Long-Term Changes in Corneal Endothelial Morphology Following Wounding in the Cat

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The cat eye was used to determine the long-term morphological changes in the corneal endothelium that occur after central endothelial wounding. Central corneal thickness was measured using ultrasonic pachometry. Specular microscopy and computer-assisted morphometry was used to quantify central and peripheral endothelial cell density (ECD), coefficient of variation (COV) and the mean and standard deviation of the shape factor (S) over an 18-month period. After endothelial wounding, there was a rapid increase in corneal thickness followed by a rapid nonlinear decline, reaching presurgical levels 35 days after wounding. Central cell density had decreased by 25% at 4 weeks after wounding. During the following 18 months, endothelial cell density in the central cornea increased slightly. The coefficient of variation had increased by 60% at 4 weeks after wounding. This recovered slowly and had reached control levels by 18 months. The mean shape factor was higher in the wounded eye throughout the 18 months, whereas the standard deviation of the shape factor recovered after 12 months. Peripheral ECD had decreased significantly by 12 months after wounding, while COV and the mean shape factor was not significantly affected. The standard deviation of the shape factor had also increased significantly in the peripheral cornea after 18 months. These findings suggest that following endothelial wounding in the cat, changes in endothelial morphology occur over the entire cornea. Endothelial cell density and the shape factor have not recovered to control values, even 18 months after wounding. This pattern of endothelial repair supports the mechanisms of cell movement suggested by Honda et al and confirm the similarities in endothelial response between cat and man. Invest Ophthalmol Vis Sci 29:1407–1412, 1988

Corneal endothelial repair processes in the rat1 and rabbit2 involve, initially, cellular enlargement and migration to cover the defect, followed by extensive cellular division at the margin of the wound. In contrast, endothelial wound repair in the cat3–4 takes place without mitosis and is more analogous to that of man5,6 or primates.7,8

Honda et al3 documented cell movements following wounding in the corneal endothelia of cats for up to 100 days. During the first stage, cells surrounding the wound underwent areal enlargement, elongated towards the wound and shifted to cover the defect. During the second stage, cells rearranged by changing neighbors in such a way that they retained their enlarged size but recovered their elongated shape: a process which spreads outward in a wave from the point of injury. A gradual reduction in the area of the large irregular cells at the wound site was observed and eventually a population of larger, uniformly sized cells would be regained. They proposed that polygonal cells achieve this pattern via the boundary shortening mechanism, whereby the total cell boundary length tends towards a minimum.

The current study was designed to test this idea by documenting the long-term changes in endothelial morphology in the cat. A number of shape factors have been used by previous investigators. Shaw et al9 used cell perimeter divided by length to obtain a quantitative measure of cell shape (polygonal versus circular), while Yee et al10 used a figure coefficient obtained by using the formula $4\pi \text{area}/\text{perimeter}^2$ to describe the degree to which a cell approximates a circle. Yee et al found no significant relationship between the figure coefficient and age. Furthermore, no correlation could be found between the figure coefficient and the frequency of hexagons, suggesting that these two parameters reflect different aspects of morphology. The shape factor ($S = \text{perimeter}/\text{area}$), as used by Colloin and Grabsch,11 appeared to be the most appropriate since it is a measure of the ratio between cell perimeter and cell area. This factor is a constant for any geometric shape, is independent of

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Supported by grants from the Optometric Vision Research Foundation (85/81) and the Contact Lens Society of Australia.

Submitted for publication: October 30, 1987; accepted April 20, 1988.

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size and represents a measure of irregularity of shape (ie, deviation from a circle). Cells with fewer sides or cell shapes with acute angles have a higher S value than those which are more circular. The average S for a group of control adult cats was 13.61 ± 0.06.\(^\text{12}\) This S value is consistent with a basic pattern of hexagonal cells with a smaller number of five-, seven- and eight-sided cells, since the theoretical predicted shape factor for a regular hexagon is 13.86.\(^\text{11}\) Endothelial cell density (ECD), coefficient of variation (COV) and the mean and standard deviation of the shape factor (S) in the central and peripheral cornea were determined over an 18-month period following central endothelial wounding in the cat.

**Materials and Methods**

**Endothelial Wounding**

Seven healthy adult domestic cats weighing between 3 to 5.5 kg and aged between 9 months and 4 years were used in this study. They were obtained from the Animal Breeding and Housing Unit, University of New South Wales. Surgery was performed on the right eye while the left eye served as a control.

