Dynamics of Corneal Epithelial Healing after an Alkali Burn

A Statistical Analysis

L. David Ormerod,*† Armando Garsd,*‡ Chittaranjan V. Reddy,*∥ Steven A. Gomes,* Mark B. Abelson,*† and Kenneth R. Kenyon*†

A precise definition of epithelial healing kinetics following chemical injury is necessary to aid the investigation of control mechanisms, potential therapeutic intervention and ophthalmic drug toxicity. Wound healing was studied photographically at frequent intervals in rabbits following IN or 4N alkali burns. Planar wound areas were determined by computerized planimetry and transformed mathematically to curved surface data. The decrease in equivalent wound radius with time was computer-modelled using two linear and three nonlinear regressions. A periodic function was also investigated. Serial photographs showed that intermittent attrition of small areas of the migrating wound edge was a common confounding variable. Although excellent coefficients of determination were found for all models, the addition of nonlinear factors gave a small advantage. A mean lag phase of 3.96 and 6.52 hr occurred after IN and 4N alkali burns, respectively; wound edge attrition was notably prevalent in early healing. Epithelial healing in the rabbit had a fundamental linear component, with mean epithelial migration rates of 76 and 80 μm/hr after IN and 4N alkali burns, respectively, and which continued to closure. A quadratic nonlinear component was also suggested. No significant circadian component was detected. Invest Ophthalmol Vis Sci 30:1784–1793, 1989

Defects of mammalian corneal epithelium heal initially by the sliding of adjacent cells,1–4 which flatten and increase in cell size and volume.5 This process requires functional actin filaments6–7 and the successive association and disassembly of focal adhesion sites,8 but is independent of cell mitosis.1 Epithelial wound closure usually occurs in an orderly manner. Cell movement at the wound margin is primarily restricted by contact with adjacent cells; considerable reorganization of the epithelial sheet occurs behind the wound edge. Qualitative differences in the underlying substrate also may influence the process.9,10

Traditional kinetic analyses have usually fitted linear models to the decrease of area with time, which implies a complex motion of the wound edge proportional to the linear areal function squared. Most studies, however, have (1) measured a planar photogrammetric area, which underestimates (with a nonlinear error) the actual curved surface of the wound; (2) modelled the kinetics on few data points; and (3) assumed that the observations are normally distributed and free of time-related errors. Studies that have attempted to reconcile most of these methodologic problems have proposed both linear11 and nonlinear12,13 models of corneal epithelial wound closure with respect to wound radius with time.

To overcome the potential bias that outlier observations and temporal errors imply in traditional models, we used a simple nonparametric model and frequent observations to describe the dynamics of corneal epithelial healing following alkali burning. The performance of the model is compared with a first-order difference equation, with first-,11 second-, and third-order12,13 least-squares regressions, and with a periodic function.

Materials and Methods

This investigation adhered to the ARVO Resolution on the Use of Animals in Research. Thirteen male New Zealand white rabbits (2.75–3.25 kg) were anesthetized with intramuscular injections of keta-
mine hydrochloride 50 mg/kg and chlorpromazine hydrochloride 10 mg/kg, and with topical proparacaine hydrochloride 0.5%. Central corneal burns were made by 2 min unilocular applications of a 7 mm diameter circular disc of Whatman #3 filter paper (Whatman, Maidstone, England) that was soaked submaximally in 1N sodium hydroxide (using a uniform technique reproducible to ± 5% by weight, unpublished data). Balanced salt solution (15 ml) (Iolab, Claremont, CA) was instilled dropwise over 60 sec.

Beginning 6 hr post-burn, photographs of the corneal surface, stained using nonpreserved fluorescein strips, were taken at ~2 hr intervals over the first 24 hr, 4 hr intervals over the second day, and 6 hr intervals over the third day, around the clock. Times were recorded to the nearest minute. A fixed-focus camera with focusing bracket was used with TRI-X Pan (Eastman Kodak, Rochester, NY) monochrome film. A 2X yellow filter (BDB, Luton, England) was fitted over the camera lens and a cobalt blue exciting filter (Kodak Wrattan 47B) over the photoflash.

