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Relationship Between Morphology and Functional Ability of Regenerated Corneal Endothelium

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Cat corneal endothelium was damaged by a methylmethacrylate segment introduced into the anterior chamber. Within 3 to 6 months after each such operation the endothelial cell density (ECD) diminished, ranging from 1933 to as low as 450 (78% to 16%, respectively, of its preoperative value [PV]). Decrease in the ECD to between 1050 and 1150 (40% to 45% of PV) made no impact on the corneal thickness (group 1: “non-swollen” corneas); further decrease was followed by an increase of the corneal thickness to a level of 11.5% to 73.5% more than its preoperative value (group 2: “swollen” corneas). The coefficient of variation of ECD increased in both groups. The number of hexagonal endothelial cells diminished, while cells with three or nine or more facets appeared. All these changes were much more pronounced and verifiable in the group 2 corneas, which were distinguished by the appearance of giant endothelial cells, 2957–4095 \( \mu \text{m}^2 \), ie, 7.5–13.5 times larger than the cells of undamaged endothelium. These data appear to indicate that the decrease of ECD to 1050 to 1150 (40% to 45% of its PV) does not provoke decompensation of the dehydrative function of the endothelium, whereas further decrease does provoke such decompensation, the giant endothelial cells probably being the main factor in this phenomenon. Invest Ophthalmol Vis Sci 29:1100–1109, 1988

The corneal endothelium is responsible for preservation of the transparency of all corneal layers. The endothelium regulates the ion composition of the various corneal layers, thereby maintaining constant osmotic pressure, permitting permanent hydration of the cornea, and thus constant thickness and transparency. Consequently, any disturbance of endothelial functions provokes corneal edema followed by partial or complete loss of transparency.

There is a loss of corneal cells throughout life. Furthermore, endothelial cells are frequently damaged or destroyed in operations involving opening of the anterior chamber. The low regenerative ability of the human endothelium, and that of nonhuman primates and carnivores such as cats and dogs, precludes complete regeneration of the endothelial layer after such operations. Therefore, damage by trauma or loss through aging is compensated by growth in size of endothelial cells which cover denuded surfaces of Descemet’s membrane.

We investigated cat corneal endothelium cells, which were modified by injury and healing. We used a quantifiable model of post-traumatic corneal reendotheliazation to measure morphological aspects of the endothelium (cell size, variability of endothelial cell density [ECD], polygonality) and relate the numerical findings to a physiological parameter of the endothelium (corneal thickness).

Materials and Methods

The experiments were performed on street cats weighing from 2.4 to 3.8 kg. The animals were anesthetized for all operations and measurements by intramuscular injection of ketamine (0.25 mg/kg) and intravenous injections of phenobarbitone sodium (2.5 mg/kg). The investigations were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

Endothelial Scraping

Endothelium was scraped from Descemet’s membrane of 57 cats by introducing into the anterior chamber a well polished methylmethacrylate sphere, pressing it against the cornea and rotating it, as previously described. Using this method the endothelium only and none of the other corneal layers, including Descemet’s membrane, was affected. Eight to 10 weeks after the operation 17 cats were reoperated. The incision was made in the postoperative scar.

Nineteen sham operations were also performed, in which no tool was inserted after the limbus incision.
Table 1. Number and size (mean ± SD) of endothelial cells in the flat preparations of the various types of corneas

<table>
<thead>
<tr>
<th>Type of corneas</th>
<th>No. of corneas</th>
<th>Total no. of investigated endothelial cells</th>
<th>No. of endothelial cells investigated in one cornea</th>
<th>Endothelial cell size (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>7470</td>
<td>498 ± 77</td>
<td>302.8 ± 50.8</td>
</tr>
<tr>
<td>After one sham operation</td>
<td>7</td>
<td>2967</td>
<td>424 ± 22</td>
<td>327.3 ± 45.5</td>
</tr>
<tr>
<td>After two sham operations</td>
<td>5</td>
<td>2071</td>
<td>414 ± 90</td>
<td>316.8 ± 66.5</td>
</tr>
<tr>
<td>After scraping: non-swollen (Group 1)</td>
<td>9</td>
<td>2169</td>
<td>241 ± 86</td>
<td>743.8 ± 271.2</td>
</tr>
<tr>
<td>After scraping: swollen (Group 2)</td>
<td>11</td>
<td>2101</td>
<td>191 ± 59</td>
<td>1223.3 ± 761.2</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>16,778</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and the wound was closed immediately. In nine cats a second sham operation was performed.