Each cat was fasted for 12 hr prior to surgery. Aspirin (325 mg/animal) was given orally 12 hr before surgery. Subcutaneous porcine heparin (1000 units/animal) and intramuscular procain penicillin (250 mg/animal) were administered 1 hr before surgery. Atropine sulphate (0.04 mg/kg) was given subcutaneously 30 min before surgery. Anaesthesia was induced by an intramuscular injection of ketamine (33 mg/kg) and xylazine (1 mg/kg). Anaesthetized animals were supported and kept in a supine position. The left eye was taped closed and a plastic surgical drape applied to the right eye. Three 5-0 silk sutures were placed through the episclera for rotation of the globe.

A 2 mm incision was made with a Beaver blade (no. 65) between 10 and 11 o’clock at the surgical limbus. A steel pellet was introduced into the anterior chamber through this opening. Although some aqueous occasionally escaped, care was taken to maintain good depth of the anterior chamber. The pellet was manipulated by moving a magnet above the outer surface of the cornea. Endothelial cells within a central 6 mm were removed by abrasion with the pellet. The average area of endothelium damaged approximated 50 mm\(^2\). The scraped area consisted of the wounded area in the center of the cornea and the area of the tract made during introduction and withdrawal of the pellet. Endothelial material could be seen floating free in the anterior chamber. At completion, the pellet was positioned near the incision using the magnet, and removed using fine forceps. One 10-0 nylon suture was placed for wound closure. Atropine sulphate drops (1%) and mycitracin ointment were applied topically for 2–3 days postoperatively.

**Measurement Procedures**

Based on measurements made on a control group of 20 cats,\(^\text{12}\) we assumed that there was no difference between the two eyes of each animal prior to surgery. All variables were analysed as the difference between the operated and control eyes using the paired student t-test (2-tailed).

**Central Corneal Thickness (CCT)**

CCT was measured on the second day after surgery and repeated every second day for the first 2 weeks and then twice weekly for 8 weeks. CCT was measured using a CILCO ultrasonic pachometer with hand-held transducer (CILCO Australia, Sydney, NSW). The pachometer was calibrated assuming an ultrasound velocity of 1590 m/sec for cat corneal tissue.\(^\text{13}\) Ten readings were taken at each session and the mean CCT recorded.

**Specular Microscopy**

Specular microscopy was performed at 4 weeks and again at 6, 12 and 18 months after surgery. Specular microscopy was performed at 1 week for two animals where the cornea had regained sufficient transparency. The animals were anaesthetised using an intramuscular injection of ketamine (10 mg/kg) and xylazine (0.7 mg/kg). Central and peripheral (2 mm from the inferior-temporal limbus) corneal endothelium were photographed using a Heyer-Schulte specular microscope with a ×20 dipping cone (Medical Optics, Irvine, CA). For photography of the peripheral endothelium, the edge of the dipping cone was placed at the limbus. Since the cone diameter is 5 mm, the area of endothelium photographed was approximately 2 mm in from the inferior-temporal limbus. The dipping cone was moved in a circular direction after each frame, covering a 2–3 mm\(^2\) area in each location. An Improved Neubauer counting chamber was photographed at the corneal plane to determine the magnification of the system. Six to eight photographs of each endothelial location were taken using Kodak Tri-X film. Based on cell density, four to eight endothelial photographs were printed and an average of 150 cells were traced onto a plastic sheet using a fine black marking pen. The tracings were analysed using an IBAS-2 Automatic Image Analysis System (Kontron, Munich, West Germany). The IBAS-2 was programmed to subject the line tracings through a thinning process to obtain the thinnest cell borders which are detectable by IBAS-2.
ECD was calculated by dividing 10^6 by the mean cell area (μm^2). The endothelial cell loss was expressed as a percentage of the cell density in the control cornea. The coefficient of variation in cell area (standard deviation of cell area/mean cell area) was used as an index of the variation in cell size (polymegethism). The shape factor of individual cells was calculated using the formula \( S = \frac{\text{perimeter}}{\text{area}} \). The mean and standard deviation of the shape factor was determined for each location on each measurement occasion.

All procedures used conformed with the ARVO Resolution on the Use of Animals in Research.

**Results**

Figure 1 shows the recovery of central corneal thickness after endothelial damage. A rapid increase in corneal thickness was followed by a rapid non-linear decrease after wounding, reaching presurgical levels by 35 days after surgery. Six cats had CCT of greater than 1250 μm (the upper limit measurable by the CILCO ultrasonic pachometer) for the first 2 days after wounding.

