelial cell morphology was analysed by using a computerised image analysis system. A quantitative index was developed to study the degree of variation in cell size. By applying this objective index we observed that pleomorphism is independent of age, and considerable alteration occurs in cell morphology during healing.

The integrity of the corneal endothelium is the most critical factor in the successful outcome of a corneal graft.[1] Most of our knowledge of human corneal endothelial cell morphology is derived from studies of corneal buttons obtained at transplantation and from post-mortem eyes. The advent of clinical specular microscopy has made it possible to study the corneal endothelium in vivo. Previous studies on corneal grafts[2–4] indicate a marked decrease in the endothelial cell density[2 4] with increase in mean cell area.[3] There is no correlation between either cell density or mean cell area and corneal thickness. From these observations it is apparent that neither cell density nor mean cell size is a decisive factor in the determination of corneal thickness.

The possibility exists that other aspects of endothelial cell morphology can influence graft survival. In the present study we photographed the endothelium of 42 clear corneal transplants using the specular microscope and studied the morphology of graft endothelium.

Materials and methods

Forty-two cases of clear corneal transplants were included in this study. The preoperative diagnosis for each case is listed in Table 1. In all instances the donor corneas were preserved in McCarey-Kaufman medium for less than 72 hours before use.

The endothelium was studied with a modified Syber clinical specular microscope. Photographs were taken and cell densities determined from at least 3 different photographic fields in each case, and the mean value was calculated. The methods employed in the determination of cell density were described by Bourne and Kaufman.[5] The central corneal thickness was determined by using a modified Haag-Streit pachometer at the time of specular microscopic examination. The intraocular pressure was also determined at the same time with a Langham type tonometer. The age of the donor is known in 37 of these cases. In 8 cases specular microscopy was performed on the donor corneas before preservation and transplantation. Statistical analyses were performed to correlate recipient and donor ages to the cell density of graft endothelium. A probability ratio of less than 0·05 (t test) was considered statistically significant.

A hand-outlined overlay in an 8×10 inch (20×25 cm) format was prepared from each photographic field by means of a pen with a felt tip of 0·35 mm width. The overlays were analysed by the Omnicon pattern analysis system. Data on area, perimeter, mean cell area, and standard deviation of cell areas were obtained.

We employed an index to quantify the degree of variation in cell size in a given cornea (polymegethism), which represents one aspect of the cellular pleomorphism. The index reflects the ratio of the standard deviation to the mean of the cell areas, and was calculated for each sample. This ratio, referred to as polymegethism quotient (PQ), is dimensionless, thus allowing direct comparisons between endothelial patterns regardless of cell size or population. The PQ approaches zero as the variation in cell size becomes minimal and reaches perfect uniformity.

Results

The pertinent clinical data for each patient included in the series are tabulated in Table 1.

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Table 1 *Details of individual patients*

Recipient age	*Donor age*	*Postoperative duration (months)*	*Endothelial cell density (cells/mm²)*	*Thickness (in mm)*	*Mean PQ*	*Rejection episodes*	*Preoperative diagnosis*
80	55	9 mo	1066	0·44	0·302	No	Fuchs's dystrophy
80	14 mo	9 mo	3710	0·78	0·354	Yes	Aphakic bullous
77	65	8 mo	1422	0·60	0·228	No	Fuchs's dystrophy
73	13	1 mo	2800	0·68	0·355	Yes	Herpes simplex kerat tis
71	10 mo	3 mo	3857	0·60	0·404	No	Aphakic bullous keratopath
70	56	5 mo	2222	0·60	0·408	Yes	Fuchs's dystrophy
70	36	7 mo	1955	0·58	0·413	Yes	Fuchs's dystrophy
68	19	6 mo	800	0·76	0·357	No	Interstitial keratitis
67	46	5 mo	1244	0·63	0·497	Yes	Fuchs's dystrophy
67	60	3 days	2133	0·76	0·464	No	Fuchs's dystrophy
66	45	10 mo	1111	0·58	0·404	No	Fuchs's dystrophy
66	43	1 day	1689	0·78	0·344	No	Fuchs's dystrophy
65	59	8 mo	1422	0·62	0·366	Yes	Fuchs's dystrophy
64	58	2 mo	2266	0·74	0·673	No	Fuchs's dystrophy
63	33	4 mo	1156	0·68	0·350	No	Fuchs's dystrophy
63	25	1 wk	2488	0·62	0·450	No	Fuchs's dystrophy
62		3 mo	756	0·66	0·504	No	Herpes simplex keratitis
61	47	11 mo	1110	0·59	0·374	No	Fuchs's dystrophy
61	58	3 mo	1333	0·66	0·286	Yes	Keratitis sicca
61	48	2 mo	800	0·66	0·388	No	Corneal oedema
60	20	3 mo	3022	0·64	0·310	Yes	Graft failure
59	54	3 mo	1111	0·64	0·311	Yes	Corneal scar
58	52	1 mo	1818	0·70	0·365	No	Aphakic bullous keratopathy
57	30	22 mo	844	0·58	0·406	No	Keratoconus
55	57	3 wk	1906	0·64	0·576	No	Aphakic bullous keratopathy
55		1 day	2887	0·68	0·582	No	Fuchs's dystrophy
55	60	4 mo	1155	0·56	0·473	No	Fuchs's dystrophy
54		4 mo	1910	0·58	0·328	No	Fuchs's dystrophy
54	60	2 mo	800	0·52	0·306	No	Interstitial keratitis
51	52	6 mo	1910	0·64	0·507	Yes	Fuchs's dystrophy
48	36	6 mo	2399	0·58	0·400	No	Graft failure
47		2 mo	2043	0·66	0·323	No	Keratoconus
44	19	1 mo	1866	0·62	0·314	No	Keratoconus
39	62	3 wk	1511	0·54	0·371	No	Keratoconus
34		2 mo	800	0·66	0·386	No	Keratoconus
29	26	6 mo	1422	0·68	0·348	No	Keratoconus
28	60	11 mo	1333	0·80	0·248	Yes	Corneal oedema post-cong. glaucoma
26	17	2 mo	1995	0·62	0·396	No	Herpes simplex keratitis
25	15	1 mo	2860	0·66	0·531	No	Keratoconus
24	46	5 mo	2710	0·61	0·393	No	Corneal scar post-traumatic
21	65	4 mo	2044	0·50	0·422	No	Keratoconus
15	35	3 mo	1910	0·60	0·342	Yes	Corneal scar post-traumatic

