Decreased Endothelial Pump Function With Aging

Melvin R. O'Neal* and Kenneth A. Poise

Endothelial function may be affected by the endothelial cell loss and increased variability in cell shape and size (polymegathism) that accompany normal aging. Endothelial function can be evaluated by monitoring corneal hydration recovery following hypoxic stress. The authors compared corneal recovery and endothelial morphology between a group of younger (x = 26.7 yr) and older (x = 65.7 yr) subjects with normal corneas. Edema (60 μm) was induced with hydrogel lenses worn with the eyes closed. Following lens removal, the decrease in corneal thickness was monitored for 4 hr with one eye open while the contralateral eye remained closed. For both age groups, corneal recovery followed a non-linear time course. The open eye required 2.5 hr and 3.0 hr to return to baseline for the younger and older age groups, respectively. Recovery during eye closure took 3.5 hr to reach the normal closed eye level for the younger subjects and was not complete at 4 hr for the older subjects. Recovery rates were significantly slower for the younger vs younger subjects during the first 2 hr of closed eye recovery, 10.5 vs 15.0 μm/hr, and for the initial 1 hr of open eye recovery, 26.5 vs 35.6 μm/hr. For both age groups combined, the rate of recovery was negatively correlated with the coefficient of variation in cell area, r = —0.62 and —0.69 (P < 0.01), for both closed and open eye recovery, respectively. When each morphological characteristic was isolated, the only significant correlation found was between the coefficient of variation in cell area and the rate of recovery during eye closure, r = —0.66 (P < 0.05). These data suggest that endothelial pump function decreases approximately 10% by age 65 and indicates a possible link between endothelial morphology and function. Invest Ophthalmol Vis Sci 27:457–463, 1986

Endothelial cell loss and increased variability in cell shape (pleomorphism) and size (polymegathism) accompany normal aging.1,2 Since aging alters many bodily functions, it might be expected that these endothelial cell changes would affect the ability of the endothelium to maintain normal corneal hydration.3 Although a low cell density can lead to corneal decompensation,4,5 the degree of cell loss accompanying aging apparently does not alter corneal hydration.6 Similarly, studies involving older patients have reported no correlation between the postoperative endothelial cell density and degree of corneal swelling following intraocular lens implant surgery.7,8 Recently, however, a relationship was found between this postoperative corneal edema and the degree of polymegathism prior to surgery,9 suggesting that endothelial polymegathism and function may be related.

The endothelium has both an active pump and passive fluid barrier functions.10 A recent study on the relationship between aging and the endothelial barrier function found no correlation between age and the endothelial permeability to fluorescein.11 Indeed, it has been reported that younger subjects have a higher endothelial fluorescein permeability compared to older subjects.12 A decreased endothelial permeability to fluorescein was found to correlate with an increase in cell size (ie, lower cell density), but not with the variation in cell size of transplanted corneas.13 These studies would seem to indicate that the endothelial barrier function is not compromised by either a moderate loss of endothelial cells or increased cell polymegathism. This suggests that it is the active pump function that may be affected by morphological changes in the endothelium. There have been no studies, however, to determine if the endothelial pump is affected during normal aging or is correlated with endothelial morphology.

We recently reported a technique to assess in vivo endothelial function by measuring changes in corneal hydration following hypoxic stress.14 Corneal recovery occurred at approximately the rate predicted for the endothelial pump, suggesting that this method provides a direct evaluation of endothelial pump function. The time course of hydration recovery was determined for a group of young subjects with normal corneas. Comparison of these recovery data to those of older subjects with normal corneas may also allow assessment of age-related changes in endothelial function. Comparison

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Table 1. Summary of selected ocular parameters for the ten subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal thickness (μm)</td>
<td>509</td>
<td>24</td>
<td>472 to 551</td>
</tr>
<tr>
<td>Sphere refractive error (D)</td>
<td>-0.54</td>
<td>1.86</td>
<td>+0.75 to -6.25</td>
</tr>
<tr>
<td>Cylinder refractive error (D)</td>
<td>-0.36</td>
<td>0.48</td>
<td>0 to -1.75</td>
</tr>
<tr>
<td>Horizontal Keratometry (D)</td>
<td>43.26</td>
<td>1.50</td>
<td>39.75 to 46.00</td>
</tr>
<tr>
<td>Corneal toricity (D)</td>
<td>0.60</td>
<td>0.47</td>
<td>0.37 against to 1.50 with</td>
</tr>
</tbody>
</table>

of corneal recovery to endothelial cell analysis should provide information on the correlation of endothelial function to morphology.