Table 1 shows the ECD, coefficient of variation and the mean and standard deviation of the shape factor in the center of the control and operated eye at various times after wounding. Mean ECD in the central cornea was reduced by 757 cells/mm^2 or 25% at 4 weeks after wounding. This difference in cell density between the operated and control eyes decreased gradually over 12 months and then showed no further change at 18 months after wounding. Central ECD was significantly reduced throughout the 18-month period (2-tailed student t-test, \( P < 0.05 \)).

<table>
<thead>
<tr>
<th>Time since wounding</th>
<th>Endothelial cell density (cells/mm^2)</th>
<th>Coefficient of variation</th>
<th>Mean shape factor</th>
<th>SD of shape factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Op</td>
<td>2315 ± 219</td>
<td>0.2557 ± 0.0437</td>
<td>13.71 ± 0.11</td>
<td>0.59 ± 0.15</td>
</tr>
<tr>
<td>Cont</td>
<td>3072 ± 191</td>
<td>0.1608 ± 0.0118</td>
<td>13.57 ± 0.07</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>Diff</td>
<td>−757 ± 308</td>
<td>0.0949 ± 0.0364</td>
<td>0.14 ± 0.10</td>
<td>0.19 ± 0.19</td>
</tr>
<tr>
<td>t Value</td>
<td>6.04</td>
<td>6.39</td>
<td>3.43</td>
<td>2.45</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.014</td>
<td>0.05</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Op</td>
<td>2438 ± 159</td>
<td>0.2054 ± 0.0596</td>
<td>13.78 ± 0.09</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>Cont</td>
<td>3038 ± 119</td>
<td>0.1515 ± 0.0265</td>
<td>13.60 ± 0.09</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>Diff</td>
<td>−600 ± 132</td>
<td>0.0539 ± 0.0632</td>
<td>0.18 ± 0.14</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>t Value</td>
<td>11.13</td>
<td>2.09</td>
<td>3.15</td>
<td>2.45</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>N.S. (0.082)</td>
<td>0.020</td>
<td>0.05</td>
</tr>
<tr>
<td>12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Op</td>
<td>2448 ± 239</td>
<td>0.1677 ± 0.0178</td>
<td>13.80 ± 0.11</td>
<td>0.49 ± 0.08</td>
</tr>
<tr>
<td>Cont</td>
<td>2862 ± 86</td>
<td>0.1417 ± 0.0178</td>
<td>13.59 ± 0.09</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>Diff</td>
<td>−414 ± 198</td>
<td>0.0260 ± 0.0195</td>
<td>0.21 ± 0.11</td>
<td>0.09 ± 0.10</td>
</tr>
<tr>
<td>t Value</td>
<td>5.11</td>
<td>3.27</td>
<td>4.45</td>
<td>2.20</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td>0.017</td>
<td>0.004</td>
<td>N.S. (0.075)</td>
</tr>
<tr>
<td>18 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Op</td>
<td>2283 ± 222</td>
<td>0.1720 ± 0.0255</td>
<td>13.82 ± 0.08</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>Cont</td>
<td>2679 ± 270</td>
<td>0.1684 ± 0.0216</td>
<td>13.63 ± 0.05</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>Diff</td>
<td>−414 ± 200</td>
<td>0.0036 ± 0.0259</td>
<td>0.19 ± 0.10</td>
<td>0.08 ± 0.11</td>
</tr>
<tr>
<td>t Value</td>
<td>5.07</td>
<td>0.35</td>
<td>4.65</td>
<td>1.78</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td>N.S.</td>
<td>0.004</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Op: operated eye, Cont: control eye, Diff: operated minus control eye, t value: student paired t-test (2-tailed), P: level of significance, NS: not statistically significant (\( P > 0.05 \)).
Following wounding, the endothelial mosaic became much more polymegathous. This was evident in the 59% increase in the COV at 4 weeks after wounding. The difference in polymegathism between the control and operated eyes showed a rapid decrease over the 18 months. No significant difference in the level of polymegathism was observed between the two eyes by 18 months after wounding.

The shape factor remained significantly elevated in the center of the cornea throughout the 18-month period. However, the variation (standard deviation) of the shape factor was significantly higher at 4 weeks and 6 months after wounding, reducing to a nonsignificant difference by 12 months after wounding. By expressing cell loss, increase in polymegathism and the change in the shape factor as a percentage of the control values, the trends with time after wounding were more evident (Table 2).

