Reassessment of the corneal endothelial cell organisation in children

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Abstract

Aim—To assess uniformity of the corneal endothelial cell mosaic in children.

Methods—36 healthy children (5–11 years old, 16 boys, 20 girls) were assessed by specular microscopy. Endothelial cell density (ECD) was calculated from measured cell areas, and the number of sides/cell noted.

Results—Average values for ECD and cell areas were 3987 cells/mm² (95% CI 3806 to 4168 cells/mm²) and 278 (SD 85) µm² respectively, with normal distribution (COV 28.2%, range 17.4 to 39.2%) and with the average percentage of six sided cells being 66.6% (8.8%). Cell area was positively correlated to number of cell sides (p < 0.01, r²=0.993), but the percentage of six sided cells was negatively correlated to ECD (p < 0.01, r²=0.493).

Conclusion—A high ECD occurs in children, but this does not mean there is a high percentage of “hexagons”.

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The clinical specular microscope¹⁻³ has been widely used on many different types of individuals with and without a range of eye diseases.¹ It has become commonplace to assess endothelial cell density (ECD) and, perhaps the percentage of six sided cells (“hexagons”), particularly within the central corneal region.¹⁶ As part of ongoing studies to assess the corneal endothelium in more detail,¹⁷ new methods were undertaken on children because reports on the corneal endothelium of children have been infrequent and the data differ somewhat. These studies confirmed that the corneal endothelium in children has a higher ECD than in young adults.¹⁵⁻²⁰

The corneal endothelium has been considered to be essentially established as a uniform monolayer at some period shortly before birth, and then undergoes a progressive change with age that introduces non-uniformity into the monolayer.¹⁴ A key element of this uniformity is the presence, in relatively large numbers, of the six sided “hexagonal” cell,⁶⁻⁸ and it is likely that many would consider the corneal endothelium in children to be composed of a very high percentage of six sided cells.¹⁵⁻¹⁷ In exploratory studies on a small group of children, a much higher than expected degree of non-uniformity within individual endothelia was encountered (unpublished results) even though the intersample variance in ECD was not substantial. These studies have thus been expanded and confirm that the endothelium of healthy white children can show quite a high degree of non-uniformity despite a high ECD, notably with respect to the percentage of six sided cells.

Materials and methods

The 36 subjects recruited were mainly children of members of staff (at Glasgow Caledonian University) and patients attending the university eye clinic. The protocol was approved by a departmental and university ethics committee. Informed consent was provided by all parents. On the basis of responses to specific questions, all subjects were considered to have no major systemic disease and no history of significant eye disease or surgery. Fourteen of the children reported having had chickenpox, five had had measles, and one rubella (German measles), although no serological confirmation of these infections was sought in this study. Two children were reported to have an intermittent asthma history, but were not regularly using medications. The children were aged between 5 and 11 years of age (mean 8.3 years, median 8 years) with 16 boys and 20 girls. Only seven of the children were habitual spectacle wearers for mild to moderate refractive errors only (average spherical refractive error = −0.125 D, range −2.75 to + 4.75D).

A Topcon SP 1000 non-contact specular microscope was used to obtain a single micrograph of the central corneal endothelium of the left eye and which was analysed as previously detailed after a scale bar was affixed to the micrograph.⁷⁻⁸ A series of other measurements were taken on the same eye, or using both eyes (refractive error, visual acuity), which were designed to provide a general perspective on the cornea and eye of the children. Refractive error was measured with a Nikon NK2000 autorefractor. The anterior radius of curvature was averaged across the two principal meridians using a Javal-Schiotz type keratometer. Central corneal thickness (average of three measures) was measured by ultrasound pachymetry (Echopach pachymeter, 3M Canada Inc) after instillation of Minims Betaneal 0.4% (oxybuprocaine, Chauvin), and a hand held non-contact tonometer (Keeler Pulsair 2000) was used for IOP measurement (average of three measures taken).

Results

The characteristics of the study group are summarised in Table 1. None of the measures reveal anything remarkable about the set of children studied. A typical micrograph is shown in Figure 1. The average area sampled from each endothelium was 0.036 mm² (range 0.025–0.048 mm²).
Evaluation of the endothelial cell density (ECD) across the 36 specular micrographs revealed a group averaged value of 3987 cells/mm² (median value 3974 cells/mm², 95% CI 3806 to 4168 cells/mm²), with the mean value for any one endothelium ranging from 2627 to 5316 cells/mm². The average ECD in the boys was 3904 (SD 545) cells/mm² and in the girls was 3906 (SD 552) cells/mm², without statistical difference. Using an unpaired Student’s t test, these sets of values were not found to be statistically different (p = 0.21). A comparison between the sets of data from those children who had experienced normal childhood ailments (chickenpox, measles, and German measles) with those who had not revealed no statistical difference (one tailed Student’s t test, p=0.38). Such endothelial characteristics are unremarkable and generally consistent with other reports (see discussion and Table 2).