In this study, the time course of corneal hydration recovery following hypoxic stress was measured on a group of older subjects and compared to the recovery profiles of the younger subjects. Endothelial photomicrographs were evaluated to determine if there is a relationship between the corneal hydration recovery response and endothelial morphology. These data suggest that endothelial pump function decreases with aging and indicates a possible link between endothelial morphology and function.

Materials and Methods

Subjects

Ten subjects (seven women, three men; mean age 65.7 ± 2.8 yr, range 62 to 71 yr) who were free of ocular disease and were not contact lens wearers participated in the study. Informed consent was given by each subject. A summary of relevant ocular parameters is listed in Table 1. The younger group, who had been measured previously under the same conditions, included ten subjects, (one woman, nine men; mean age 26.7 ± 4.8 yr, range 23 to 37 yr) with similar ocular parameters. In a previous study involving six women and eight men, we found no difference in the rate of recovery between the sexes.

Corneal Swelling

Corneal hydration was increased by exposing the anterior corneal surface to a hypoxic environment. Subjects wore a piggyback combination of a B4 and U4 Bausch & Lomb hydrogel lens (Bausch & Lomb Inc.; Rochester, NY) with a total thickness of 0.20 mm and an oxygen transmissibility (Dk/L) of 4.5 × 10^-8 (cm/sec) (ml O2/ml × mm Hg). This contact lens combination reduced the oxygen level at the lens-cornea interface to approximately 4 mm Hg, which is below the oxygen level required to prevent corneal swelling.

Endothelial Cell Morphology

Endothelial photomicrographs were obtained prior to lens insertion using the Nikon Non-contact Endothelial Microscope (Nippon Kogaku Inc.; Garden City, NY) and Kodak Ektachrome 200 slide film (Kodak; Rochester, NY). Each photomicrograph was projected onto a screen at known magnification, and only cells in which the complete cell margin could be visualized were then traced onto paper. From this outline each cell boundary was again traced on a digitizer table connected to a computer. For each tracing, computer analysis determined the mean and standard deviation of cell area, from which the coefficient of variation in cell area (SD/ Mean cell area × 100) was calculated. This coefficient expresses the standard deviation as a percentage of the mean cell area, and provides an index of the variation of cell size (polymegathism). The closer the value to zero, the more uniform the cell size; the closer the value to 100, the greater the variation in cell size.

Procedure

Corneal hydration changes were monitored by measuring central corneal thickness using an optical pachometer which had been modified to increase accuracy. Each measurement included ten readings with a standard deviation of ±4.0 μm. Baseline corneal thickness was measured at least 3 hr after awakening to eliminate any influence sleep may have on thickness. The hydrogel lens combination was then inserted in each eye and worn for 1.5 hr with both eyes closed. This procedure resulted in approximately 60 μm of corneal swelling. The lenses were then removed and corneal thickness was monitored for 4 hr. During the recovery period one eye was kept open and measurements were made every 30 min, while the contralateral eye remained closed (except for brief 30 sec measurement every hour). In a separate session, the normal physiologic closed eye swelling (23.3 ± 5.1 μm) was measured after 3 hr of eye closure when no lens was worn. The procedures used in the session for these older subjects were the same as those used in the session for the younger subjects measured previously. The instruments and environmental conditions were identical for the two groups, and the same experimenter (MRO) made all the measurements at both sessions.

Results

Figure 1 shows the progressive decrease in central corneal swelling (recovery) over the 4 hr time period.
for the open and closed eye conditions from 58.8 ± 4.8 μm of induced edema. The recovery was nonlinear, with the rate of recovery decreasing as the cornea thins.

For the open eye, the cornea returned to baseline thickness in about 3.0 hr; while with the eyes closed, the cornea did not reach the normal physiologic closed eye edema (dotted line) in the 4-hr recovery period.

The recovery curves were fitted by the method of least squares to third order polynomial equations:

\[
CS\ (\text{closed}) = 58.8 - 11.9t + 0.3t^2 + 0.1t^3 \quad (5.1)
\]

for recovery with the eyes closed (n = 50, r = 0.923), and

\[
CS\ (\text{open}) = 58.8 - 32.0t + 8.2t^2 + 0.09t^3 \quad (5.2)
\]

for recovery with the eyes open (n = 110, r = 0.867); where CS is the corneal swelling in μm, and t is the time in hours since lens removal (recovery).

