Correlation of Corneal Endothelial Pump Site Density, Barrier Function, and Morphology in Wound Repair


After transcorneal freezing, physiologic function (pump and barrier) of the regenerating rabbit corneal endothelium was evaluated and compared with the morphologic differentiation that occurs during wound healing. Endothelial pump function was investigated utilizing the specific binding of tritiated ouabain to endothelial Na⁺/K⁺ ATPase (pump sites); the permeabilities of isolated de-epithelialized corneas to labeled inulin and dextran were measured to determine endothelial barrier function. Endothelial recovery after transcorneal freezing can be described as a three-stage process. Stage one is characterized by the establishment of an initial coverage of the wound by pleomorphic spindle-shaped cells which form a functional but incomplete barrier and minimal pump site density. In stage two, the cells assume a flattened configuration consisting of irregular polygons and establish nearly normal pump capacity. In stage three, a significant remodeling of the monolayer continues despite the layer's early physiologic recovery. Invest Ophthalmol Vis Sci 26:1191-1201, 1985

The regenerative capacity of the rabbit endothelium after various forms of injury is well known. During unstressed conditions, no mitosis occurs in the rabbit endothelial monolayer. However, when the endothelium is damaged, cells at the wound periphery effect healing by migration and mitosis. Histologic and ultrastructural studies have documented the morphologic appearance of the endothelium during the regeneration process. Although endothelial morphology during wound healing is well documented, little is known about the ability of the corneal endothelium to regain its normal physiologic function after wounding. Khodadoust and Green and Van Horn et al., using corneal thickness as an index of endothelial function, followed the time course of corneal swelling and deturgescence after transcorneal freezing of rabbit corneal endothelium. They reported that the functional capacity of the regenerated endothelium lags behind the apparent morphologic repair of the injured area by several days. Minkowski et al., in addition to observing corneal thickness, measured endothelial barrier function using fluorophotometry after transcorneal cryoinjury. They suggested endothelial pump function recovered after endothelial permeability had returned to normal.

The corneal endothelium functions both as a pump, which requires metabolic energy to remove fluid from the stroma, and as a physical barrier to retard aqueous flow back into the stroma. While corneal barrier and pump functions are reflected by corneal thickness, alterations in either or both of these functions can affect thickness. Previous studies have demonstrated that endothelial permeability can be measured in vitro using radioactive labeled substances of ³H-inulin and ¹⁴C-dextran. More recently, measurement of endothelial Na⁺/K⁺ ATPase "pump sites" has provided a new parameter for the in vitro measurement of endothelial transport capacity. Using these methods it is possible to independently quantitate both barrier and pump functions. The functional development of the regenerating endothelium can thus be evaluated and compared with the morphologic differ-
entiation that occurs during wound healing. The present study was undertaken to correlate the reestablishment of endothelial function (pump site density and permeability) and morphology during the wound healing process in the rabbit cornea.

Materials and Methods

Corneal Freezing

Two- and six-mm diameter brass probes, machined to approximate the curvature of the cornea, were used to wound the corneas of 2–2.5 kg New Zealand White rabbits. The use of rabbits in this study adheres to the ARVO Resolution on the Use of Animals in Research. After an intramuscular injection of ketamine HCl (10–15 mg/kg) and xylazine (6 mg/kg) to sedate the rabbit, two drops of proparacaine were applied topically to the cornea. The brass probes were cooled in liquid nitrogen and placed on the central corneal surface for 5 sec. The 5-sec application was selected because it provided consistent endothelial injury with the least amount of morbidity (inflammation, retrocorneal membrane, and stromal scarring). After freezing, the eyes were left propsected until the ice ball was completely thawed. The total endothelial area damaged by the freezing procedure was approximately 7 mm² and 39 mm², respectively, for the 2- and 6-mm probes.

Measurement of Corneal Thickness

Central corneal thickness was measured using a modified Haag-Streit pachymeter. Prior to freezing and at regular intervals from 1–30 days after transcorneal freezing, corneal thickness was taken as the mean of three readings.