Analyses of the cell areas found within and across the set of 36 micrographs revealed average values for any particular endothelium to range from 208 to 396 µm² and a group averaged mean area value of 282 µm². Over the entire set of endothelial images, the cell areas ranged from 39 to 787 µm² and the overall median area was 275 µm² from the entire set of 4834 cells (which was almost identical to the group averaged median area of 280 µm²). The distribution of cell areas is shown in Figure 2, both as pooled (A) and as group averaged histograms (B). These histograms indicate that there is little gross irregularity (polymegethism) in the endothelia analysed, as judged by the normal distribution of cell area values. The overall coefficient of skewness on the distribution averaged 0.409 (range −0.867 to 1.424) and the kurtosis averaged 0.904 (range −0.356 to 6.922). The normalised standard deviation of cell area values (the coefficient of variation, COV) is often used as a measure of uniformity, or non-uniformity, in the endothelial mosaic and these values averaged 28.2%, range from 17.4 to 39.2%. The endothelium depicted in Figure 1 is thus representative of the overall average data from the set of 36 children in that its ECD was 4050 cells/mm², the average cell area was 275 µm² (median 285 µm²) and its COV was 28.6%. A more detailed evaluation of the composition of the samples of endothelial mosaic provides a rather different perspective however.

The overlay generated from the micrograph of Figure 1 is shown in Figure 3. The six sided cells are indicated and comprised 80 of 131 cells in this example—that is, 61.0%. Overall, the 36 endothelial samples contained an average of 66.6% (SD 8.8%) six sided cells, with these cells having an average area of 285 (34) µm². Similar proportions of five sided and

### Table 1 Study group characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Average value</th>
<th>Range of values</th>
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<tbody>
<tr>
<td>Age</td>
<td>8.3</td>
<td>5 to 11 years</td>
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<tr>
<td>Refractive error (D)</td>
<td>−0.125</td>
<td>−2.75 to +4.75</td>
</tr>
<tr>
<td>Central corneal thickness (SD mm)</td>
<td>0.529 (0.036)</td>
<td>0.441 to 0.605</td>
</tr>
<tr>
<td>Anterior corneal curvature (SD mm)</td>
<td>7.73 (0.20)</td>
<td>7.05 to 8.35</td>
</tr>
<tr>
<td>IOP (SD mm Hg)</td>
<td>15.4 (2.4)</td>
<td>10 to 21</td>
</tr>
</tbody>
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**Figure 1.** Typical non-contact specular micrograph of the corneal endothelium of a child. The ECD is 4050 cells/mm² compared with the group averaged value of 3987 cells/mm² (see Results).

**Table 2 Summary of corneal endothelial studies in children**

<table>
<thead>
<tr>
<th>Ref No</th>
<th>Year</th>
<th>Age of children</th>
<th>ECD (cells/mm²)</th>
<th>Range of ECD values (cells/mm²)</th>
<th>Percentage six sided cells (% hexagons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1986</td>
<td>3–21 days</td>
<td>6100</td>
<td>3450–5600</td>
<td>82 (4.5) (X (SD))</td>
</tr>
<tr>
<td>16</td>
<td>1987</td>
<td>&lt;12 months</td>
<td>4425</td>
<td>3000–5700</td>
<td>59 (6.7) (X (SD))</td>
</tr>
<tr>
<td>18</td>
<td>1989</td>
<td>&lt;12 months</td>
<td>4252</td>
<td>3947–5305</td>
<td>73 (1.4) (X (SD))</td>
</tr>
<tr>
<td>19</td>
<td>1992</td>
<td>&gt;5 weeks</td>
<td>4291</td>
<td>3800–5700</td>
<td>66.6 (8.8) (X (SD))</td>
</tr>
</tbody>
</table>

