Epithelial metabolism of the corneal graft is abnormal

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SUMMARY Both eyes of 16 patients who had undergone unilateral penetrating keratoplasty were examined in this study. The mean corneal oxygen uptake rate in the centre of the graft was 3.88 (SD 0.55) mmHg/s and in the control eye was 4.29 (SD 0.62) mmHg/s. In the temporal host periphery the results for the grafted and control eyes were 4.52 (SD 0.54) mmHg/s and 4.26 (SD 0.52) mmHg/s respectively. The differences in the oxygen uptake rate between the centre of the graft cornea and the contralateral control eye and between the centre and periphery of the graft eye were statistically significant ($p < 0.01$). The central corneal touch threshold of the graft eye showed good recovery in four patients, while the remaining 12 had no or minimal recovery. The central and temporal epithelial and corneal thicknesses of the graft and the control eyes were not significantly different. The central endothelial cell density was 1383 cells/mm² in the graft and 3053 cells/mm² in the control eye. As corneal oxygen uptake reflects changes in aerobic epithelial metabolism, it is possible that there is a link between loss of neural integrity of graft epithelium and lowered epithelial aerobic metabolism following penetrating keratoplasty.

The cornea of the aphakic eye swells less with a hypoxic stimulus than does the cornea of the paired normal eye.¹⁻³ However, neither the removal of the crystalline lens nor the endothelial changes produced by surgery appear to be related to the difference in hypoxic corneal swelling response. Rather, the difference has been attributed to the lower aerobic metabolic activity of the epithelium due to partial denervation of the cornea following surgery.⁴ Differences in epithelial aerobic metabolism are reflected in the corneal oxygen uptake rate when corneal thickness, the main factor influencing diffusion of oxygen, remains approximately the same.⁵

The present study was initiated to investigate further the effects of denervation on aerobic metabolic activity. Both the central and peripheral corneal oxygen uptake rates and sensitivities in both eyes of 16 patients with unilateral penetrating keratoplasty were measured.

Materials and methods

SUBJECTS

Sixteen subjects who had undergone unilateral penetrating keratoplasty participated in the study after

their informed consent was obtained. The patient characteristics and details of the surgery are listed in Table 1. The average age of the patients was 43 years (range 24-60 years) and the time after surgery averaged 29 months (range 10-102 months). All the

Table 1 Details of subjects

Subject	Sex	Age	Operated eye	Reason for surgery	Time since surgery (months)
1	M	28	R	K	10
2	M	54	R	H	10
3	F	39	L	S	13
4	M	60	L	H	14
5	F	54	R	K	15
6	M	36	L	K	21
7	F	56	R	S	22
8	M	38	L	K	24
9	M	44	L	K	24
10	F	39	R	K	27
11	M	24	L	K	28
12	M	47	R	K	32
13	M	29	R	K	36
14	M	33	L	H	38
15	F	47	L	K	52
16	M	60	L	B	102
Mean		43			29
SD		12			22

K = Keratoconus. H = Herpes simplex. S = Past corneal trauma.
B = Bullous keratopathy.

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operations were performed by the one surgeon using the same technique. The diameter of the grafts was 8 mm. The running 10-0 nylon suture was removed after six months. Local corticosteroid therapy was also stopped at this time. Inflammatory changes were not observed in any of the corneas at the time of measurement.

METHODS

Corneal oxygen uptake rate. Corneal oxygen uptake rate was measured with a polarographic oxygen sensor^{6,7} applied directly to the anterior cornea. The oxygen sensor (Radiometer E5057/0, 8 mm in diameter with a 25 μ m diameter cathode) was covered with a 12.5 μ m thick polypropylene membrane. The sensor current was amplified by a radiometer amplifier (PHM 73) linked to a microcomputer (Apple II Plus). The sensor was calibrated periodically at 34°C in air (155 mmHg PO₂) and nitrogen-saturated water (0 mmHg PO₂) baths. The corneal oxygen uptake rate was determined by recording the time required for the oxygen tension at the membrane to drop from 140 to 100 mmHg. The result is expressed in mmHg/s.

Three readings of the central and the temporal peripheral corneal oxygen uptake rate of each eye were taken. The average variability (SD) for three readings taken at each measurement session was 0.22 mmHg/s.