Measurement of Pump Site Density

At various times after freezing, rabbits were killed, their eyes were enucleated, and the corneas were excised. Matched pairs of de-epithelialized corneas were incubated in tritiated ouabain for the determination of Na⁺/K⁺ ATPase pump sites densities, using methods previously described. Briefly, corneas were incubated in 10⁻⁷ M carrier-free ³H-ouabain (18 μCi/ml, New England Nuclear; Boston, MA) and 1.05 μCi/ml inulin (³H, MW 5,000, New England Nuclear) was added to the endothelial surface of the corneas. After addition of the isotopes, tissues were allowed to equilibrate for 1 hr. At the end of the equilibration the chamber facing the de-epithelialized surface or “cold” chamber (1.8-ml volume) was rinsed with 2.0 ml of GBR. A second 2.0-ml rinse was collected immediately in a tared vial, weighed, and counted to provide a background or residual count. At 30-min intervals for 3 hr, the “cold” chamber was evacuated, collected, and analyzed in a similar manner. After the final collection of the “cold” chamber, the “hot” chamber was evacuated, collected, and assayed. All samples were counted by liquid scintillation, and the counts were corrected for the 10.5% ¹⁴C energy overlap into the ³H channel and the 0.5% ³H overlap into the ¹⁴C channel. The permeability coefficient, \( K_{\text{trans}} \), for each tracer was calculated according to Maffly et al as follows:

\[
K_{\text{trans}} = \frac{\text{increase in counts on the unlabeled side}}{\text{concentration of counts on labeled side}} \times \text{area of membrane} \times \text{time}
\]

Morphologic Evaluation

Rabbits were killed at various intervals after transcorneal freezing. The corneas were excised and stained.
Fig. 1. Changes in corneal thickness and pump site density with time after transcorneal freezing of central cornea with a 6-mm brass probe. (Each point represents the mean ± SEM; pump site density was measured on 5–6 isolated endothelial sheets at each point.)

with 1% alizarin red and 0.25% trypan blue. The central wounded area was photographed. An additional group of frozen corneas was fixed at similar intervals in 2.7% phosphate-buffered glutaraldehyde and processed for scanning electron microscopy (SEM).

Specular Microscopy and Computer-Assisted Morphometry

At various times after freezing, wide-field specular photomicrographs of the central corneal wound were taken with a Keeler-Konan specular microscope.
Fig. 3. Scanning electron microscopic appearance of the central endothelium after 6-mm transcorneal freezing: A, 1 day; B, 2 days; C, 3 days; D, 4 days; E, 7 days; F, 30 days (bar = 50 μm, A-D; bar = 100 μm, E and F).

(Keeler Instruments Inc.; Broomall, PA) using Tri-X pan film (ASA 400, Eastman Kodak Company; Rochester, NY). The black and white film was developed in Acufine (Acufine, Inc.; Chicago, IL) for 5 min at 75°F. Based on cell boundary clarity, the best frame of each eye at various intervals after freezing was selected and enlarged (×400) on grade 4 Kodabromide RC paper (Eastman Kodak Company; Rochester NY). Prior to printing, a calibration negative of a micrometer scale (0.01 mm) was placed in
the enlarger to assure accurate cornea-to-print magnification.

One hundred individual cells in a cluster per specular micrograph were outlined and numbered consecutively to minimize sampling and analyzing errors. Individual cells were digitized by touching cell apices with a Hewlett Packard 9111A graphics tablet pen (Hewlett-Packard Company; Brookfield, WI). The coordinates entered onto the digitizer tablet were analyzed by a Hewlett Packard 85-B computer (Hewlett-Packard Company; Brookfield, WI) using endothelial analysis software as previously described.

Computer-assisted analysis of each individual cell determined cell size parameters (perimeter, mean cell area, standard deviation of the cell area, cell density, and coefficient of variation in cell area) and cell shape parameters (the relative frequency of each cell shape) at various intervals after transcorneal freezing. The cell shape can be described by the number of sides of each cell, and the frequency of each cell shape was counted automatically by numbering each apex that permitted quantitation of variation in cell shape (pleomorphism). Cell density was calculated by dividing $10^6 \text{ mm}^2/\text{mm}^2$ by the mean cell area (um$^2$). The coefficient of variation (CV) was calculated by dividing the standard deviation of the cell area by the mean cell area. The CV is a dimensionless index independent of cell area which provides a quantitative measurement of cell area variation (polyemegethism).