Table 3 shows the ECD, COV and the mean and standard deviation of the shape factor in the periphery of the control and operated eyes at 12 and 18 months after wounding. Endothelial cell density had fallen significantly in the periphery of the cornea at 12 and 18 months, while polymegathism and the mean and standard deviation of the shape factor showed an increase with time after wounding. Figure 2 shows the changes in endothelial morphology in the central and peripheral cornea in one cat during the 18-month period.

**Discussion**

We have described the long-term changes in corneal endothelial morphology that take place after wounding in the cat. Cell loss in the center of the cornea resulted in areal expansion of the cells adjacent to the wound. During the first stage of wound healing, drastic changes to cell morphology took place. Polymegathism and polymorphism as indicated by the COV and the standard deviation of the shape factor increased dramatically. This was followed by a period of cellular rearrangement which resulted in a population of larger, more uniformly sized cells over the entire cornea. These findings support the long-term pattern of cell movement predicted by Honda et al and is similar to the first stage of cellular migration after wounding in the rabbit. Such movements have been attributed to microfilaments located in a circumferential region along cell boundaries.

From a geometric and physical point of view, a regular hexagonal shape is the most stable arrangement to form a tessellated plane because it keeps the total perimeter at a minimum and therefore produces minimum surface tension energy. Thus, cellular migration following endothelial wounding would be undertaken to achieve maximal hexagonality over the entire endothelial surface. A surprising finding from this work is that following endothelial wounding the mean shape factor (13.80) approached the theoretical predicted shape factor for a regular hexagon (13.86) rather than the value of 13.61 found in a group of control adult cats. Perhaps this altered morphology...
is necessary to achieve maximal efficiency to compensate for irrecoverable cell loss. This is in agreement with Matsuda et al.\textsuperscript{6} who found a gradual increase in hexagonal cells after intraocular surgery, once the cornea returned to its presurgical thickness.

After an 18-month period, ECD decreased by approximately 400 cells/mm\textsuperscript{2} in both the central and peripheral cornea. Central ECD was 2697 cells/mm\textsuperscript{2}, while peripheral ECD was 2908 cells/mm\textsuperscript{2}, that is, an 8\% disparity in cell density between central and peripheral cornea. This corresponds closely to the mean difference in central and peripheral cell density of 265 cells/mm\textsuperscript{2} or 9\% found in a group of 25 control adult cats.\textsuperscript{18} Therefore, even the vertical disparity in ECD is regained 18 months after wounding.

The changes in endothelial morphology with time following wounding in the peripheral cornea are characteristic of healing by migration rather than mitosis. The average area of endothelium removed by scraping was estimated to be 50 mm\textsuperscript{2}. Since posterior corneal surface area in the cat is 2.83 cm\textsuperscript{2},\textsuperscript{19} we calculated that approximately 18\% of the endothelial surface was damaged. This was only slightly higher than the average cell loss of 14\% found at 18 months after wounding and supports the conclusion that damaged areas are covered by mobilization of the
remaining cells without significant cell proliferation. However, this is only a rough estimation and further investigation with tritiated thymidine is required to confirm this.

The rapid recovery of corneal thickness over the first few days following wounding is consistent with the reestablishment of an intact endothelial monolayer and with the return of endothelial function reported by Yee et al. Maximal edema occurred at 24 hr and this started to subside after 2 or 3 days. In the rabbit, pump site density was restored to near normal levels between 4 to 7 days after endothelial wounding and normal corneal thickness was found by the eighth day. Perhaps complete recovery of corneal thickness was slower in the cat due to the lack of endothelial cell mitosis.

This study has documented the long-term changes in endothelial morphology in the cat following wounding. It has shown that feline corneal endothelial cells are capable of cellular movement that results in changes in cell morphology in regions of the cornea that were distant from the initial site of wounding. Cellular rearrangement after wounding resulted in a population of larger, more uniformly sized cells with an average shape factor approaching that predicted for a regular hexagon. The vertical disparity in ECD (central versus peripheral) found in control cats were also regained after 18 months. These results confirm the species differences in response to endothelial wounding reported by other workers and suggest that the monkey and cat are better models for further studies of endothelial response to injury in humans.

Key words: wound repair, endothelium, cat, cell density, polymegathism, shape

Acknowledgments

The authors wish to thank Ms. Helen Swarbrick for assistance with programming of the IBAS-2 and Dr. Daniel O’Leary, Arthur Ho and Michelle Madigan for critical review of the manuscript. Ms. Sue Donahue and Denise Lawler provided excellent technical assistance.

References