Clinical assessment of corneal endothelial cell density: an original system of grading using a slit-lamp biomicroscope

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SUMMARY An original system of grading of the corneal endothelial specular reflection, as assessed with a Haag-Streit 900 slit-lamp biomicroscope, has been shown to have a very highly significant relation to the endothelial cell density measured by contact specular photomicroscopy. The grading, though subjective and therefore not a substitute for the detailed record of photomicroscopy, is readily applicable to clinical practice and is a useful method of comparing and recording endothelial cell densities when assessed by the same observer. The intraobserver, but not the interobserver, variability has been examined, and the theoretical aspects of the grading system and a clinically applicable interpretation of the grading are presented.

With the introduction of specular microscopy of the corneal endothelium into clinical practice,[1] there came a widespread recognition of the loss of endothelial cells that could occur with anterior segment surgery,[2-4] and of the development of corneal oedema and bullous keratopathy as a consequence of lower endothelial cell densities (ECDs). The ECD below which corneal decompensation becomes probable is poorly documented but is thought to be approximately 500 cells/mm^2.[5-8]

There still remains a need to assess the ECD of an eye prior to surgery despite markedly improved techniques of microsurgery and surgical instrumentation over the last decade. This need is not only to detect those eyes at risk of postoperative corneal decompensation but also to plan and monitor the effects of surgery.

Specular endothelial photomicroscopy has demonstrated that age is a poor predictor of ECD[9-13] and that corneal thickness, though a useful measure of endothelial function, is a poor predictor of cell density.[2-14] Other signs of endothelial abnormality include the presence of guttae or an increase in the cellular pleomorphism[14-15]; both of these changes tend to be associated with lower ECD. Illustrations of the corneal endothelial mosaic in health and disease, as demonstrated on the Gullstrand slit-lamp,

have been published by Vogt.[16] Although the matching of slit-lamp visualisation of the mosaic to a series of standardised grids or a graticule has been described,[17-20] there has been no report of a comparison between the ease of slit-lamp visualisation of the mosaic and the ECD measured by specular photomicroscopy.

This paper reports a study of the validity of a clinical assessment of corneal endothelial cell density, considers theoretical aspects, and derives guidelines for clinical management. The aims of this study were (*a*) to increase the clinical awareness of the facility of slit-lamp specular illumination and thereby to increase its practice; (*b*) to provide a method of conveniently recording approximate cell densities for clinical practice and for future reference by the same observer; and (*c*) to highlight the fact that low endothelial cell densities, of particular concern surgically, are readily recognisable at the slit-lamp microscope.

Materials and Methods

The corneas of 236 subjects were examined by the writer on two occasions, these examinations being separated by at least 10 weeks and the second being without reference to the results of the first; the work was completed within one year. Subjects on long-term eye medication were omitted from the study.

At each examination the ease of visualisation of

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the endothelial mosaic was graded by means of a Haag-Streit 900 slit-lamp microscope (by a technique detailed later), the density of corneal guttae was assessed (subjective scale, range 0–5), the degree of corneal arcus was graded (subjective scale, range 0–3), and the presence of stromal opacity or disease was noted. The central corneal thickness was measured with a Haag-Streit pachymeter (mode of three or more readings, with avoidance of instrument 'backlash' errors). After the clinical assessment of a cornea, specular photomicrographs of the central corneal endothelium were made with a Pocklington contact specular microscope (Keeler–Konan).

ECD was assessed by the projection of the photomicrographs to a known magnification and by counting of the number of cells in an area of known size.[21] The system was calibrated by the use of a haemocytometer, allowance being made for a corneal thickness of 0·55 mm. Made by a single independent observer, the number of counts for each examination was normally three or more (range 1–6).

PRACTICE OF SPECULAR ILLUMINATION

To visualise the low-reflectance corneal endothelial mosaic without the image being saturated by extraneous light from the high-reflectance epithelium requires considerable practice. The fundamental basis of the technique, namely *specular* (as opposed to diffuse) reflection of light, dictates that, on a convex reflecting surface (such as the cornea), there is, for a given position of light source and observer, only a *single point* that will produce a specular reflection. The practical implication is that the area illuminated should be kept as small as possible to reduce extraneous reflected light; a slit-beam 0·5×2 mm is in general adequate. An additional restriction, again dictated by this 'three-point' optical model, is that the specular reflection can be viewed only monocularly. Although the use of a second ocular (as available on the slit-lamp microscope) can provide a slightly larger field of view, one or both of the images must by definition be suboptimal. Furthermore with wider beams of incident light the low reflectance endothelial image is increasingly spoilt by light from the high reflectance epithelial surface (Fig. 1).