Results

6-mm Endothelial Wound

After transcorneal freezing, corneal thickness (mean ± SEM) rapidly increased from 0.40 ± 0.004 mm to 1.2 ± 0.005 mm at day 2 (Fig. 1). This marked increase in thickness was due to loss of the endothelial barrier function as the cells sloughed from Descemet's membrane. Associated with the endothelial cell loss, the pump site density also decreased from a prefreeze value of $8.2 \pm 0.6 \times 10^9$ sites/mm$^2$ to $2.3 \pm 0.3 \times 10^9$ sites/mm$^2$ at day 2 (Fig. 1). It is important to note that, after transcorneal freezing, the time point of minimum pump site density is also the point at which corneal thickness was greatest.

Endothelial morphology early in the regenerative period was evaluated by preparing corneal flat preparations stained with 1% alizarin red and 0.25% trypan blue, and by SEM since specular microscopy during this period was not possible because of marked corneal edema. Immediately after transcorneal freezing, all endothelial cells over the frozen area were lost except for some areas covered with degenerating endothelial cells and debris (Fig. 2, top). The cells outside the wounded area appeared not to be damaged by the freeze, nor was any damage observed in Descemet's membrane (Figs. 2, 3A). The diameter of the wounded area was approximately 1 mm larger than the diameter of the 2- and 6-mm probes.

At both 1 and 2 days after freezing, both vital staining and SEM demonstrated that the denuded area was smaller than immediately after freezing. The cells at the wound margin were elongated and stretched toward the center of the wound (Figs. 2, 3A). Between 2 and 3 days, the 6-mm wound was completely covered with pleomorphic spindle-shaped cells having extensive cytoplasmic processes which overlapped neighboring cells (Figs. 2, 3B, 3C).

Within the 3- to 7-day time period (in the 6-mm wounded cornea), the random pleomorphic spindle-shaped cells rapidly flattened and retracted as a monolayer of cells forming a confluent polygonal mosaic (Figs. 2, 3C-E). These morphologic transfor-
Fig. 5. Specular microscopic appearance of the central endothelial monolayer after 6-mm transcorneal freezing. A, prefreeze; B, day 7; C, day 14; D, day 30 (bar = 50 μm).

The endothelial monolayer could not be visualized in vivo with specular microscopy because of stromal edema, but they could be observed with vital staining (Fig. 2) and SEM (Figs. 3A–D).

Once Descemet’s membrane was covered by the pleomorphic spindle-shaped cells on days 2 to 3, barrier function was reestablished as measured by the endothelial permeability to inulin and dextran (Figs. 4A, 4B). Prior to wounding, the endothelial permeability, \( K_{\text{trans}} \) (mean ± SEM), for inulin and dextran were 3.57 ± 0.163 \( \times 10^{-6} \) cm/sec and 0.270 ± 0.012 \( \times 10^{-6} \) cm/sec, respectively (Figs. 4A, 4B). For comparative purposes, complete removal of the endothelium by scraping increased the \( K_{\text{trans}} \) to 6.40 ± 0.33 \( \times 10^{-6} \) cm/sec for inulin and 0.538 ± 0.051 \( \times 10^{-6} \) cm/sec for dextran. After 6-mm transcorneal freezing, the \( K_{\text{trans}} \) at day 1 increased to 5.3 ± 0.20 \( \times 10^{-6} \) cm/sec for inulin (Fig. 4A) and 0.444 ± 0.03 \( \times 10^{-6} \) cm/sec for dextran (Fig. 4B). By day 2, as the wounded area was covered with migrating and dividing cells, endothelial barrier function was partially restored as manifest by a decrease in the endothelial permeability to both inulin and dextran (Figs. 4A, 4B).

Although barrier function was partially restored by day 2, the pump site density remained low (Fig. 1). Pump function did not begin to increase until complete endothelial coverage was achieved at day 2. Pump site density then rapidly increased as the spindle-shaped endothelial cells reorganized into polygonal cells (Figs. 2, 3D). During this period (days 3–7) pump site density increased from 3.0 ± 0.3 \( \times 10^{9}/\text{mm}^2 \) to 6.6 ± 0.7 \( \times 10^{9}/\text{mm}^2 \).