Corneal touch threshold. Corneal sensitivity was measured with a Cochet-Bonnet aesthesiometer,

which consists of a 0.12 mm diameter nylon monofilament encased in a cylinder. The aesthesiometer was mounted on a triaxis holder so that its position and movement could be controlled in the x, y, and z meridians in an arrangement similar to that used by Millodot.⁸ The length of the monofilament can be varied from 6.0 to 0.5 cm. This range corresponds to pressures of 1 to 20 g/mm² (cross-sectional area of the filament, 0.0113 mm²). To obtain a measurement the aesthesiometer was moved slowly toward the cornea at a constant velocity until visible bending of the filament occurred. The subject was asked to tap on the table if touch was felt. Presence or absence of the blink reflex was also noted. The aesthesiometer was first set at a maximum thread length of 6.0 cm and the length was then decreased by 0.5 cm increments until the patient felt three of the six stimuli. This filament length was recorded as the value corresponding to the corneal touch threshold. The central and temporal regions of the graft and control corneas were measured.

Epithelial thickness. The Payor-Holden micro-pachometer, consisting of a Haag-Streit pachometer adapted to a Rodenstock Model 2000 biomicroscope, was used to monitor central and temporal epithelial thickness. The design and operation of the system have been previously described.⁸ Ten readings of central and temporal epithelial thickness were obtained; the average standard deviation for the 10 readings was 5 μ m (10% of epithelial thickness).

Corneal thickness. Total corneal thickness was measured centrally and temporally with the micro-

Table 2 Central and temporal corneal characteristics of the graft patients

Subject	Oxygen uptake rate (mmHg/s)						Corneal touch threshold (mg/mm ²)								
	Central cornea			Temporal cornea			Central cornea			Temporal cornea			Graft	Control	Diff.
	Graft	Control	Diff.	Graft	Control	Diff.	Graft	Control	Diff.	Graft	Control	Diff.			
1	4.45	5.26	-0.81	4.33	4.35	-0.02	200	11	189	12	11	1			
2	3.14	3.55	-0.41	5.01	3.62	1.39	200	11	189	11	11	0			
3	4.28	5.09	-0.81	5.23	4.85	0.38	11	11	0	11	11	0			
4	4.21	4.25	-0.04	4.70	3.88	0.82	200	11	189	16	11	5			
5	3.40	4.48	-1.08	4.17	4.42	-0.25	36	16	20	21	13	8			
6	4.14	4.35	-0.21	4.35	4.15	0.20	200	11	189	12	11	1			
7	3.23	3.36	-0.13	3.99	3.30	0.69	145	11	134	21	11	10			
8	4.03	4.51	-0.48	4.39	4.56	-0.17	200	12	188	11	13	-2			
9	4.04	4.64	-0.60	4.33	4.26	0.07	200	11	189	11	11	0			
10	4.11	4.48	-0.37	4.21	4.45	-0.24	145	11	134	11	11	0			
11	4.39	4.73	-0.34	4.44	4.62	-0.18	200	11	189	11	11	0			
12	3.78	3.80	-0.02	4.15	5.17	-1.02	13	11	2	21	11	10			
13	2.93	3.01	-0.08	3.77	3.35	0.42	200	11	189	11	13	-2			
14	4.51	4.68	-0.17	4.63	4.60	0.03	200	12	188	6	12	-6			
15	3.02	3.83	-0.81	6.03	4.09	1.94	11	11	0	11	11	0			
16	4.47	4.68	-0.21	4.54	4.54	0.00	200	11	189	21	45	-15			
Mean	3.88	4.29	-0.41	4.52	4.26	0.25	148	11	136	14	13	1			
SD	0.55	0.62	0.33	0.54	0.52	0.70	80	1	80	5	6	6			
t value			4.81			1.38			6.58			0.65			
p			0.001			ns			0.001			ns			

pachometer. Ten readings of corneal thickness were obtained on each measurement occasion; the average standard deviation for 10 readings was 4 μ m (0.8% of corneal thickness).

