Predicting Endothelial Cell Loss and Long-Term Corneal Graft Survival

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PURPOSE. To evaluate a biexponential decay model for describing the loss of corneal endothelial cells with age as well as the increased loss of cells after cataract surgery and penetrating keratoplasty.

METHODS. Data from previous studies were identified and the sum of two exponentials, \( d = p \cdot \exp(-at) + q \cdot \exp(-bt) \) (where \( d \) is cell density at time \( t \), \( p \) and \( q \) are constants the sum of which is equal to the initial cell density, and \( a \) and \( b \) are exponential rate constants), fitted to each data set by a nonlinear least-squares algorithm. Goodness of fit was indicated by the residual standard deviation. Half times were calculated from the exponential rate constants.

RESULTS. The model identified in each instance a rapid and a slow component to the cell loss. The half time for the slow component of the loss with age was 224 years, underlining the excess endothelial capacity in normal eyes. After surgery, the rapid component of the cell loss was probably due to surgical trauma and, after penetrating keratoplasty, cell-mediated rejection and other complications. The half times of the slow component were only 26 years after cataract surgery and 21 years after penetrating keratoplasty.

DISCUSSION. The loss of endothelial cells followed a biexponential decay and could thus be described by a single equation. The half times of the slow component of the cell loss after surgery were substantially less than for the loss with age, indicating a markedly increased rate of cell loss that persisted for many years after surgery. A mechanism for this accelerated cell loss is suggested that involves a nonspecific, innate response initiated by the breakdown of the blood–ocular barrier. The model used to calculate endothelial cell loss in the long term after penetrating keratoplasty and to predict when cell density would reach levels that are incompatible with maintenance of transparency and graft function. Thus, a rationale is presented for the setting of minimum donor cell densities by eye banks. (Invest Ophthalmol Vis Sci. 2003;44: 3326–3331) DOI:10.1167/iovs.02-1255

Human corneal endothelial cells undergo mitotic division only rarely in situ, and there is a well-documented, gradual decline in endothelial cell density with increasing age.1–3 As cell density decreases, there is a corresponding increase in cell surface area as the remaining cells spread to maintain the barrier properties of the endothelial mosaic. After penetrating keratoplasty, the loss of endothelial cells is greatly exacerbated. Although especially marked in the short term, this abnormal cell loss persists, albeit at a much reduced rate, for many years after transplantation.4 This potentially jeopardizes the long-term survival of grafts as the cell density declines toward levels, variously reported to range from 250 to 500 cells/mm2 5,6 that are inconsistent with the control of stromal hydration and maintenance of corneal transparency. It has been suggested that a low donor endothelial cell density is a risk factor for long-term endothelial failure.4 If that is indeed the case, then a mathematical description of the postoperative cell loss in corneal grafts may allow long-term survival to be predicted and provide a rational basis for setting a minimum donor endothelial cell density compatible with long-term survival.

Because many biological processes are first-order, changing with time in an exponential manner, Redmond et al.7 modeled endothelial cell loss in grafts with up to 4 years’ follow-up, with the equation, \( D_t = D_0e^{-\lambda t} \), where \( D_0 \) and \( D_t \) are, respectively, the initial cell density and cell density at postoperative time \( t \), and \( \lambda \) is the exponential rate constant. The half-time for cell loss, calculated from the exponential rate constant, was only 3.4 years. This suggests that cell density could approach critical levels in only 7 to 10 years, which is somewhat sooner than longer term observations indicate.4 Use of a single phenomenologic exponential coefficient therefore appears unable to account for the likelihood that different mechanisms of cell loss predominate in the early and late postoperative phases. Intuitively, the initially high loss of endothelium could be attributed to adverse events such as surgical trauma, cell-mediated rejection episodes, and other postoperative complications. With time, these early mechanisms of cell loss would have a diminishing influence, and the underlying slow attrition of cells, albeit at a higher than normal rate, would predominate. Similarly, after cataract surgery, surgical trauma would cause and early rapid loss of cells, but an accelerated cell loss also persists for many years after surgery.8 Thus, two components to the cell loss may be defined after surgery: a rapid component that dominates the early postoperative period and a slow component that persists for many years.