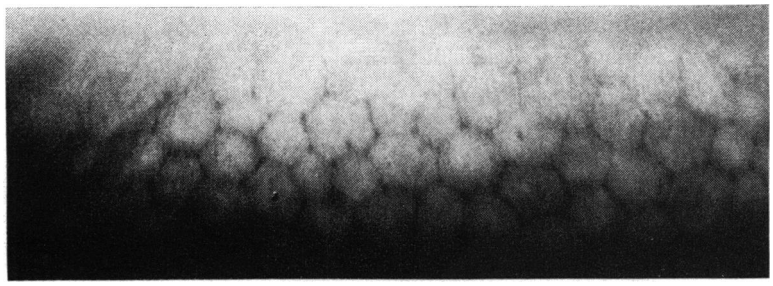

Fig. 1 *Endothelium of the donor cornea obtained from a 52-year-old donor. Cell density 2128 cells/mm². Polymegethism quotient 0·288.*

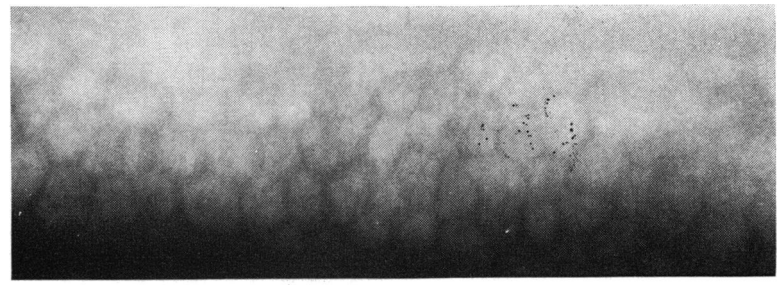

Fig. 2 *Postoperative appearance of the graft endothelium 1 month after surgery at which donor cornea in Fig. 1 was used. Cell density 1818 cells/mm². Polymegethism quotient 0·365. Recipient age 58 years.*

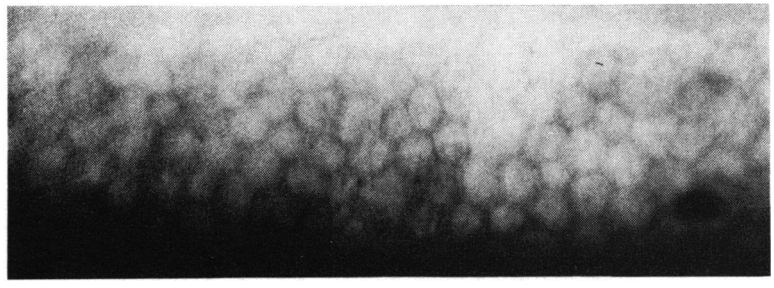

Fig. 3 *Endothelium of the donor cornea obtained from a 15-year-old donor. Cell density 2860 cells/mm². Polymegethism quotient 0·445.*