Endothelial morphology. The central endothelium was photographed with Kodak Tri-X pan film using a non-contact specular microscope as described previously.^{9,10} Fifteen photographs were taken of each eye. The photographs were printed at 13 $\times$ magnification from negatives which contained images of endothelial cells at approximately 30 $\times$ magnification. A precision microscopic grid of known dimensions was photographed at the corneal plane to enable exact quantification of the endothelial mosaic. Five photographs of good quality were chosen from the 15 photographs of each eye. Technicians traced 50 cells from each photograph on to a plastic overlay using a fine black marking pen, giving a total of approximately 250 cells traced per eye. The cell tracings were analysed with an IBAS-2 automatic image analysis system (Kontron, West Germany). This instrument measured individual cell areas (adjusted for the width of the pen lines) and calculated endothelial cell density (ECD) and the coefficient of variation of mean cell size, which was used as an index of endothelial cell polygathism.

Data analysis. The difference between the two eyes were tested for statistical significance by a *t* test for matched pairs. A 95% confidence level was chosen as the criterion for accepting a difference as being significant.

Results

The corneal characteristics of all the subjects are listed in Table 2. The central corneal oxygen uptake rate of the graft eye (3.88 (SD 0.55) mmHg/s) was significantly depressed in comparison with the contralateral control eye (4.29 (SD 0.62) mmHg/s, $p < 0.001$, Student's two-tailed paired *t* test). In addition, the corneal oxygen uptake rate was depressed in the centre of the graft in comparison with the temporal host periphery (3.88 (SD 0.55) mmHg/s vs 4.52 (SD 0.54) mmHg/s. The mean reduction was 9% and this was also statistically significant (< 0.05 , Student's two-tailed paired *t* test). The difference, however, between the central (4.29 (SD 0.62) mmHg/s) and temporal (4.26 (SD 0.52) mmHg/s) measurements of the control eye (0.7%) was not statistically significant.

Mean corneal touch threshold for all subjects was also significantly reduced in the centre of the graft eye in comparison with the centre of the control eye and with the temporal host periphery ($p < 0.001$, Student's two-tailed paired *t* test).

There was no statistically significant difference in mean epithelial or total thickness between the graft and control central and temporal corneas.

The average central endothelial cell density in the graft eye was 1383 cells/mm² and in the control eye 3053 cells/mm² (Table 3). The difference in the endothelial cell density between the graft and control eyes was 55%. This reduction was statistically significant ($p < 0.001$, Student's two-tailed paired *t* test).

Epithelial thickness (μ m)						Corneal thickness (μ m)					
Central cornea			Temporal cornea			Central cornea			Temporal cornea		
Graft	Control	Diff.	Graft	Control	Diff.	Graft	Control	Diff.	Graft	Control	Diff.
61	73	-12	40	57	-17	593	555	38	345	608	-263
71	62	9	77	34	43	488	564	-76	663	514	149
57	52	5	90	57	33	590	553	37	688	626	62
50	76	-26	33	72	-39	457	569	-112	395	670	-275
53	47	6	58	52	6	608	427	181	293	550	-257
51	55	-4	50	68	-18	523	500	23	662	590	72
66	68	-2	38	90	-52	526	540	-14	322	661	-339
42	54	-12	76	47	29	508	421	87	663	462	201
59	42	17	55	60	-5	497	455	42	566	571	-5
60	46	14	30	36	-6	547	517	30	616	385	231
71	72	-1	68	67	1	535	496	39	498	645	-147
54	52	2	58	83	-25	580	494	86	594	589	5
48	59	-11	56	47	9	502	460	42	530	415	115
55	69	-14	52	68	-16	468	474	-6	523	633	-110
52	49	3	40	20	20	523	431	92	594	319	275
60	59	1	43	61	-18	544	604	-60	550	695	-145
57	58	-2	54	57	-3	531	504	27	531	558	-27
8	11	11	17	18	26	45	56	71	129	110	197
0.70			0.45			1.47			0.53		
ns			ns			ns			ns		

Table 3 Central endothelial cell density (ECD) and polymegathism

Subject	EDC cells/mm ²			Polymegathism		
	Central cornea			Central cornea		
	Graft	Control	Diff.	Graft	Control	Diff.
1	2237	3799	-1562	0.291	0.243	0.048
2	1031	2747	-1716	0.458	0.263	0.195
3	2531	3524	-993	0.327	0.283	0.044
4	1780	3201	-1421	0.276	0.316	-0.039
5	463	3058	-2595	0.280	0.247	0.033
6	1091	3499	-2408	0.312	0.285	0.036
7	1443	1788	-345	0.303	0.286	0.017
8	1402	3084	-1682	0.356	0.319	0.037
9	1173	2834	-1661	0.295	0.319	-0.024
10	2085	2792	-707	0.276	0.271	-0.002
11	1679	4095	-2416	0.344	0.280	0.065
12	1122	3348	-2226	0.298	0.275	0.024
13	1162	3268	-2106	0.321	0.229	0.092
14	1176	3571	-2395	0.257	0.312	-0.054
15	782	2892	-2110	0.221	0.295	-0.074
16	973	1354	-381	0.301	0.333	-0.031
Mean	1383	3053	-1670	0.307	0.285	0.011
SD	552	693	733	0.052	0.030	0.121
t value			8.83			0.35
P			0.001			NS