In a separate experiment with eight rabbits, we investigated the duration of the initial lag phase of wound closure by taking approximately hourly photographs from hours 1 to 9 after unilateral 1N or 4N NaOH alkali burning, performed in an identical manner. Photographs also were taken at 34 hr and then at 2 hr intervals between 48 and 58 hr after 1N or 4N alkali burning to study the final phases of wound closure.

Analysis of Wound Healing

Photographic prints (10" X 7") were made with a final magnification of X10.8 (camera X1.46, printing X7.4). Serial photographs from each animal were carefully examined using a divider. For each time point, the area of the epithelial defect was digitized on a Zeiss Videoplan 2 Tablet (Rainin Instruments, Woburn, MA) and the mean of three area measurements taken. Planar wound areas (Ap) were treated as equivalent circles and the equivalent planar wound radii (rp) determined. However, planar equivalent wound radii must be transformed to curved surface data12"14 (Fig. 1). Assuming the curvature of the central and paracentral rabbit cornea to be spherical, and the radius of curvature (R) to be 7.3 mm,15 the spherical wind radii (rs) and spherical wound areas (As) can be derived simply as follows: (a) rs = \int drp; and (b) drp = \sqrt{R^2 - r_p^2} + dh^2, by Pythagoras's theorem, where \int represents the integral and d the differential. Regarding the cornea as a sphere, it can be shown that

h = R - \sqrt{R^2 - r_p^2},

and similarly the spherical area (As) is given by

As = 2\pi R(R - \sqrt{R^2 - r_p^2})

Two-Point Slope Nonparametric Modelling

Let t be the time in hours from the beginning of the experiment and Yt a measure of the epithelial wound radius at time t. We can model epithelial healing by the linear function

Yt = \beta_0 + \beta_1 t + \epsilon_t

\epsilon_t represents the random error, which is usually assumed to be normally distributed, and \beta_0 (Y-intercept) and \beta_1 (linear healing rate or slope) are unknown parameters to be estimated from the data.

The nonparametric two-point slope [NON-PAR(2PS)] method assumes that the distribution of \epsilon_t is unknown and therefore may contain outlying observations, and also that t may be subject to error. Nonparametric estimates of \beta_0 and \beta_1 were calculated\textsuperscript{16,17} as follows. For any two observations, Y_{ti}, Y_{tj}, made at times t_i and t_j (i < j), a two-point slope is determined as
\[ \hat{\beta}_t(i, j) = \frac{(Y_u - Y_b)}{(t_i - t_j)} \]

and a two-point Y-intercept as

\[ \hat{\beta}_0(i, j) = Y_u - \hat{\beta}_t(i, j) t_i, \]

where the symbol * represents the fitted value. We can calculate two-point slopes and two-point intercepts between each datum point and every other point in the time course of the epithelial healing process. If \( S_1 \) is the set of all the slopes and \( S_0 \) the set of all the Y-intercepts so obtained, and \( M(S_1) \) and \( M(S_0) \) define their respective sample medians, then we can take these as the best estimates of \( \beta_1 \) and \( \beta_0 \), respectively.

As the ordinary coefficient of determination, \( r^2 \), is not resistant to outliers, we introduced Kvålseth’s nonparametric resistant coefficient of determination, \( rr^2 \). For each observation \( Y_t \), we defined its nonparametric fitted value \( \hat{Y}_t \).

\[ \hat{Y}_t = b_0 + b_1 t \]

The nonparametric error (\( e_t \)) was defined as

\[ e_t = Y_t - \hat{Y}_t \]

The resistant coefficient of determination is then

\[ rr^2 = 1 - \frac{M(|e_t|)}{M(|Y_t - M(Y_t)|)^2} \]

where the symbols \( | \) represent absolute values.

**Modelling by the Difference Equation**

A weaker alternative to a linear model is the first-order difference equation

\[ Y_t = \beta_0 + \beta_1 Y_{t-1} + \epsilon_t \] (2)

that establishes a linear relation only between two consecutive values of \( Y_t \). This equation is stochastic, incorporating errors as it proceeds through the time course. Therefore, the difference equation is not linear as an overall function of time.19

**Least-Squares Modelling**

For comparative purposes, the data sets also were investigated by three least-squares analyses.20 We incorporated the first-order least-squares model (FOLS) based on Equation (1) as used by Crosson and colleagues14 and the cubic (third-order) model (TOLS) of Kwok and Madigan,12,13 as well as a second-order least-squares (SOLS) analysis.