Even with the decline in cell density with age in nondiseased corneas, there is evidence of more than one mechanism for the decrease. Møller-Pedersen9 found a higher rate of loss in the younger age groups. His data suggest an annual loss of 2.9% up to 14 years of age but only 0.3% after 14 years. Clearly, the mechanisms are different from those responsible for the cell loss after surgery; but, if it is assumed that there are indeed two components to the cell loss, both after surgery and with increasing age, then a biexponential decay model may be considered more appropriate than the monoexponential equation used by Redmond et al.7 Our purpose, therefore, was to evaluate a biexponential decay model for describing the normal endothelial cell loss with age, as well as the postoperative cell loss observed after both penetrating keratoplasty and cataract surgery.

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Supported in part by National Eye Institute Grant EY02037 (WMB).

Submitted for publication December 9, 2002; revised February 21, 2003; accepted February 26, 2003.

Disclosure: W.J. Armitage, None; A.D. Dick, None; W.M. Bourne, None.

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Endothelial cell loss with age in nondiseased eyes. Biexponential model fitted to data from cadaveric eyes (▲: Møller-Pedersen) showing 95% prediction interval. The coefficients are shown with their respective standard error (se) and the corresponding values for τ and p. The half times for the fast and slow components of the model, calculated from the relevant exponential rate constants, are 3.1 and 224 years, respectively. The residual standard deviation was 113.9 cells/mm². Also shown for comparison are data from live subjects (○: Yee et al.). The corresponding half times for the fast and slow components of the decay are 3.5 and 277 years, respectively.

Methods

Studies were identified from the literature that described changes in endothelial cell density. Data in three groups were sought: the typical change in cell density with age in nondiseased eyes, the loss of cells after cataract surgery, and the loss of cells after penetrating keratoplasty. Nonlinear least squares curve-fitting algorithms (Marquardt-Levenberg) were applied to the data (Axum ver. 5.0C Technical Graphics and Data Analysis software; MathSoft, Inc., Cambridge, MA).

A decay model was evaluated that fitted the sum of two exponentials to each set of published data: \( d = p \cdot \exp(-at) + q \cdot \exp(-bt) \), where \( d \) is cell density at time \( t \), \( p \) and \( q \) are constants the sum of which is equal to the initial cell density, and \( a \) and \( b \) are exponential rate constants. For each fitted parameter, the standard error and the probability for the \( t \)-test are given. The goodness of fit is indicated by the residual standard deviation. The 95% confidence and prediction intervals were calculated based, respectively, on the SE of the estimated values and the SD of the differences between the actual data and the estimated values. Half times for the two components of the decay were calculated as 0.693/exponential rate constant.

Results

Change in Endothelial Cell Density with Age

Møller-Pedersen reported cell densities measured in 178 corneas from 88 cadaveric donors ranging in age from 6 months to 90 years and from one 30-week fetus. No abnormalities were apparent in these eyes. The biexponential curve fitted to the mean cell density for each decade is shown in Figure 1. Fast and slow components to the decay were evident with half times, respectively, of 3.1 and 224 years. For comparison, data from an earlier study by Yee et al. are also shown in Figure 1. These measurements were made on 60 eyes from live subjects ranging in age from 12 to 85 years. Previous ocular surgery, elevated intraocular pressure, external ocular disease, contact lens wear or diabetes were exclusion criteria for the study. Measurements were made on 50 eyes up to 10 years after cataract extraction and implantation of an intracocular lens. Three types of lens implant were used: a medallion iris suture lens with intracapsular cataract extraction, a transiridectomy clip lens with extracapsular cataract extraction, and a posterior chamber lens with extracapsular cataract extraction. No differences in endothelial cell density were reported for the different lens types, justifying the pooling of these data. Figure 2 shows the biexponential curve fitted to the data. The rapid and slow components of the model had half times of 0.5 months and 315 months, respectively.