Comparison of the time course of closed eye recovery for these older subjects to the younger subjects is shown in Figure 2. The younger age group recovers faster compared to the older age group from a similar (60 μm) level of initial swelling. Using least squares linear analysis, the rate of recovery (μm/hr) over the first 2 hr was computed for each subject and are listed in Table 2. The recovery rate was significantly slower for the older subjects (10.5 ± 2.9 μm/hr, range 5.5 to 14.0 μm/hr) than for the younger subjects (15.0 ± 2.2 μm/hr, range 11.5 to 19.0 μm/hr), (Wilcoxon rank-sum, P < 0.001).

There is, however, a difference in the mean normal physiologic closed eye swelling of 17.2 μm vs 23.4 μm for the younger and older subjects, respectively. In the younger age group, the closed eye recovery rate was related to the amount of swelling above the physiologic (no lens) swelling that occurs with eye closure.14 To give the same amount of initial swelling above the normal closed eye level (35 μm) the mean starting edema for the younger age group was adjusted to 52 μm.

Using these adjusted data, Figure 3 was constructed.
to compare the mean recovery during eye closure for both study groups. For the younger group, recovery took 3.5 hr to reach the normal closed eye level; while for the older group, recovery was not complete at the end of the 4-hr test period. Figure 3 also shows that the younger group had 1.5 μm of swelling remaining after 3 hr, while the older group did not reach this level of recovery until 4 hr. From the adjusted edema level, the mean rate of recovery over the first 2 hr for the younger subjects was 13.5 μm/hr (range 10.0 to 17.5 μm/hr), which is also significantly faster compared to the older age group recovery rates (Wilcoxon rank-sum, \( P < 0.022 \)).

Comparison of open eye recovery for both groups of subjects is shown in Figure 4. From approximately 60 μm of swelling, recovery to baseline thickness required 2.5 hr and 3.0 hr for the younger and older age groups, respectively. The main difference in recovery profiles between the two groups occurred over the first hour. Using least squares linear analysis, the rate of recovery (μm/hr) over the first hour was computed for each subject and is listed in Table 2. The recovery rate was significantly faster for the younger subjects (35.6 ± 3.4 μm/hr, range 31.0 to 41.0 μm/hr) compared to the older subjects (26.5 ± 6.9 μm/hr, range 17.0 to 40.0 μm/hr), (Wilcoxon rank-sum, \( P < 0.003 \)).

Endothelial cell photomicrograph tracings showed that the younger subjects tended to have fairly uniform cell size, while the older subjects had considerable variation in cell size. Analysis of these photomicrographs for mean values and ranges of cell area and coefficient of variation in cell area is compared between the two groups in Table 3. Mean cell area in the older subjects was 420 ± 106 μm² (range 313 to 747 μm²) and was 341 ± 40 μm² (range 300 to 432 μm²) in the younger subjects. The difference between the two groups was significant (Wilcoxon rank-sum, \( P < 0.01 \)). This corresponds to an endothelial cell density in the older subjects of from 1339 to 3195 cells/mm² (mean = 2381) and from 2315 to 3333 cells/mm² (mean = 2933) in the younger subjects. The coefficient of variation in cell area ranged from 30.9 to 44.4 (mean = 36.0) in the older subjects, whereas in the younger subjects the range was from 26.3 to 35.1 (mean = 29.9). The difference between the two groups was significant (Wilcoxon rank-sum, \( P < 0.001 \)).

The correlation between endothelial cell morphology and recovery rate with respect to age for the two age groups both separately and combined is shown in Table

**Table 3.** Endothelial cell morphology of the older age group (mean age, 65.7 yr) and younger age group (mean age, 26.7 yr) subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Older age group</th>
<th>Younger age group</th>
</tr>
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<tbody>
<tr>
<td>Mean cell area (μm²) (range)</td>
<td>420 (313–747)</td>
<td>341 (300–432)</td>
</tr>
<tr>
<td>Mean cell density (cells/mm²)</td>
<td>2,381 (1339–1195)</td>
<td>2,933 (2315–3333)</td>
</tr>
<tr>
<td>Mean coefficient of variation</td>
<td>36.0 (30.9–44.4)</td>
<td>29.9 (26.3–35.1)</td>
</tr>
</tbody>
</table>

Fig. 3. Comparison of the mean decrease in central corneal swelling vs time between the older (triangles) and younger (circles) subjects during recovery with the eyes closed from the same amount of edema, 35 μm, above the closed eye corneal thickness.