In this study the following technique was developed, based on the reasons detailed:

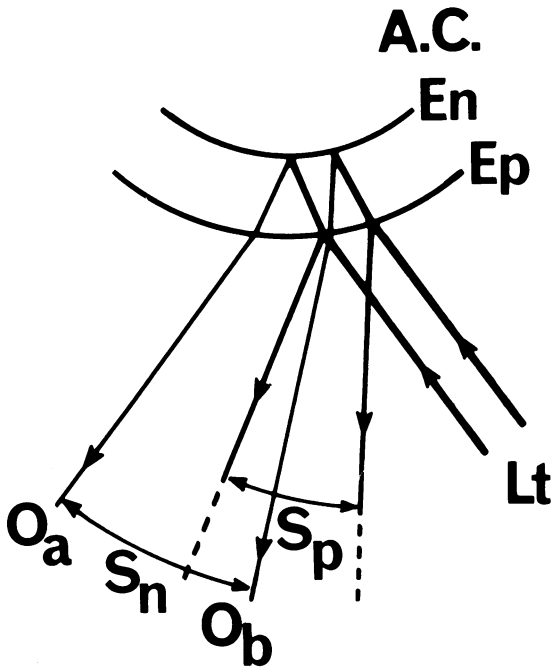It is imperative to adjust the eyepieces on the

Fig. 1 *Ray diagram of the specular corneal reflections produced by a collimated light source* (Lt); *only extreme rays are represented. The endothelium* (En) *with low reflectance, produces a zone of low-intensity specular endothelial reflection* (S$_n$), *this zone often being overlapped by a high-intensity specular reflection* (S$_p$) *from the epithelium. The degree of overlap is dependent on illumination beam width, corneal thickness (and refractive index), and the angle between the light-source beam and the observer. Observation at position* O$_a$ *would provide an endothelial image of optimum quality, whereas that at* O$_b$ *would be saturated by light reflected from the epithelium.* A.C. *denotes anterior chamber.*
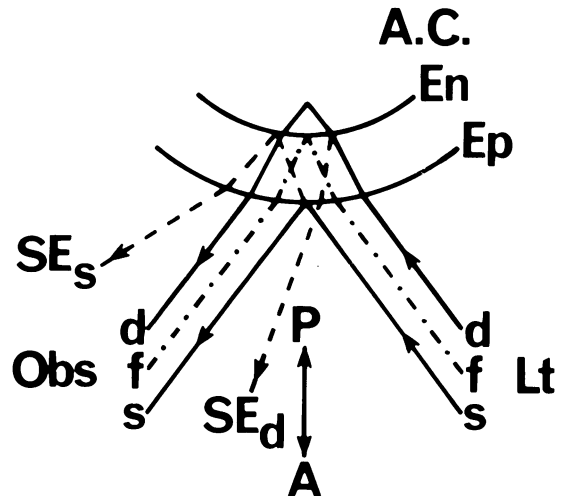

Fig. 2 *Effect of adjustment of the slit-lamp microscope, along the anteroposterior* (AP) *axis, on the specular endothelial reflection; ray diagram illustrating only single rays, passing from the light source* (Lt) *to the observer* (Obs). *When focused deeply* (d-d) *to the ideal position* (f-f), *the specular endothelial reflection bypasses the observer to reach* SE$_d$; *when focused superficially* (s-s), *the reflection bypasses the observer to* SE$_s$. A.C. *denotes anterior chamber.*
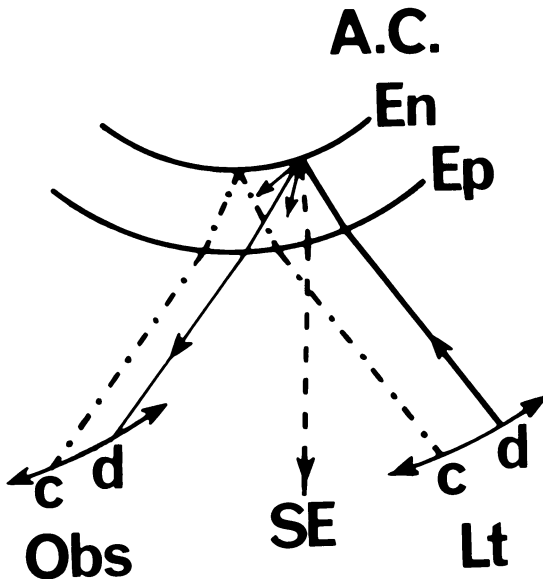
Fig. 3   *Effect of adjustment of the slit-lamp microscope, along a line* (cd) *parallel to the plane of the corneal endothelial surface; ray diagram illustrating only single rays from light source* (Lt) *to observer* (Obs). *When displaced* (d-d) *from the ideal position* (c-c), *the specular reflection from the endothelium bypasses the observer to reach* SE. *The image of the endothelium viewed by the observer at position* d *is that due to diffuse (non-specular) reflection at the endothelial surface (thin and short arrows).* A.C. *denotes anterior chamber.*

slit-lamp microscope to observer emmetropia. Ametropic settings, frequently found, contribute to a poor visualisation.