Change in Endothelial Cell Density after Cataract Surgery

Bourne et al. presented data on the change in endothelial cell density after cataract extraction. Patients with uveitis or proliferative diabetic retinopathy or those who had undergone previous ocular surgery were excluded from the study. Measurements were made on 50 eyes up to 10 years after cataract extraction and implantation of an intracocular lens. Three types of lens implant were used: a medallion iris suture lens with intracapsular cataract extraction, a transiridectomy clip lens with extracapsular cataract extraction, and a posterior chamber lens with extracapsular cataract extraction. No differences in endothelial cell density were reported for the different lens types, justifying the pooling of these data. Figure 2 shows the biexponential curve fitted to the data. The rapid and slow components of the model had half times of 0.5 months and 315 months, respectively.

Biexponential Decay Model for Endothelial Cell Loss

The biexponential model we have presented is not put forth as definitive, rather the goal was to present an approach that may, as more data become available, lead to a robust mathematical
description of postoperative cell loss. Other mathematical models could have been chosen. However, when the monoexponential used by Redmond et al.\(^7\) was applied to long-term follow-up data, it underestimated early cell loss and significantly overestimated cell loss in the long term (data not presented). Alternatively, Møller-Pedersen\(^3\) used a two-phase linear model that required dividing the data into two groups to which separate lines were fitted by linear regression. The advantage of using the biexponential model is that a single curve is fitted to the entire data set without the need arbitrarily to divide the data.

Clearly, there are deficiencies in the fitting procedure for the corneal graft data. Each time point does not represent a series of independent measurements, nor, given that these are repeated measures, are the same grafts represented at every time point. These factors could have two adverse effects on the reliability of the model. First, the early phase of cell loss may be exaggerated by cell counts in grafts that undergo rapid cell loss and early failure. Second, the data for the longest postoperative time are the least reliable (i.e., lowest number of observations), yet are likely to have a disproportionately large influence on the fitted curve and, hence, on the estimates for the rate constants. This means that extrapolation much beyond the final measured time point could become increasingly unreliable. It is also uncertain how the undoubted variations in the distributions of both patient age and diagnosis at each time

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**Figure 2.** Endothelial cell loss after cataract surgery. Biexponential model fitted to data from Bourne et al.\(^8\) showing 95% prediction interval. The coefficients are shown with their respective standard error (se), and the corresponding values for t and p. The half times for the fast and slow components of the model, calculated from the relevant exponential rate constants, are 0.5 and 315 months, respectively. The residual standard deviation was 37.0 cells/mm\(^2\).

**Figure 3.** Endothelial cell loss after penetrating keratoplasty. Biexponential model fitted to data from Bourne\(^4\) showing 95% confidence interval (inner dotted lines) and 95% prediction interval (outer dashed lines). The coefficients are shown with their respective standard error (se) and the corresponding values for t and p. The half times for the fast and slow components of the model, calculated from the relevant exponential rate constants, are 8.6 and 257 months, respectively. The residual standard deviation was 109.6 cells/mm\(^2\).
point would influence the overall pattern of decreasing cell density.

Earlier data from Bourne et al.10 and Ing et al.11 who reported measurements on cell densities in grafts for which the same patients were measured at every time point, were therefore compared with the cell densities predicted by the model (Table 1). Notably, both sets of data included only grafts with no reported rejection episodes and no reoperations affecting the corneal endothelium. These two cohorts are subsets of the cohort used to create the model. Despite this overlap, the agreement appears to be good, with the largest differences evident at two months after surgery. At all other time points, the deviations from the predicted values varied by only a few percentage points and both sets of data were well within the 95% prediction limits.

### Endothelial Cell Loss

The changes in endothelial cell density in nondiseased eyes reported by Møller-Pedersen3 demonstrate the nonlinear nature of the change in cell density with age and the faster decline in infants and children (Fig. 1). Møller-Pedersen fitted two straight lines by linear regression with a break point at approximately 14 years, giving an initial decline in cell density of 2.9% per annum, which then slowed to 0.3% per annum. Our model, which fitted a single equation to the complete data set, also suggests that the rapid component becomes negligible after approximately 15 years and that the underlying slow component, with its half time of approximately 225 years, thereafter predominates. Removing the first data point, which was just a single observation from a 30-week fetus, had little impact on the fitted curve, reducing the half time for the slow component to 200 years. Such a long half time indicates a substantial excess capacity of the endothelium compared with a typical human life span. The agreement with the data of Yee et al.2 from live subjects, which gave a half time for the slow component of 277 years, is good (Table 2).