Biomicroscopy and Specular Microscopy

The corneas were investigated biomicroscopically as well as by specular microscope with a built-in digital pachometer (Heyer Schulte Medical Optics Center, Irvine, CA; Model CEM-4) to determine the central ECD and central corneal thickness. This was done before the operation and 1, 2, 3, 4 and 6 months afterwards. The number of endothelial cells was counted on the TV screen of the specular microscope in six random 0.01 mm² squares. Only cells half of whose area was found to be included within the borders of the squares were counted. The ECD, ie, the number of cells per 1 mm², was calculated as the mean ± SD of endothelial cells over the squares multiplied by 100.

Coefficient of variation of ECD was defined as the ratio of the standard deviation of ECD to its mean values. The coefficient of variation is for the ECD of the six cell counts from the six individual 0.01 mm² squares. This coefficient reflects the comparative variability in size of the endothelial cells. We present the coefficient of variation of the ECD rather than that of the cell area because the former is easier to calculate and thus is clinically more significant.

In addition to the sham operated eyes, 19 normal, nonoperated eyes were also investigated, using the same methods and at the same time intervals.

For better comparison of results the postoperative values of both ECD and corneal thickness were presented as percents of their preoperative value, and in normal corneas as percents of values recorded at the first investigation.

Histology

Most of the investigated eyes were enucleated. Eleven, 18 and 21 eyes were enucleated 1, 3 and 6 months, respectively, after either one or two endothelial scrapings. Six, six and seven eyes were enucleated after similar periods following the sham operations. In the case of normal eyes, seven, seven and four eyes were enucleated after 1, 3 and 6 months after the first ophthalmological investigation. The corneas were cut together with a strip of sclera 1 mm in width. Endothelial flat preparations were performed by the impregnation method.

Cell Size and Polygonality

The endothelial cell size and cell polygonality (Table 1) were determined on photomicrographs of the corneal endothelial flat preparations taken from 3 to 6 months after test operations, sham operations, or initial investigation (in the case of normal corneas), using digital planimeter Planix-9 (Tamaya, Tokyo, Japan). These counts were made from the flat preparations since in the specular microscopy photographs the corneal center with its maximal changes could not be accurately localized. Since the silver impregnation process causes some shrinkage of the cells, counts thus obtained were compared only with values similarly obtained and not with specular microscopy data.

For this purpose randomly chosen plots of endothelium with a 2.5 mm radius (at X320 magnification in the microscope) were photographed in the central part of the endothelium. Each normal or sham-operated cornea was photographed in five different plots. The endothelium of the operated corneas, whose cells were larger than normal ones, was photographed eight to 15 times. In order to exactly determine the magnification for each photomicrograph, the eyepiece micrometer scale was also photographed.

In all, 16,778 endothelial cells in 47 corneas were investigated (Table 1). The data were processed in the Visual-50 (Northstar Co., San Leandro, CA) computer with the help of the microstate program, through which endothelial cell distribution according to cell size can be obtained (Table 2, Fig. 9).

Results

Corneal Thickness (Fig. 1)

The operated corneas were divided into two groups. Group I included those corneas that did not
Table 2. Distribution of endothelial cells 3 to 6 months after operations (number of cells and area they occupy [as percentage of total cell number and total cell area respectively]) according to cell size in the various types of corneas

<table>
<thead>
<tr>
<th>Type of corneas</th>
<th>No. of corneas</th>
<th>Distribution of cells</th>
<th>Cell size in (\mu m^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>52.5–105</td>
<td>157.5</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>Number</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>—</td>
</tr>
<tr>
<td>One sham operation</td>
<td>7</td>
<td>Number</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>—</td>
</tr>
<tr>
<td>Two sham operations</td>
<td>5</td>
<td>Number</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>—</td>
</tr>
<tr>
<td>Scrapping: non-swollen</td>
<td>9</td>
<td>Number</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>0.03 ± 0.06</td>
</tr>
<tr>
<td>Scrapping: swollen</td>
<td>11</td>
<td>Number</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>0.02 ± 0.05</td>
</tr>
</tbody>
</table>