With the illumination beam locked at 20°–30° to the microscope boom, the endothelial reflection is focused in the anteroposterior axis (AP axis; AP on Fig. 2). If the focused deep to the endothelium, the specular image bypasses the observer (Obs) on the side of the illumination and vice versa (Fig. 2). In practice one maximises the intensity of reflected light by altering the AP position and brings into focus the mosaic if visible.

If the instrument AP axis does not correspond with the anteroposterior axis of the convex corneal surface (Fig. 3), then the specular reflection will again bypass the observer (Obs) even though, viewed by diffuse illumination, the endothelial mosaic might be in focus. To remedy this situation the slit-lamp mounting must be moved in an arc paralleling the corneal convexity (line cd; Fig. 3), until maximum specular reflectance of a focused endothelial image is achieved.

GRADING OF ENDOTHELIAL VISUALISATION
By means of this method the appearance of the mosaic was graded thus:

Grade 0:   Mosaic not visible at high-power objective setting on the slit-lamp ($\times$16).
Grade 1:   Mosaic barely visible ('grainy' image) at $\times$16.
Grade 2:   Mosaic visible at $\times$16 after >15 sec-. onds.
Grade 3:   Mosaic readily visible at $\times$16, within seconds, but not visible at low-power setting ($\times$10).
Grade 4:   Mosaic barely visible ('grainy' image) at $\times$10.
Grade 5:   Mosaic visible at $\times$10, after >15 seconds.
Grade 6:   Mosaic readily visible at $\times$10, within seconds.

ANALYSIS
By the use of generalised linear models which incorporate the statistical techniques of multiple regression and analysis of variance,[2] it was possible to determine the relation between the factors assessed clinically and the ECD derived from specular photomicrography.

The first group (group I) consisted of single eyes from 122 subjects, these eyes being unoperated upon. In all cases the right eye was chosen unless either the right eye had been previously operated upon or the subject's left eye was to undergo cataract extraction.

In the second group (group II) single eyes from 192 subjects were examined between 10 and 30 weeks after cataract extraction, with or without lens implantation. Seventy-six of these postoperative eyes were also examined preoperatively in group I.

In the third group (group III) were 53 eyes not operated upon. These eyes were twice assessed, in a masked manner, at between 10 and 30 weeks.

Results

The groups were well matched with respect to age: group I, 68·7±12·7 years (mean±standard deviation); group II 69·8±11·2 years; and group III 68·2±11·7 years. Similarly, they were matched for the proportion of diabetics (group I 10%, group II 10%, and group III 8%). The number of subjects within each of the visual gradings is summarised in Table 1.

Both in group I and in group II visual grading proved to be statistically a very highly significant predictor of cell density (F test; $p \ll 0.001$ for both groups). Within the preoperative group (group I), the presence of stromal opacity or disease proved also to be a significant additional predictive factor ($p < 0.01$), while in the postoperative group (group II), the grading of guttae appeared to be a weak, but sig-

Table 1 *Mean cell density for each clinical grading (with standard error of mean (SEM) and the number of values) within group I (preoperative) corneas, group II (post-operative) corneas, and within the combined groups I and II. Endothelial cell densities expressed as cells/mm²*

Group		Visual grading of corneal endothelium							Total
		0	1	2	3	4	5	6	
Pre-	Mean	2553	2289	2106	1861	642	—	—	
operative	SEM	60	40	61	220	—	—	—	
(Group I)	Number	28	53	34	6	1	0	0	122
Post-	Mean	2204	2225	1948	1694	1226	614	552	
operative	SEM	79	46	58	75	123	.56	—	
(Group II)	Number	22	74	63	22	8	2	1	192
Combined	Mean	2400	2252	2003	1730	1161	614	552	
(Groups	SEM	54	32	44	74	126	56	—	
(I&II)	Number	50	127	97	28	9	2	1	314

nificant, additional factor ($p<0.05$). In neither group was there a significant relation between ECD and the age of the subject, a history of previous eye disease or of diabetes, the degree of corneal arcus, or the central corneal thickness. Moreover within group II subjects the implantation of an intraocular lens was not a significant factor in predicting ECD after the significance of the visual grading was taken into account.