Fig. 4. Comparison of the mean decrease in central corneal swelling vs time between the older (triangles) and younger (circles) subjects during recovery with the eyes open. Error bars equal ±1 SD and lines were fitted by polynomial equation.
4. For the combined groups, the rate of recovery was better correlated (negatively) to the coefficient of variation in cell area, \( r = -0.62 \) \((P < 0.01)\) and \(-0.69 \) \((P < 0.01)\), than was the mean cell area, \( r = -0.27 \) and \(-0.55 \) \((P < 0.05)\), for both closed and open eye recovery, respectively. Within the younger age group, however, the coefficient of variation in cell area showed little correlation to the rate of recovery with the eyes closed \( (r = -0.16) \) or open \( (r = -0.22)\). None of the correlation coefficients for each subject group alone was significant.

The correlation between recovery rate and an individual endothelial morphological characteristic was studied by forming two groups of subjects, without regard to age, in which one characteristic remained within selected limits while the other varied over a wide range (Table 5). The subject groups were (1) similar coefficient of variation, \(32 \pm 3.5\), mean cell area \(301\) to \(525\) \(\mu m^2\) \((n = 11, 4\) older, \(7\) younger); and (2) similar mean cell area, \(330 \pm 25\) \(\mu m^2\), coefficient of variation \(26.3\) to \(38.6\) \((n = 11, 5\) older, \(6\) younger). Recovery rate was not significantly correlated with mean cell area, \(r = -0.27\) and \(-0.50\) for closed and open eye recovery, respectively; nor with the coefficient of variation \((r = -0.48)\) for open eye recovery. The only significant correlation was between the coefficient of variation and closed eye recovery rate \((r = -0.66, P < 0.05)\). The endothelial cell morphological data on each subject can be found in the author's dissertation.\(^{20}\)

**Discussion**

The rate of corneal recovery following induced edema was slower in the older age group compared to the younger subjects. These recovery rate differences are apparently related to a reduced endothelial function, which could result from a decrease in the active pump or barrier mechanisms. Our analysis of these recovery data suggests that the most likely cause of this reduced function is a decrease in the endothelial pump rate.

This conclusion is based on the following analysis. First, we previously found that after lens removal the rate of recovery with the eyes closed was in close correspondence to the calculated recovery rate due the endothelial pump.\(^{14}\) However, for these older subjects, the measured rate of recovery was slower than that calculated for the pump. For instance, at the initial 60 \(\mu m\) of swelling the calculated recovery rate is 19 \(\mu m/h\) hr; however, the measured rate of recovery was only 12 \(\mu m/hr\). After 1 hr of recovery, the difference in rates was still 14 vs 10 \(\mu m/hr\) for the calculated and measured recovery rates, respectively. This finding suggests that on the whole the endothelial pump of the older subjects is moving fluid out of the cornea at a reduced rate in comparison to the younger subjects.

Second, the rate of recovery with the eyes open was also slower in the older group compared to the younger subjects, but only until their normal physiologic closed eye swelling level was reached. From this point the open eye recovery to baseline thickness was the same for both groups. Both the endothelial pump and evaporation contribute to the initial portion of recovery,\(^{14}\) while the later phase of thinning is due entirely to evaporation.\(^{22}\) Had there been a decreased barrier function, we might expect this later portion of recovery to also be slower in the older subjects. This was not the case, suggesting that the endothelial barrier function remains largely intact with normal aging, a finding which is consistent with studies on the endothelial permeability to fluorescein.\(^{11,12}\)

The reduction in endothelial pump rate can be estimated using the closed eye recovery data. Assuming the endothelial hydraulic conductivity remains normal with aging and given that the mean baseline corneal thickness \(509\) \(\mu m\) was the same for the two age groups, then the fluid leak into the cornea due to the stromal swelling pressure would also be equal. Since the leak

| Table 4. Correlation (r) between endothelial cell morphology and the rate of recovery over the first 2 hr during eye closure and 1 hr with the eyes open for both age groups separately and combined |
|---------------------------------|-----------------|-----------------|
|                                | Closed eye recovery | Open eye recovery |
|                                | \((r = \text{Coef var})\) | \((r = \text{Coef var})\) |
| Younger age group              | +0.47             | -0.16           |
| Older age group                | -0.12             | -0.32           |
| Combined age groups            | -0.27             | -0.62           |
|                               | \((P < 0.01)\)    | \((P < 0.05)\)  |
|                               | \((P < 0.01)\)    | \((P < 0.01)\)  |