After cataract surgery (Fig. 2), the rapid component of the cell loss, which is presumably due to surgical trauma, becomes negligible after 6 months. With the use of viscoelastics and small-incision procedures, this trauma is likely to be reduced, which is reflected in a lower cell loss over the first year after surgery.12,13 In marked contrast to the cell loss with age, the half time of just 26 years for the slow component after cataract surgery was approximately 10-fold shorter (Table 2). Whether viscoelastics and small-incision surgery also reduce this impact on the underlying slow component of the cell loss remains to be determined. The data from Bourne et al.8 used in Figure 2 came from measurements on 50 eyes with three different types of lens implant. The type of implant had no influence on postoperative cell density. Moreover, they also presented data from a further 17 eyes with 10-year follow-up after cataract extraction, but without lens implantation. The postoperative cell densities in this group were not different from the lens implant group, suggesting that the continued, long-term cell loss was not simply due to the presence of a lens implant.

After penetrating keratoplasty, the rapid component of the cell loss lasts longer than after cataract surgery, becoming negligible only after 4 years, and reflecting more severe surgical trauma and postoperative complications, including cell-mediated immunologic reactions. The underlying slow component of the loss, although substantially higher than in nondiseased eyes, is intriguingly similar to that after cataract surgery, 21 versus 26 years (Table 2). Bourne3 argued that, in the majority of grafts, there was little evidence to suggest that cell-mediated rejection contributes significantly to late endothelial failure (i.e., to the slow component of cell loss). This raises the prospect that there may be a common mechanism of long-term cell loss after cataract surgery and penetrating keratoplasty, perhaps stemming from a nonspecific, innate response initiated by breakdown of the blood–ocular barrier.

### Mechanism of Postoperative Cell Loss

Although the reasons for the exacerbated, long-term cell loss after surgery are unknown, it is likely that the surgical intervention alters in some way corneal and/or anterior chamber homeostasis. The breakdown of the blood–ocular barrier and subsequent inflammatory response would cause a transient increase in proinflammatory cytokines, the potential mobilization of dendritic cells, and create a proapoptotic milieu. Given that all three cell types of the cornea express Fas and Fas-ligand as well as regulators of apoptosis, Bcl-2, Bcl-XL, and Bax,14 these changes in the anterior chamber could potentiate endothelial loss. There could also be disruption of control mechanisms that render endothelial cells less susceptible to apoptosis, such as that reported by Li et al.15 who observed

<table>
<thead>
<tr>
<th>Postoperative Time (mo)</th>
<th>Calculated (95% PI)*</th>
<th>Study A†</th>
<th>Δ (%)‡</th>
<th>Study B§</th>
<th>Δ (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>2854 (2499–3210)</td>
<td>2956 (546)</td>
<td>+3</td>
<td>3019 (594)</td>
<td>+6</td>
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<tr>
<td>2</td>
<td>2630 (2276–2985)</td>
<td>2417 (756)</td>
<td>−8</td>
<td>2366 (801)</td>
<td>−10</td>
</tr>
<tr>
<td>12</td>
<td>1909 (1557–2260)</td>
<td>1998 (705)</td>
<td>+5</td>
<td>1969 (735)</td>
<td>+3</td>
</tr>
<tr>
<td>36</td>
<td>1348 (1003–1693)</td>
<td>1418 (600)</td>
<td>+5</td>
<td>1373 (551)</td>
<td>+2</td>
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<tr>
<td>60</td>
<td>1199 (858–1540)</td>
<td>1214 (555)</td>
<td>+1</td>
<td>1171 (489)</td>
<td>−2</td>
</tr>
<tr>
<td>120</td>
<td>1010 (666–1553)</td>
<td>—</td>
<td>—</td>
<td>958 (471)</td>
<td>−5</td>
</tr>
</tbody>
</table>

* Calculated cell densities from biexponential equation fitted to data from Bourne4 (see Figure 3) with 95% prediction interval (PI).
† Mean (SD) cell densities in 129 grafts with 5-year follow-up from Bourne et al.10
‡ Percentage difference between measured and predicted cell densities.
§ Mean (SD) cell densities of a subset of 72 grafts from Study A with 10-year follow-up from Ing et al.11