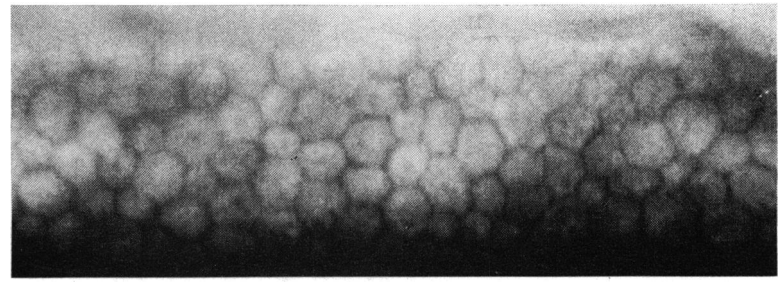

Fig. 4 *Postoperative appearance of the graft endothelium 1 month after surgery at which donor cornea in Fig. 3 was used. Cell density 2533 cells/mm². Polymegethism quotient 0·480. Recipient age 25 years.*

The endothelial cell density ranged from 444 to 3857 cells per square millimetre in this series. The cell density was found to have no correlation with corneal thickness. No correlation was observed between the cell density of the graft endothelium and the preoperative clinical status of the recipient cornea. Donor age was found to have an inverse correlation with cell density of the graft endothelium. No correlation was found between recipient age and cell density. In corneas where the donor tissue was obtained from adults, there was a decrease in cell density during the postoperative period (Figs. 1, 2, 3, 4).

However, in the 2 corneas in which the donor ages were 10 and 13 months an increase in cell density was observed in the postoperative period. This increase was consistently apparent at each specular examination during the postoperative period (Figs. 5, 6, 7, 8) and is observed only in these 2 corneas.

Light microscopic examination of the pathological corneal buttons in the 2 cases in which infant

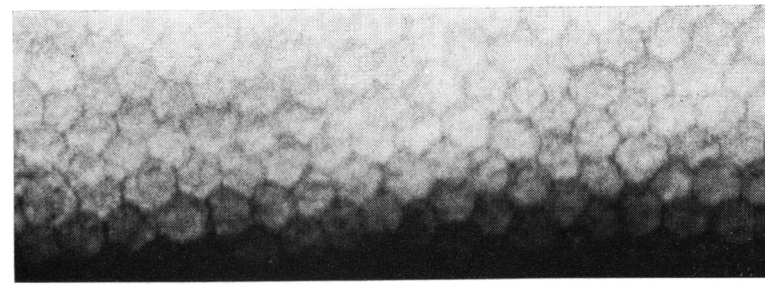

Fig. 5 *Endothelium of the donor cornea obtained from a 14-month-old donor. Cell density 3187 cells/mn.². Polymegethism quotient 0·182.*

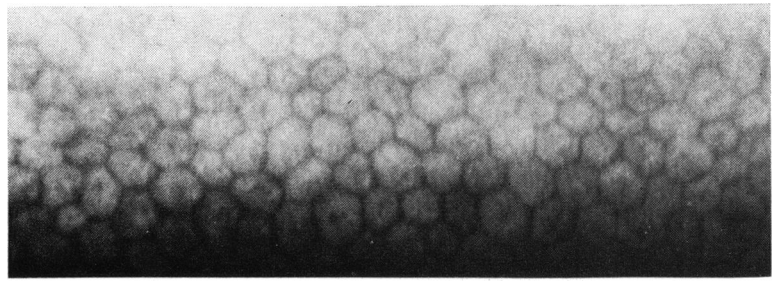

Fig. 6 *Postoperative appearance of the graft endothelium 9 months after surgery at which donor cornea from Fig. 5 was used. Cell density 3710 cells/mm². Polymegethism quotient 0·312. Recipient age 80 years.*

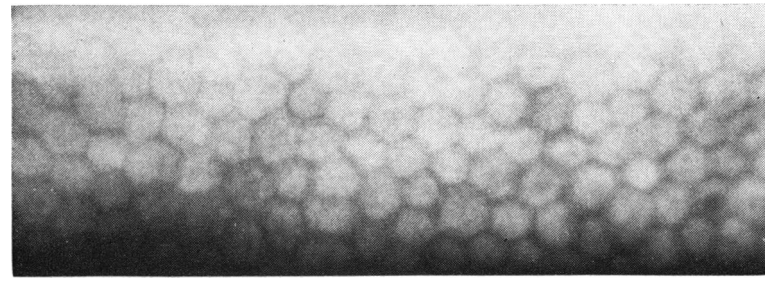

Fig. 7 *Endothelium of the donor cornea obtained from a 10-month-old donor. Cell density 3200 cells/mm². Polymegethism quotient 0·316.*