There was no statistically significant difference in the endothelial polymegathism of the graft and control eyes.

Discussion

The results of this study show that the corneal oxygen consumption of the graft epithelium is not only significantly lower than the normal contralateral cornea but also lower than the host periphery. The periphery of the host cornea was subjected to the same post surgical conditions as the graft, yet retained normal oxygen uptake characteristics. This suggests that the depressed oxygen uptake of the graft is not due to postsurgical treatment such as steroids. As differences in corneal oxygen uptake rates are primarily caused by differences in epithelial metabolic activity,⁵ these results indicate that the epithelial metabolic activity of the graft cornea is depressed despite the long postoperative period. Even in the same eye the epithelium at the centre and periphery has different levels of metabolic activity following penetrating keratoplasty. This reduction, however, does not influence epithelial thickness, as measurements were similar in the graft and control eyes.

After penetrating keratoplasty the properties of the graft cornea are altered. Bourne and Brubaker¹¹ showed that endothelial permeability to fluorescein is reduced with reduced endothelial cell density. This study showed a marked reduction of the endothelial

cell density in the graft eye. Similar findings have been reported by others.¹²⁻¹⁶ A relevant question is whether endothelial changes influence corneal oxygen uptake rate. In our patients corneal oxygen uptake differences did not correlate with endothelial cell loss (Pearson correlation, $r=0.14$, $p=0.60$). This finding is not unexpected, as calculations with a computer model¹⁷ show that a two-fold increase or decrease in endothelial oxygen consumption has no significant effect on the uptake rate.¹⁸

There was no statistically significant difference between the mean central cornea thickness of the graft and control eyes. However, in individual cases, the thickness of the graft and control eye did vary (Table 2). As reported previously, significant differences in corneal thickness can alter the rate of passive diffusion of oxygen from the atmosphere to the aqueous humour.⁵ To test whether this may have influenced our findings we used the technique described by Holden *et al.*⁵ to recalculate the oxygen uptake rates to allow for this difference. After recalculation the differences between the graft and control eye central oxygen uptake rates and between the central and peripheral oxygen uptake rates of the graft cornea were still statistically significant. Thus the overall results were not influenced by corneal thickness changes in individual cases.

Problems with neovascularisation, punctate epithelial lesions, and infections with extended contact lens wear following keratoplasty have been reported.^{19,20} Epithelium with a lower than normal aerobic metabolism must be regarded as compromised and as a result may be more prone to develop infections during contact lens wear.^{19,21} The compromised epithelium could also be more prone to secrete neovascularogenic substances²² causing superficial neovascularisation observed in many graft patients with contact lens wear.

The central corneal sensitivity of the graft cornea was significantly reduced in 12 patients in agreement with previous studies.^{23,24} However, three of our patients (3, 5, and 12) had normal sensitivity post-operatively, with one patient having normal sensitivity only 13 months after penetrating keratoplasty. In another study Ruben and Colebrook²⁴ found no case of normal central sensation until at least three years after surgery.

The reduction in the graft eye sensitivity could partly be explained by an abnormal nerve regeneration pattern. Nerve fibres in the graft cornea are single axons running in the basal epithelial plexus, where they give rise to a profuse plexiform network with very few stromal nerves evident.^{23,25} Stromal nerves find it difficult to regenerate, as Schwann cell channels are not in apposition within the incision and incomplete sensory recovery in humans results

despite epithelial reinnervation.²³ There is also evidence of some neurological control of the corneal epithelium in both the resting state and when it is physiologically challenged.^{4 26 27} Our findings of depressed epithelial metabolic activity in association with incomplete recovery of corneal nerves following keratoplasty lend further support to the hypothesis that normal corneal epithelial metabolism partly depends on normal innervation.

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