### Table 2. Summary of Half Times for the Rapid and Slow Components of Exponential Cell Loss with Age, after Cataract Surgery and after Penetrating Keratoplasty

<table>
<thead>
<tr>
<th>Component</th>
<th>Rapid (years)</th>
<th>Slow (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3.00</td>
<td>224</td>
</tr>
<tr>
<td>Møller-Pedersen3</td>
<td>4.00</td>
<td>277</td>
</tr>
<tr>
<td>Cataract*</td>
<td>0.04</td>
<td>26</td>
</tr>
<tr>
<td>Penetrating keratoplasty†</td>
<td>0.70</td>
<td>21</td>
</tr>
</tbody>
</table>

* Bourne et al.8
† Bourne4
upregulation of Bcl-2, a suppressor of apoptosis, in corneal endothelial cells by factors, produced by iris/ciliary body cells, normally present in the aqueous humor. In addition to direct responses on endothelial cells, a response could be mediated, perhaps through keratocytes. It has been reported that in Fuchs’ endothelial dystrophy, there is a decrease in Bcl-2 in keratocytes, suggesting increased susceptibility to apoptosis which, in turn, may have a deleterious effect on the morphology and function of endothelial cells. Alternatively, it has been proposed that the accumulation of advanced glycation end products in Descemet’s membrane affects the attachment of endothelial cells and may contribute to the loss of cells with age and after cataract surgery. There remains, however, the question of the longevity of these responses and the presumed failure of the altered immunologic environment of the anterior chamber and cornea to correct itself.

A Rationale for a Minimum Donor Endothelial Cell Density for Penetrating Keratoplasty

The loss of endothelial cells and indeed graft survival will initially be dominated by complications such as vascularization, inflammation and cell-mediated rejection. The indication for the graft will itself strongly influence graft survival, especially in the first few years after transplantation. In the longer term, however, Bourne found that grafts that had endothelial failure had not lost cells any faster than grafts that did not fail; rather, the failed grafts had lower initial donor endothelial cell densities and simply reached cell densities incompatible with graft function sooner. The time taken to reach a putative critical cell density may be estimated by extrapolation of the current model (Fig. 3). If the critical density is taken to be 500 cells/mm², it can be seen from Figure 4 that corneas with initial cell densities lower than 2000 cells/mm² could reach the critical density in less than 20 years. With initial densities above 2500 cells/mm², however, the grafts should remain functioning for at least 50 years. These extrapolations are based on the upper limit of critical cell density reported in the literature by endothelial cell density rather than by donor age, which allows corneas from older donors (>70 years) to be made available for transplantation. Minimum donor cell densities are typically 2000 to 2500 cells/mm². Provided that grafts survive beyond the early complications, the model suggests that donor cell densities within this range should provide sufficient endothelial capacity for at least 20 years of graft survival before the upper limit (i.e., 500 cells/mm²) of the critical range of cell density is reached (Fig. 4).

In summary, a biexponential decay model was used to describe the change in endothelial cell density with increasing age in nondiseased eyes, and the postoperative decline in cell density after cataract surgery and penetrating keratoplasty. The intention was to suggest an approach that may in time produce a robust mathematical description of endothelial cell loss. Only with the gathering of more extensive data can the central assumptions of the model be properly tested, principally that the underlying slow rate of attrition is independent of the initial cell density. The impact of other factors, such as donor age and storage method of corneal grafts, also should be evaluated. Even in its present form, however, insights may be gained and questions posed about the mechanisms of cell loss and the similarity between the loss after cataract surgery and penetrating keratoplasty. Moreover, the 10-fold decrease suggested by the model in the half time of the slow component of cell loss after surgery compared with that of nondiseased eyes emphasizes that this accelerated cell loss is evident for many years after the surgical intervention. Finally, although acknowledging the constraints of the current model and the uncertainty of extrapolation, it provides a rationale for setting a minimum donor endothelial cell density for use in eye banks.

Acknowledgments

The authors thank Stephen Kaye, Monica Berry, and Andrew Tullo for helpful comments and stimulating discussion.

References