*These ECD estimates were obtained by using the authors’ own scaling factor (>210) to change counts/cornea into cells/mm².
†From regression analysis.
‡Postmortem, histological study.
seven sides cells were present in all the endothelia (that is, 15.2% (3.9%) and 14.6% (3.7%) respectively) with their average areas being 202 (31) µm² and 356 (44) µm². Most of the endothelia (27 of 36) also contained at least one four sided cell (average 1.9% (1.4%) of the cells assessed), while 26 of 36 endothelia contained at least one eight sided cell (average 1.8% (1.0%)). The average area of the four sided cells was very small (at 94 (21) µm²) while the eight sided cells were much larger (442 (99) µm²). Only two endothelia contained a solitary nine sided cell (average area 608 µm²). The relation between the cell areas, with respect to the number of sides from four to eight inclusive, was linear (Fig 4). A linear regression analysis across the group averaged mean cell areas for each cell type (Fig 4A) was highly significant (p <0.01; r²=0.993; 4 dF, F ratio=543). The average area of the nine sided cells (at 441 µm²) also fits on the same regression line. A Kruskal-Wallis analysis across the sets of average cell area values (Fig 4B) was also highly significant (χ² =142, 4 dF, p <0.01). Further analyses also revealed that the relation between the percentage six sided cells and the ECD was similarly significant (p <0.01), but with a substantial negative correlation (Fig 5). A linear regression analysis yielded a Pearson correlation coefficient of r= 0.492 (p <0.01, F ratio = 10.92). Plots of the coefficient of skewness, on the cell area distributions for any one endothelial micrograph, against either ECD or the percentage six sided cells in the micrograph revealed no significant correlations (p >0.2). Such a lack of correlation confirms that the set of endothelial samples examined were relatively uniform and that the negative correlation between ECD and percentage six sided cells was not due to mosaic samples with a skewed cell distribution (that is, outlier effects).

Discussion

The corneal endothelial monolayer, when fully developed, is considered vitally important for maintaining corneal clarity.21 22 It has still however to be quantitatively established in what way the organisation of the corneal endothelial mosaic is related to its function.482 3 A decrease in cellularity and increase of the corneal surface to be covered results in changes in endothelial cell organisation, size, shape, and thickness and, by the eighth month, the endothelial cells are considered to be active and fully functional.13 24 High ECD values (>4200 cells/mm²) have been reported just after birth or in infancy (Table 2).
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Thereafter, ECD values have been reported to be lower (Table 2), although the kinetics of the change in ECD with time have yet to be well defined—for example, as a linear or logarithmic function.7

The present studies reveal an unexpected extent of non-uniformity in cell shape (pleomorphism) in the endothelia of normal healthy children. As noted earlier, some consider the corneal endothelium in children to be composed of a very high percentage of six sided cells, 1, 5, 15–17 but there are few published data to support such an idea. There have certainly been quite a few studies on the corneal endothelia in children, 5, 6, 20, 25–30 but few details on the actual number of six sided cells (the “hexagons”) in these endothelia have been published (see Table 2). A number of investigators have presented data to indicate that the percentage of hexagons decreases as a function of age. 21, 25 Kim et al25 report that children aged <10 years had 88% hexagons, while an average value of 82% was reported by Tusukuhara et al26; both studies are, however, on non-white eyes. Others have provided regression analysis of age dependent changes from which the percentage of hexagons could perhaps be estimated—for example, the regression analyses reported Carlson et al26 would yield a value of around 82% for 5–10 year olds, while a study by Suda, 27 would yield an estimate of 73–75%. A range of values for the percentage of six sided cells in children has thus been reported, and such a range is important to note. Some studies would be consistent with a concept that children’s endothelia are regular in appearance, and include a very high percentage of hexagons.7 20, 20 However, other studies have considered that the corneal endothelia in any very young individuals (including human) should contain cells that were smaller and more pleomorphic compared with those in adults (although specific data for human endothelia were not provided).14 Such an early life pleomorphic appearance is especially remarkable for feline endothelia.27

The consistent finding of subtle non-uniformity in the endothelia of apparently healthy young children suggests therefore that this is a normal attribute of these endothelia and that a further reorganisation has yet to occur to realise the highest values for percentage hexagons (and after which an age dependent decline could then occur). A fairly wide range of ECD values were found (2627–5316 cells/mm²) and it is readily acknowledged that there are a variety of factors that might be responsible for the differences. Such factors could include eye size, refractive error, corneal curvature and corneal thickness. For example, an analysis of the possible relation between horizontal corneal diameter reveals a negative correlation that just reaches statistical significance (p = 0.05, F ratio = 4.111, r²=0.332). Further and more extensive studies are thus currently in progress to try to establish which, if any, of these factors might play the most important role in determining the uniformity of the corneal endothelium in children.

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