VISUAL GRADING
The mean ECD within each of the clinical visual gradings is represented in Fig. 4. As reasonably expected, the ease of visualisation (grading scale) increases as the ECD decreases (cell size increases).

The similarity between the gradings of the preoperative and the postoperative eyes is notable. In addition the observation that the mean ECDs in grades 0–3 are consistently lower in the postoperative compared with the preoperative corneas suggests that some postoperative factor—perhaps, for example, minimal corneal oedema or irregularities in Descemet's membrane—is acting to reduce slightly the visibility of the corneal endothelial mosaic (but not to a statistically significant degree).

REPRODUCIBILITY
In the 53 corneas tested for intraobserver variation (group III) there was no significant change in corneal thickness measurements; the mean difference between initial and repeated measurements was 0·0038 mm (initially larger; standard deviation of difference=0·0065, $p>0.3$). Similarly, the mean difference between ECDs (18 cells/mm², initially larger) was not significant (SD difference=64, $p>0.5$).

Table 2 *Reproducibility of the grading of 53 corneas. The number within each combination of initial and repeated grading is indicated; values on the line of identity are underlined*

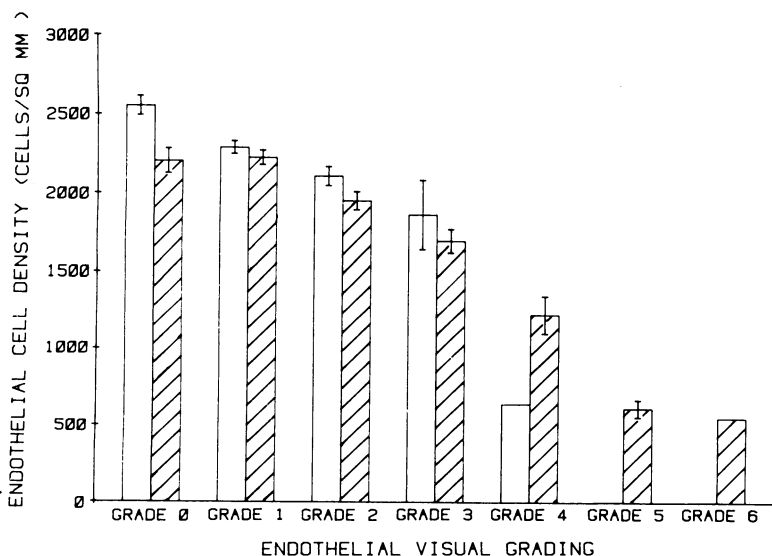| Initial visual grading	Repeated visual grading			
	0	1	2	3
0	4	6	1	0
1	1	20	3	2
2	0	5	8	1
3	0	0	1	1

Fig. 4 *Mean endothelial cell densities for each visual grading of preoperative corneas (open bars) and postoperative corneas (cross-hatched bars); error bars represent mean ± one standard error. Number of corneas in each group is summarised in Table 1.*

Table 3 *Percentage probabilities that a cornea of known endothelial visual grading would lie within each of four endothelial cell density ranges (see text for example); cell densities expressed as cells/mm². With the exception of grade 6, for each visual grading the probabilities within each grade are calculated from estimates of the mean and standard deviation for that grade (values in Table 1) and the distribution of the Student's t statistic; estimates were derived from a 'pooling' of group I and group II corneas (total number=314). The estimates for grade 6 were derived from a pooled estimate of standard deviation and the distribution of the z statistic*

Range of cell density	Visual grading of corneal endothelium							Surgical comment
	0	1	2	3	4	5	(6)	
>1500	>99%	98%	88%	72%	20%	3%	(>1%)	'Safe.' Unlikely to decompensate, even after major surgery
1500–1000	<1%	2%	11%	24%	46%	4%	(11%)	'Safe' for majority of surgery. 'At risk' for procedures with major or continued cell loss
999–500	0%	0%	1%	3%	28%	73%	(39%)	'At risk' for majority of surgery and major procedures likely to decompensate cornea
<500	0%	0%	0%	<1%	6%	20%	(49%)	Corneal decompensation inevitable

There was, however, some variation on repeated visual grading of the corneas (Table 2). Most notable is the slight tendency towards increased visualisation (higher grading) on repeating the assessment. Thirty-three corneas had the same initial and repeated grading, more than the 20 agreements expected by chance alone.