By day 7, the endothelial cells had reorganized into a complete polygonal array (Figs. 3E, 5B) and endothelial barrier function was not significantly different from prefreezing values (Figs. 4A, 4B). Directly correlated to the polygonal endothelial morphology was the return of the endothelial pump site density (Fig. 1). With both the barrier and pump functions

Table 1. Cell size analysis (x ± SEM)

<table>
<thead>
<tr>
<th>Wound size</th>
<th>Day after freezing</th>
<th>Cell density (cells/mm²)</th>
<th>Mean cell area (μm²)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mm</td>
<td>pre</td>
<td>3646 ± 95</td>
<td>274.6 ± 7.0</td>
<td>0.19 ± 0.014</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2227 ± 328*</td>
<td>465.8 ± 65*</td>
<td>0.36 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2461 ± 202*</td>
<td>499.1 ± 33.6*</td>
<td>0.33 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3042 ± 188</td>
<td>331.5 ± 21.4</td>
<td>0.16 ± 0.007</td>
</tr>
<tr>
<td>2 mm</td>
<td>pre</td>
<td>3591 ± 43</td>
<td>278.6 ± 3.3</td>
<td>0.17 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1883 ± 83*</td>
<td>532.3 ± 23.4*</td>
<td>0.33 ± 0.023*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2252 ± 294*</td>
<td>459.1 ± 57.7*</td>
<td>0.37 ± 0.021*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2835 ± 65*</td>
<td>353.1 ± 8.2*</td>
<td>0.34 ± 0.010*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3227 ± 231</td>
<td>315.1 ± 24.4</td>
<td>0.29 ± 0.036</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3500 ± 41</td>
<td>283.8 ± 3.4</td>
<td>0.20 ± 0.037</td>
</tr>
</tbody>
</table>

* Statistical significances refer to comparison with normal values. \( P < 0.05 \).
of the endothelium restored to control values, there was a concomitant decrease in corneal thickness.

The return of corneal thickness to 0.7 mm enabled endothelial specular microscopy to be performed (Fig. 5B). Computer morphometric analysis of the central endothelium showed that significant remodeling of the individual cells occurred after a confluent monolayer was established (Tables 1, 2; Fig. 6). It was not possible to perform quantitative computer analysis of the regenerating cells prior to the establishment of the monolayer because of the morphological nature of the early regenerating cells (Figs. 2, 3A–D).

Although the endothelial monolayer had been established at day 7, endothelial cell density and the frequency of hexagons were still decreased while the coefficient of variation in cell area (CV) and relative frequency of non-six-sided cells were increased (Tables 1, 2). Analysis of endothelial specular micrographs at day 14 (Fig. 5C) showed that endothelial cell density was still decreased below the prefreeze values, the CV was high, and the original hexagonal array was significantly compromised (Tables 1, 2; Fig. 6). However, by day 30 complete endothelial remodeling had occurred. Cell density, CV, and percent hexagonal cells (70%) had all returned to their respective prefreeze values (Tables 1, 2; Fig. 6).

### 2-mm Endothelial Wound

After transcorneal freezing, there was a twofold increase in corneal thickness on day 1 and a significant decrease in the number of measured pump sites, which corresponded to the area of lost endothelial cells (Fig. 7). By day 2, corneal thickness decreased. This decrease was associated with the reestablishment of the endothelial barrier when the endothelial cells migrated from the wound edge (Fig. 8A). Complete coverage of Descemet’s membrane occurred earlier in the 2-mm wound (between 1 and 2 days) than in