Specifically, Equation (1) can be extended to include nonlinear terms:

- Quadratic function (SOLS)
  \[ Y_t = \beta_0 + \beta_1 t + \beta_2 t^2 + \epsilon_t \] (3)
- Cubic function (TOLS)
  \[ Y_t = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \epsilon_t \] (4)

**Modelling a Periodic Component**

Equation (3) can be extended to incorporate sin and cos functions of a periodic component

\[ Y_t = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 \sin(\omega t) + \beta_4 \cos(\omega t) + \epsilon_t \]

that can be rewritten21 to

\[ Y_t = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 \cos(\phi) \epsilon_t \] (5)

where \( A \) is the amplitude, \( \omega \) is the angular frequency (in radians), and \( \phi \) is the phase (in radians); \( \omega \) is related to the period, \( p \), through \( \omega = p/2\pi \). We investigated a 24 hr periodicity. The spherical radial data and the incremental wound edge velocities, \( \Delta r/\Delta t \), were modelled according to Equation (5) with determinations of \( A \), \( \phi \), and the real-time peak; the models were fitted by standard multiple linear regression analysis.21

Computer programs were written for all methods using RS/Explore software.22 The transformed data points and the regression lines obtained by the nonparametric two-point slope method and the least-squares analyses were plotted separately for all rabbits. Linear and multiple linear statistical analyses were performed.

**Results**

Even, circular alkali burns were achieved by standard submaximal soaking of the filter discs. Epithelial healing was first studied in 13 rabbits at 18 time points over the 75 hr period following central 1N NaOH alkali burning, or until wound closure or the onset of late epithelial breakdown. To avoid the potential bias implicit in the existence of an initial lag phase prior to epithelial migration, the (approximate) 6 hr post-burn point was the first observation time. Data were corrected for conical sphericity.

The kinetic parameters and significance levels for four of these models are shown in Table 1. Excellent overall coefficients of determination \( (r^2) \) were found for the application of all the models, often with differences only in the third decimal place. As expected from tightly linear data, the ordinary \( r^2 \) is slightly higher for the FOLS than for the NONPAR(2PS) method. The nonparametric resistant \( rr^2 \) appears to put the nonparametric method on equal footing.
Measured by $r^2$ alone, the predictive performance of the TOLS is superior by a small margin.

In general, Table 1 shows good agreement in the estimation of $\beta_0$ and $\beta_1$ by the nonparametric and the first-order regressions. However, these estimates differ from those obtained from second- and third-order least-squares; many of the differences in the $\beta_1$ are due to underlying collinearities between the linear, quadratic, and cubic components (particularly in the cubic models) as shown by the lack of significance of one or more $\beta$s in the presence of a high $r^2$. Sixty-two percent (8 of 13) of the rabbit data undergo reduction in the significance of $\beta_1$ within the third-order regression. Only 23% (3 of 13) and 31% (4 of 13) of the rabbits show partially significant $\beta_2$ and $\beta_3$, respectively.

Graphs of spherical wound radii against time were plotted for all data sets with the fitted regression slopes (dashed lines) superimposed from each of the mathematical models. Three examples are shown with FOLS, NONPAR(2PS), and TOLS regression lines in Figure 2.

Visual analysis of the photographs using a divider showed the symmetrical healing of epithelial wounds to be complicated by instability of the migrating wound edge. Small, localized areas of migrated epithelium (Fig. 3) broke away from the leading edge during epithelial closure in all but two animals (nos. 11 and 13) (Fig. 4). All quadrants were involved, but the superior quadrant most frequently. This epithelial attrition was commonly multiple and recurrences were sometimes contiguous. Epithelial loss did not occur behind an intact epithelial margin. In several eyes, the lag phase of epithelial healing was unduly prolonged (for a number of hours) over small segments compared with the rest of the wound periphery, but then healed symmetrically; these areas appeared unrelated to later marginal attrition.

To determine whether the observed nonlinearity might be entirely explicable by the episodes of wound edge attrition, we investigated individual data sets by the first-order difference equation [Equation (2)].$^{19}$ The coefficients of determination ($r^2$) were high but somewhat inferior (data not shown) to the $r^2$ calculated from the other mathematical models, suggesting a true deterministic nonlinear component, exclusive of wound edge attrition.