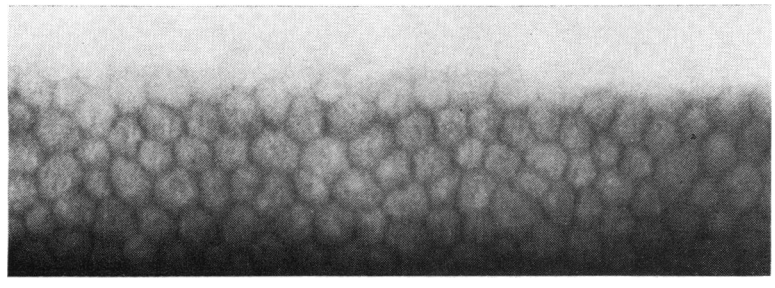

Fig. 8 *Postoperative appearance of the graft endothelium 3 months after surgery. Cell density 3857 cells/mm². Polymegethism quotient 0·404. Recipient age 71 years.*

corneas were grafted showed total absence of recipient endothelium. The preoperative clinical diagnosis in both cases was aphakic bullous keratopathy.

When we applied PQ to study the degree of variation in cell size to these 42 corneas, the value ranged from 0·179 to 0·673 with a mean of 0·389 (Table 1). We found no apparent correlation between the PQ and either donor or recipient age, indicating that pleomorphism is not influenced by age.

The results in Table 2 reflect the great alteration that occurs in corneal endothelium in the transition from donor to postoperative graft. The PQ values differed significantly between the donor endothelium and the graft endothelium.

Discussion

All corneas in the present study remained clear in spite of the wide variation in cell density. This clearly shows a lack of correlation between cell

Table 2 *Alteration in corneal endothelium*

Donor age	Donor endothelium		Graft endothelium	
	Cell density (per mm²)	*PQ*	*Cell density (per mm²)*	*PQ*
57	2527	0·411	1906	0·576
52	2128	0·288	1818	0·365
48	3059	0·554	800	0·388
17	2361	0·390	1995	0·396
15	2860	0·445	2860	0·480
13	3228	0·346	2800	0·355
14 months	3187	0·182	3710	0·354
10 months	3200	0·316	3857	0·404

density and corneal thickness and is in agreement with earlier reports.[2,4]

Two cases in the present series showed an increase in cell density in the graft endothelium from that of the donor endothelium. This increase was evident in all postoperative specular microscopic examinations and is seen only in these 2 corneas. In both instances, the preoperative clinical diagnosis in the recipient was aphakic bullous keratopathy. The corneas were obtained from infant donors. This increase in cell density can only be explained by either a replication in the graft endothelium or the spread of cells from the recipient on to the graft. The total absence of corneal endothelium in the excised corneal buttons makes it very unlikely that the recipient endothelium has contributed to the increase in cell density. The morphological appearance of the graft endothelium is unlike that seen in patients with aphakic bullous keratopathy or in the normal corneas of the same age group. Although observations on a small sample of cases cannot be conclusive, the demonstration of an increase in cell density with time, in the endothelium of only these 2 corneas, strongly suggests the possibility of replication in infant corneal endothelium. However, confirmation of this phenomenon is possible only on histological examination.

The morphological appearance of the endothelial cells in these 2 grafts also supports the concept of persistence of the donor endothelium in the corneal graft. One of these cases was reported in an earlier publication.[6]

The study of pleomorphism and its role in corneal hydration has hitherto received very little attention. We examined this aspect of endothelial cell morphology by measuring the area of individual cells using an automated image analysis system.[7] An index referred to as polymegethism quotient (PQ) was developed to quantitate the degree of variation in cell morphology in terms of variation in cell size. This represents our attempt to quantitate one aspect of pleomorphism.

When we applied this index to the present series of cases, certain interesting observations emerged. The lack of correlation between PQ and either donor or recipient age contradicts earlier observations, which indicate an increase in pleomorphism with advancing age.[8–10] The precision and the objectivity of the methods used in this study were lacking in the previous reports. The other striking feature that has come to light is the dynamic alteration that occurs in endothelial cell morphology in the transition from donor endothelium to graft endothelium.

Our observations in the present study make it apparent that endothelial cell morphology of corneal grafts presents a great degree of variability.

PQ is a dimensionless ratio developed from measurements of cell areas by the application of a mathematical relationship. A dimensionless ratio is independent of cell size, cell density, and the age of the patient. Thus, the PQ index offers the opportunity to analyse objectively endothelial cell morphology per se in our efforts to quantitate the changes which occur during the healing process.

A comprehensive analysis of all morphological parameters is essential to understand fully the role of endothelial cell morphology in corneal transparency, and to study objectively the healing processes in the corneal endothelium.

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