Discussion

It has been demonstrated that the corneal endothelial mosaic can usefully be graded, albeit with some variability, and that this grading is closely related to the central corneal ECD as determined by specular photomicrography. This fast method requires neither special grids or graticules nor the processing and time-consuming assessment of specular photo-micrographs. However, the grading not only requires the observer to have a normal visual acuity, but also can be difficult in the presence of stromal opacity or of small eye movements. Indeed, the author considers it to be a wise precaution in clinical practice to increase a grading by one category in the presence of persistent small eye movements.

The grading is subject, without doubt, not only to variability when used by one observer (visualisation improves markedly with practice of specular illumination), but also to considerable variation between observers. Because of this the grading is *not* intended as a substitute for the objective record of photo-microscopy, but rather as an encouragement for the neglected clinical art of specular illumination, as a method of recording approximate ECDs prior to surgery and as a means of assessing endothelial losses when repeated measurements are made by any one observer.

VISUAL ACUITY AND THE LIMIT OF CELL RESOLUTION
By calculating the angular visual resolution required to see those cells at the limit of slit-lamp resolution (i.e., those cells in grade 1), this calculated angular resolution is found to be in close agreement with the generally accepted optical resolution of the healthy eye. This calculation, demonstrating concordance between observation and theory, is presented in Appendix I.

CLINICAL USAGE OF THE GRADING SYSTEM
Not only does the system of grading provide an indication of the mean ECD in both preoperative and postoperative corneas (Fig. 4), but also the deductions may be extended to provide a guide more useful in clinical practice (Table 3).

From the mean cell density and variance estimates from the combined results of groups I and II (Table 1), for each visual grading the probability of a subject lying within one of four surgically relevant ECD ranges (>1500, 1500–1000, 999–500, and <500 cells/mm²) can be estimated (Table 3). Thus, if a patient has a clinical grade 4 corneal endothelium, he has (based on the present series) a 20% probability of having a cell density of greater than 1500 (and tolerating major anterior segment surgery), a 46% chance of having a density of 1000–1500 (where major surgery may jeopardise the corneal compensation), a 28% chance of having a cell density of 500–1000 (where corneal decompensation becomes likely after surgery), and a 6% probability of having a cell density below 500 cells/mm².

In clinical practice these 'risk' factors can be weighed against the necessity of the surgery and the life expectancy of the patient.

Appendix I

From the mean ECD of grade 1 visualisations a grading at the limits of resolution at 16-fold magnification—

D, the mean ECD (pooled estimate group I and group II, $n = 127$) is given by:

$$D = 2250 \text{ cells/mm}^2;$$

A, the mean cell area is thus given by:

$$A = 1/D$$
$$= 4 \cdot 4 \times 10^{-4} \text{ mm}^2.$$

If the shape of the cells is taken to be circular, then diameter, d, is given by:

$$d = 2\sqrt{A/\pi}$$
$$= 2 \cdot 4 \times 10^{-2} \text{ mm}.$$

At the usual slit-lamp microscope image distance of 250 mm, the angle G subtended in the vertical meridian* by such a cell, unmagnified, is given by:

$$G = d/250$$
$$= 9 \cdot 5 \times 10^{-5} \text{ radians}$$

But, with ×16 (high-power) magnification, the image subtends an angle, G', given by:

$$G' = 16 \times G$$
$$= 1 \cdot 5 \times 10^{-3} \text{ radians}.$$

However, the limit of resolution is set by the width of the cell boundaries as contrasted on specular illumination. From analysis of specular micrographs the cell boundary width is found to be between approximately one-seventh and one-tenth of the cell 'diameters' (personal observation).

Thus the angular visual acuity, V, required to resolve the endothelial mosaic under the defined conditions is given by:

Range of $V = G'/7$ to $G'/10$

$$= 2 \cdot 2 \times 10^{-4} \text{ to } 1 \cdot 5 \times 10^{-4} \text{ radians}$$
$$= 0 \cdot 75' \text{ to } 0 \cdot 5' \text{ arc}.$$

This calculated angular visual resolution ($0 \cdot 5' - 0 \cdot 75'$) is of the same order of magnitude as that generally accepted to be the optical resolution of the healthy eye.

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* The obliquity of slit-lamp microscope specular illumination results in a small reduction in image sizes in the horizontal meridian. This 'scaling factor' approximates to the cosine of A/2, where A is the angle subtended by the illumination and the observer axes at the corneal surface. In practice this factor varies between the negligible values of 0·98 and 0·96 if specular illumination is practised as detailed.

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