* Cells ranged from 420–525 \(\mu m^2\) only.

swell permanently after surgery and group 2 included corneas that did swell permanently. In group 1 ("non-swollen corneas"), 1 month after scraping a 16.4% increase in thickness was observed compared with normal corneas and a 13.4% and 13.6% increase, respectively, as compared with once and twice sham operated corneas \((P < 0.001)\) for all corneas. Two months after operation the differences were negligible (4.4% for normal and 3.7% for twice sham-operated corneas) but nonetheless significant \((P < 0.001\) and \(P < 0.05\), respectively). In later observations (3 and 6 months after operation), no differences in thickness between operated, sham-operated and normal corneas were observed.

In group 2 ("swollen corneas") the increase of corneal thickness after scraping was prolonged and significant \((P < 0.001)\). At all stages of observation the difference in corneal thickness between the two

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**Fig. 1.** Central corneal thickness in percentage of preoperative value in normal, sham-operated and endothelial scraped corneas. Numbers in rectangles—months after the first investigation (normal corneas) or after the operations. Each column signifies mean ± standard deviation.

<table>
<thead>
<tr>
<th>Type of corneas</th>
<th>Months after first investigation (normal) or operations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>19</td>
</tr>
<tr>
<td>After one sham operation</td>
<td>19</td>
</tr>
<tr>
<td>After two sham operations</td>
<td>9</td>
</tr>
<tr>
<td>Non-swollen corneas</td>
<td>35</td>
</tr>
<tr>
<td>Swollen corneas</td>
<td>30</td>
</tr>
</tbody>
</table>

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groups of operated corneas (from 22.5% to 28.9%) was significant ($P < 0.01 - P < 0.001$).

A slight but significant difference in thickness was observed between normal and sham-operated corneas 1 and 4 months after sham operations (3.3%, 5.7%; $P < 0.005$, $P < 0.01$, respectively).

Endothelial Cell Density (Fig. 2)

In the group 1 corneas the decrease in ECD throughout all stages of observation ranged from $1454 \pm 219$ to $1575 \pm 200$ (56.0% ± 13.2 to 58.2% ± 10.8, respectively, of preoperative values). Nuclei of the enlarged cells were oval and of greater size (Fig. 3). In group 2 the decrease was even sharper: 709 ± 185 to 842 ± 237 (26.1% ± 7.2 to 30.5% ± 7.8 of preoperative values). In the latter group endothelial cells and their nuclei were markedly enlarged and one or two nucleoli were seen inside the nuclei (Fig. 4). Within each group no significant difference in ECD was observed between various observation stages.

There was no difference between ECD of sham-operated and normal corneas (2261 ± 480 and 2499 ± 211, respectively); in both the endothelial nuclei were bean-shaped (Fig. 5).

Coefficient of Variation of Endothelial Cell Density (Fig. 6)

This was significantly greater for group 1 than for the normal corneas ($P < 0.001$) in all observation
stages and for sham-operated corneas only 1 and 2 months after sham operations. There was no significant difference in this parameter between the corneas of group 1 and twice sham-operated corneas.

Coefficient of variation of ECD for group 2 was significantly greater than that for normal corneas ($P < 0.001$), for sham-operated corneas ($P < 0.05-0.01$) and for all group 1 corneas ($P < 0.05-P < 0.001$). Great variability in endothelial cell size was observed on limited parts of the central zone (Fig. 7).

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**Fig. 4.** Endothelium of the swollen corneas. Very enlarged endothelial cells with big nuclei and one or two nucleoli. Left, 1 month after two endothelial scrapings. Right, 6 months after two endothelial scrapings. Endothelial flat preparation (silver-hematoxylin). Original magnification $\times 320$.

**Fig. 5.** Endothelium of the sham-operated corneas. The cells are normal in size and their nuclei are not changed. Left, 6 months after one sham operation. Right, 6 months after two sham operations. Endothelial flat preparation (silver-hematoxylin). Original magnification $\times 320$. 