| Table 5. Correlation (r) between isolated endothelial cell morphology (one characteristic held within limits while other varied) and the rate of recovery over the first 2 hr during eye closure and 1 hr with the eyes open, without regard to age |
|---------------------------------|-----------------|-----------------|
| Morphological Character        | Closed Eye      | Open Eye        |
| MEAN CELL AREA \((301 - 525\) \(\mu m^2\)) | -0.27           | -0.50           |
| (Coef Var = 32 \pm 3.5)        |                 |                 |
| COEFFICIENT OF VARIATION \((26.3 - 38.6)\) \((Area = 330 \pm 25\) \(\mu m^2\)) | -0.66           | -0.48           |
| \((P < 0.05)\)                |                 |                 |
is the same for both groups, any decrease in recovery rate would be due to a reduced endothelial pump rate. At the initial 60 μm of swelling, the recovery rate for the older subjects is 7 μm/hr less than for the younger group. This corresponds to a decrease in pump rate of 0.7 μl/cm² × hr. The endothelial pump rate reported for the rabbit is 6.7 μl/cm² × hr²; taking this rate as normal for the younger subjects gives an endothelial pump rate of approximately 6.0 μl/cm² × hr, or a 10% decrease, for the older subject group.

To investigate the aspect of endothelial morphology that is affecting pump function, we correlated the results of the endothelial photomicrograph analysis with recovery rate. Within age groups, the mean cell area was found to be both positively and negatively correlated with recovery rate for the closed and open eye conditions, respectively, in the younger subjects and had only slight to no correlation within the older subjects. The coefficient of variation in cell size was not correlated with either open or closed eye recovery rates for the younger subjects; however, it was the best indicator of the rate for the older subjects. When the two subject groups were combined, the coefficient of variation in cell size showed good correlation to recovery rate for both the open and closed eye conditions. When age was disregarded and recovery correlated over a range of values for one morphological characteristic while the other remained within selected limits, mean cell area was again not significantly correlated with the recovery rate, while the only significant correlation was between the coefficient of variation and the rate of recovery during eye closure (ie, when recovery is due only to the pump mechanism). These findings suggest that endothelial pump function is affected more by an increase in the relative variability in cell size (ie, polymegathism) than by the average cell size, although an interference with function by the latter characteristic cannot be excluded.

Our finding that mean cell area (and cell density) is not correlated with pump function is in agreement with recent studies on pump site density. The endothelial transport mechanism appears to be based on the Na,K-ATPase pump located in the lateral cell membrane. Geroski and Edelhäuser quantitated the density of these pump sites in the rabbit corneal endothelium and calculated that the lateral cell membrane could accommodate many more pump sites than measured. This would allow a decrease in membrane area without necessarily resulting in a loss in pump sites. In addition, Geroski et al report that in human donor tissue the pump site density remained relatively constant with age, although the number and age distribution of the tissues were not listed. Our results are in agreement with these findings since the decrease in cell density that occurred in our older subjects had little effect on pump function.

The rate of corneal recovery following induced swelling was slower in the older group of subjects compared to the younger subjects. It is possible that measuring the recovery for each group at different times may have affected the response; however, care was taken to insure that the instruments and environmental conditions were identical for both groups. Analysis of these recovery data suggests that endothelial pump function decreases with aging, and that this change may be linked to alterations in endothelial morphology, particularly polymegathism. For instance, in the analysis where each morphological characteristic was isolated, the only significant correlation occurred between closed eye recovery rate and coefficient of variation (r = -0.66); however, within this analysis group, the correlation was r = -0.81 for the five older subjects, while no correlation (r = -0.01) was found for the six younger subjects. Although a small subject number was involved, this suggests that age plays a role in the susceptibility of the pump mechanism to interference from morphological changes. The complete pump mechanism is complex and involves cell membranes and pump sites other than the Na, K-ATPase sites, and the combination of age and polymegathism may be affecting one of the pump components and causing the decrease in overall pump function that we found in the older age group.

The difference in recovery rates was apparent after only 1 hr, indicating only a short monitoring period is needed to evaluate recovery ability. Additional studies on larger populations are needed to establish the normal range of recovery responses and to investigate endothelial pump function in corneas demonstrating endothelial disease.

Key words: cornea, human, aging, corneal recovery, endothelial pump, polymegathism

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References