### Table 2. Cell shape analysis: percentage (± SEM) of cells having 4–9 sides at various times after freezing

<table>
<thead>
<tr>
<th>Wound size</th>
<th>Day after freezing</th>
<th>4-sided</th>
<th>5-sided</th>
<th>6-sided</th>
<th>7-sided</th>
<th>8-sided</th>
<th>9-sided</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>0</td>
<td>15.3 ± 0.7</td>
<td>72.0 ± 1.5</td>
<td>12.3 ± 2.3</td>
<td>0.33 ± 0.3</td>
<td>0</td>
</tr>
<tr>
<td>6 mm</td>
<td>7</td>
<td>2.0 ± 0</td>
<td>32.3 ± 2.3*</td>
<td>37.3 ± 4.7*</td>
<td>24.3 ± 2.4*</td>
<td>2.7 ± 1.2</td>
<td>1.3 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.5 ± 0.5</td>
<td>27.5 ± 3.5*</td>
<td>48.0 ± 8.0*</td>
<td>19.5 ± 0.5*</td>
<td>4.0 ± 3.0</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0</td>
<td>15.3 ± 0.3</td>
<td>70.3 ± 0.7</td>
<td>14.3 ± 0.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 mm</td>
<td>pre</td>
<td>0</td>
<td>12.7 ± 0.3</td>
<td>75.3 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.0 ± 2.0</td>
<td>27.5 ± 2.5*</td>
<td>36.0 ± 0*</td>
<td>27.5 ± 1.5*</td>
<td>4.0 ± 1.0*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.7 ± 2.7</td>
<td>27.0 ± 3.5*</td>
<td>36.0 ± 1.2*</td>
<td>27.0 ± 2.5*</td>
<td>4.0 ± 1.5*</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.3 ± 0.9*</td>
<td>25.0 ± 1.0*</td>
<td>45.0 ± 1.7*</td>
<td>22.0 ± 0*</td>
<td>3.3 ± 1.3*</td>
<td>1.3 ± 0.3*</td>
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<tr>
<td></td>
<td>14</td>
<td>1.3 ± 0.6</td>
<td>25.8 ± 1.6*</td>
<td>48.8 ± 2.4*</td>
<td>19.5 ± 1.2</td>
<td>3.0 ± 0.9*</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.7 ± 0.7</td>
<td>16.3 ± 0.7</td>
<td>67.0 ± 1.5</td>
<td>14.3 ± 0.7</td>
<td>1.3 ± 0.7</td>
<td>0.33 ± 0.3</td>
</tr>
</tbody>
</table>

* Statistical significances refer to comparison with normal values, P < 0.05.

Fig. 6. Changes in the cell density, coefficient of variation in cell area (polymegethism) and hexagonality (pleomorphism) after transcorneal freezing of central cornea with 6-mm brass probe (dotted curve is the corneal thickness data from Figure 1; each point represents the mean ± SEM of 6–8 determinations).
the 6-mm wound. Although the decrease in thickness occurs as the barrier is reestablished, the pump site density remained decreased until after the reestablishment of the monolayer, as in the 6-mm wound (Fig. 7). By day 4, the endothelium was covered by large, variable-shaped polygonal cells. Though cell density was low (Table 1), corneal thickness decreased to 0.5 mm. The large pleomorphic polygonal cells are illustrated in Figure 8B. Quantitation of these cells by computer-assisted morphometry showed that only 36% of the cells are hexagonal compared with 75% for the prefreeze values (Table 2).

As endothelial cells became polygonal (day 4), there was a marked increase in the number of pump sites. Pump site density returned to normal by day 5. During the period from day 7 to 30, the corneal endothelium remodeled to the extent that 67% of the endothelial cells returned to their hexagonal shape, and cell density returned to its normal prefreeze value (Tables 1, 2). There was no further change in endothelial pump site density during this time.

Discussion

Results of this study indicate that, after transcorneal freezing, the normalization of central corneal thickness, endothelial pump site density, and endothelial permeability precede the establishment of a normal endothelial mosaic. The functional characteristics of the regenerating endothelium correlate with its morphologic appearance (Fig. 9).

Though a 2-mm wound heals more quickly than a 6-mm wound, the general scheme of wound healing is applicable to both small and large wounds. Based on endothelial morphology, the wound healing process after a 2-mm and 6-mm freezing can be divided into three stages: I, the denuded area is covered by dividing and migrating endothelial cells; II, the spindle-shaped cells form a flattened monolayer and assume a pleomorphic polygonal pattern; and III, the cells gradually remodel to a normal hexagonal configuration (Fig. 9). Corresponding to these three morphologic stages of wound healing are changes in corneal thickness, Na⁺/K⁺ ATPase pump site density, and barrier function.