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Y.
Relationship Between Endothelial Cell Density and Corneal Thickness

As shown above, the mean value of ECD for the group 1 corneas was approximately twice that of the group 2 corneas. Nevertheless, these mean values are insufficient for determination of ECD values characteristic for distinguishing between endothelial cells from compensated and decompensated corneas. In order to determine these values the graph of relationship between ECD and corneal thickness was established (Fig. 8). The respective data were obtained before enucleation—3 and 6 months after endothelial scrapings (38 eyes) or one or two sham operations (13 eyes), and in the case of normal, nonoperated eyes (12 eyes), 3 and 6 months after initial investigation.

As can be seen on the graph (Fig. 8), the endothelium is able to support the normal dehydration of the cornea when ECD decreases to as low as 1050–1150 (40% to 45% of its normal [preoperative] value). Further decrease induces disturbances of the dehydration function of endothelium and thus corneal swelling.

Distribution of Endothelial Cells According to Cell Size (Fig. 9)

In normal and sham-operated corneas, the size of the vast majority of endothelial cells was between 210–367.5 μm² (median 297.5–320 μm²) (Table 2), with a slightly increased quantity of large cells (420–577.7 μm²) in the sham-operated corneas. No larger cells were observed in these corneas.

The distribution curve for endothelial cells in the non-swollen corneas (group 1) became flatter and moved to the right. The proportion of cells of characteristic size (210–367.5 μm²) decreased from 95.9%
in normal and 92.6% in sham-operated corneas to
11% in non-swollen corneas (Table 2). The number
of larger cells increased markedly: their size reached
2205 \( \mu \text{m}^2 \), ie, 7.3 times greater than average size of
endothelial cells for normal corneas, while the me-
dian increased up to 577.5 \( \mu \text{m}^2 \) (Table 2) and average
cell size reached 743.8 \( \mu \text{m}^2 \) (Table 1).

The distribution curve for the swollen corneas
(group 2) became even flatter and moved further to
the right. The median rose to 850 \( \mu \text{m}^2 \) (Table 2) and
the average cell size reached 1223.3 \( \mu \text{m}^2 \) (Table 1).
Giant cells of 2257.5-4095 \( \mu \text{m}^2 \), ie, 7.5-13.5 times
the average size for the cells of non-damaged endo-
thelium emerged. These cells constituted 2.5% of the
total cells of the swollen corneas, while the area occu-
pied by them was 8.1% of the endothelial surface
(Table 2).

**Polygonality (Fig. 10)**

There was no significant difference in polygonality
of the endothelial cells between normal and sham-
operated corneas. These cells were predominantly
hexagonal, with half as many five- and seven-faceted
cells and a negligible number of four- and eight-fac-
eted cells.
The number of hexagonal cells in both groups of operated corneas decreased sharply, with fewer in group 1 than in group 2 \((P < 0.001)\), whereas the number of five- and seven-faceted cells increased significantly \((P < 0.001)\), more pronouncedly in group 2 than in group 1 \((P < 0.05, P < 0.001)\). In both groups there were three-, nine- and more-faceted cells, which were not observed in normal and sham-operated corneas; their number was significantly greater in the corneas of group 2 \((P < 0.001)\).

**Discussion**

The endothelial cells present following regeneration either after a trauma of the corneal endothelium in mammals or humans, or following the decline in the number of these cells due to ageing are different from normal ones in density, size and shape.6-19

In the present investigation a wide range of ECD variability was obtained: from 1933 to 450 (16% to 78% of the preoperative values). This is due to the use of street cats of unknown age and varied size (weight range from 2.4 to 3.8 kg) in our experiments. Thus, their corneas were of different sizes and scraping of the endothelium with a methylmethacrylate segment of constant diameter yielded portions of the denuded endothelium which differed from one another in size. The procedure was performed once in some cases and in others it was repeated 8 to 10 weeks later. The various combinations of these parameters (age, corneal size and number of scrapings) undoubtedly contributed to the range of ECD in our experiments.

The experimental corneas fall postoperatively into two distinct groups according to the degree of hydration, as determined by the changes in their thickness. The thickness of the non-swollen corneas (group 1) was not different 3 to 6 months after the endothelial scraping, when ECD was somewhat less than normal, but not lower than 1050 to 1150 (40% to 45% of the initial value). The ECD in group 2 was less than that in group 1 and the corneas were swollen during all observation periods. The 1050 to 1150 "demarcating line" found in our experiments is in line with findings in experiments on other species and in clinical observation. In rabbits, very rapid regeneration of the corneal endothelium has been observed, with full reestablishment of the endothelium and of corneal thickness. However, there is a certain period within the regeneration process when ECD is still diminished even though corneal thickness has been reestablished.21,22 Reduction of ECD to 55%-75% of preoperative value following endothelial damage caused by intraocular surgery in man14,23 or by transcorneal freezing in the monkey24 also did not evoke change in corneal thickness.

It is noteworthy that in some clinical observations corneal edema was observed when ECD diminished to the 1000 cells per mm² level or less.25,26 This is in accord with our data, since ECD of the normal human cornea is approximately 3000 cells per mm².27

Besides changes in ECD values, there were morphological changes in the regenerated endothelium; these were more clearly pronounced in group 2 corneas. There was a decrease in the number of hexagonal cells from 66.5% in normal corneas to 49% in group 1 and to 33% in group 2. The number of cells with three, four, eight or more facets, almost unobserved in normal eyes (0.6%), was increased in groups 1 and 2 to 8.7% and 21.1%, respectively. Recently, it was shown that decrease of hexagonal cells to 45%-58% is not accompanied by corneal swelling.14,23,28 It is possible that the more profound decrease of hexagonal cells to 33%, together with the considerable increase of endothelial cells with extreme polygonality (three or nine and more sides) is associated with the corneal swelling in our experiments.

There was a clear difference in cell size between experimental and normal groups. In both experimental groups there were many more large cells, especially in group 2. In the latter group the giant cells...
were 7.5–13.5 times larger than the average normal cell. The giant cells comprised 2.5% of the total number of endothelial cells of the second group corneas, whereas they occupied 8.1% of the endothelial surface. The presence of giant cells in the endothelial layer is probably one of the reasons for corneal swelling in this group as well. Rao et al.29,30 found that in clinical cases endothelial dysfunction is associated with variations of cell size rather than cell density. The dysfunction in their cases occurred even at ECD values higher than 50% of preoperative values. In their studies the endothelium contained many exceedingly large cells which might have contributed to the corneal edema. It has previously been noted that edema of rabbit corneas during regeneration of damaged endothelium is probably affected by large, flat endothelial cells present in this period, which are unable to function normally.31 The giant cells probably participate in the formation of the edema in group 2 corneas by three potential mechanisms: (1) It is possible that the junctions of giant cells are abnormal. It is known that endothelial cell junctions impede fluid diffusion from the anterior chamber to the cornea since damage to the junctions produces increased diffusion.32,33 Therefore, abnormalities of cell junctions can cause an increase in the permeability of the intercellular spaces, hence the freer diffusion of fluid toward the cornea. (2) It is also possible that the density of organelles in the giant cells (eg, mitochondria and rough endoplasmic reticulum) is diminished. These organelles are indispensable for the functioning of the biological pump and pump damage leads to insufficient removal of fluid from the corneal stroma to the anterior chamber.34 (3) Functional changes in the external membranes of the giant endothelial cells due to their overextension cannot be excluded. Overextension of the membranes can influence density of the biological pump sites on the external cell membrane and decrease in availability of the endothelial pump site leads to corneal swelling.35

In conclusion, we wish to emphasize that increase in the size of corneal endothelium cells and change in their polygonality up to a certain measure had no impact on their ability to regulate the volume of corneal hydration. Disturbances in endothelial function leading to corneal edema occur only when ECD falls below 1050–1150 (40% of normal value), hexagonality falls to 33%, coefficient of variation of endothelial cell density increases three- to four-fold, and when giant endothelial cells appear which are over 7.5 times larger than normal endothelial cells.

**Key words:** cornea, corneal endothelium, wound healing, morpho-functional relationship, cat

**References**