Stage I begins immediately after freezing with complete destruction and loss of the endothelial cells without rupture of Descemet's membrane. As long as a wound is present, endothelial barrier function is compromised as is evidenced by a rapid rise in corneal thickness and endothelial permeability. The passage of inulin and dextran occurs freely through the endothelial defects if these defects are no smaller than the effective molecular radius of inulin (14 Å) and dextran (38 Å).19 Pump site density declines to a minimum at 1–2 days after freezing, and it is also at this time that corneal thickness is greatest. The reduction in endothelial transport capacity is proportional to the damaged area produced by either the 2- or 6-mm freezing.
Coverage of the central wounded area occurs by cell migration and mitosis in the rabbit cornea. Studying endothelial incorporation of tritiated thymidine, previous authors have demonstrated endothelial DNA synthesis within 24 hr after injury. Endothelial maximal incorporation is seen at 24-48 hr. Once a confluency of cells is formed, DNA synthesis and cell migration stop and contact inhibition of cell growth or density-dependent growth inhibition occurs. The migrating and proliferating fibroblastic-like cells appear to function solely to cover the denuded area and to reestablish a barrier. Hence, no increase in pump site density occurs during stage I of healing. In contrast, corneal thickness and endothelial permeability begin to decrease as the wound becomes smaller, and they approach prefreezing values when Descemet's membrane is completely covered with cells. The regenerating cells form primary contacts with their neighboring cells by lateral expansion. Although large intercellular spaces exist, apical cytoplasmic extensions bridge over these spaces forming secondary contacts. Together the primary and secondary contacts form a functional but incomplete endothelial barrier. Permeability studies using a smaller molecule such as fluorescein (4Å effective radius) have been performed by Minkowski and associates and presumably would reflect the endothelial barrier function more accurately. Unfortunately,
SUMMARY

<table>
<thead>
<tr>
<th>STAGE</th>
<th>DAY</th>
<th>STATE OF HEALING</th>
<th>CORNEAL thickness</th>
<th>PUMP</th>
<th>BARRIER</th>
<th>MORPHOLOGY</th>
</tr>
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<tr>
<td>0-1 day</td>
<td>wound present</td>
<td>++</td>
<td>----</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1-2 days</td>
<td>cells sliding, mitosis</td>
<td>+++++</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wound in size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>wound completely covered</td>
<td>+++</td>
<td>----</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4-6 days</td>
<td>establishment</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>of monolayer</td>
<td>+</td>
<td>NL</td>
<td>NL</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>8-30 days</td>
<td>remodeling of monolayer</td>
<td>NL</td>
<td>NL</td>
<td>NL</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 9. Summary of the stages of endothelial wound healing and correlation between corneal thickness, pump site density, permeability, and morphology after transcorneal freezing (changes relative to prefreeze values are expressed as follows: I = decrease; + = increase; NL = normal, i.e., no change).

the authors were not able to evaluate the endothelial barrier function during the first 7 days postcryoinjury due to stromal edema which precluded fluorophotometry. Stage II of the regenerative process does not begin until the wound is repopulated with cells and presumably after contact inhibition has occurred. Once an endothelial layer is reformed, the cells become less spindle-shaped, and over the next several days an intact endothelial mosaic comprised of pleomorphic polygonal cells is formed. During this time period, a significant cellular differentiation occurs as intercellular spaces narrow and apical junction and lateral intercellular contacts are reestablished. Associated with these morphologic changes are also changes at the membrane level as evidenced by the increase in pump site density to prefreeze values.

Although endothelial permeability and pump site density are near normal once the endothelial mosaic is present, corneal thickness remains elevated. This may be due to the stromal damage with the loss of stromal proteoglycans and glycoproteins that occurs during corneal edema. Similarly, pump site density may be normal, but total pumping capacity (turnover) may still be decreased at this time.

Stage III of endothelial healing begins as a flattened but pleomorphic endothelium is established. This is accompanied by the gradual return of pump site density, permeability, and corneal thickness to control values. While these parameters of endothelial function are approaching normal, the regenerated mosaic pattern is still morphologically quite abnormal. During this healing period, the cells undergo significant remodeling. As cell density increases, the cells become more uniform in size (reduced CV) and assume a normal hexagonal configuration as shown by the increase in the frequency of hexagonal cells. By 30 days, all the morphologic parameters have returned to prefreeze values.

These observations demonstrate that rapid functional recovery of the regenerated endothelium occurs once a monolayer is reestablished over the injured area. Although the newly regenerated monolayer has normal endothelial function (permeability and pump site density) early in the course of wound healing (stage II), normal structural appearance of the monolayer is not seen until later (stage III).

Key words: corneal endothelium, Na/K pump site density, barrier function, transcorneal freezing, morphology, wound healing, sodium potassium adenosine triphosphatase

Acknowledgments

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References