Restricting the data sets to spherical wound radii of $\leq 3.6$ mm (from 12–17 hr post-burn) removed 42% of the episodes of epithelial attrition (and might be expected to avoid the prolonged effects of corneal alkalinization). The modelled kinetic parameters and significance levels for this limited time course (data not shown) failed to improve the mean coefficients of determination, except for the NONPAR(2PS) mean.

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>$r^2$</th>
<th>$\beta_0$</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
<th>$\beta_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6505</td>
<td>0.0990</td>
<td>0.946</td>
<td>4.425</td>
<td>-0.0583</td>
</tr>
<tr>
<td>2</td>
<td>0.4957</td>
<td>0.0724</td>
<td>0.9955</td>
<td>4.515</td>
<td>-0.0772</td>
</tr>
<tr>
<td>3</td>
<td>0.5751</td>
<td>0.1050</td>
<td>0.9878</td>
<td>4.745</td>
<td>-0.0650</td>
</tr>
<tr>
<td>4</td>
<td>0.7451</td>
<td>0.1096</td>
<td>0.9898</td>
<td>4.875</td>
<td>-0.1050</td>
</tr>
<tr>
<td>5</td>
<td>0.8251</td>
<td>0.1120</td>
<td>0.9851</td>
<td>4.925</td>
<td>-0.1050</td>
</tr>
<tr>
<td>6</td>
<td>0.8851</td>
<td>0.1150</td>
<td>0.9814</td>
<td>4.975</td>
<td>-0.1050</td>
</tr>
<tr>
<td>7</td>
<td>0.9251</td>
<td>0.1180</td>
<td>0.9784</td>
<td>4.985</td>
<td>-0.1050</td>
</tr>
<tr>
<td>8</td>
<td>0.9651</td>
<td>0.1210</td>
<td>0.9751</td>
<td>4.995</td>
<td>-0.1050</td>
</tr>
<tr>
<td>9</td>
<td>0.9851</td>
<td>0.1240</td>
<td>0.9721</td>
<td>4.995</td>
<td>-0.1050</td>
</tr>
<tr>
<td>10</td>
<td>0.9951</td>
<td>0.1270</td>
<td>0.9691</td>
<td>4.985</td>
<td>-0.1050</td>
</tr>
<tr>
<td>11</td>
<td>0.9951</td>
<td>0.1300</td>
<td>0.9661</td>
<td>4.975</td>
<td>-0.1050</td>
</tr>
<tr>
<td>12</td>
<td>0.9951</td>
<td>0.1330</td>
<td>0.9631</td>
<td>4.965</td>
<td>-0.1050</td>
</tr>
<tr>
<td>13</td>
<td>0.9951</td>
<td>0.1360</td>
<td>0.9601</td>
<td>4.955</td>
<td>-0.1050</td>
</tr>
</tbody>
</table>

Note: $\beta_0$ = intercept; $\beta_1$ = linear healing rate; $\beta_2$ = quadratic healing rate; $\beta_3$ = cubic healing rate; $r^2$ = coefficient of determination; $r^2$ = $p < 0.05$; $p < 0.01$.
Fig. 2. Plots of the decrease of equivalent epithelial wound radius over time following 1N alkali burning of rabbits 3, 5 and 8. Regression lines from first-order least-squares (FOLS), nonparametric two-point slope (NONPAR [2PS]), and third-order least-squares (TOLS) models are superimposed.

Fig. 3. Individual episodes of corneal epithelial wound edge attrition following 1N NaOH alkali burn with preceding photograph for comparison. (A) Rabbit 2 at 17 hr. (B) Rabbit 3 at 31 hr. (C) Rabbit 5 at 25 hr. All times are post-burn.

rr² that increased from 0.9904 to 0.9936. The linear component remained unchanged. Mean linear epithelial migration rates of 70 and 74 μm/hr were calculated for the complete and restricted data sets, respectively, for both the NONPAR[2PS] and FOLS methods (P > 0.05). However, the colinearities underlying Equation (4) (TOLS) could no longer be resolved statistically for the restricted data of five animals. Plots of the time-restricted data sets from the three animals least affected by episodes of epithelial wound edge attrition are shown with three regression slopes in Figure 5.

A sensitive analysis of wound healing is to investigate the temporal aspects of the incremental wound edge velocities, that is, to plot \( \frac{\Delta r}{\Delta t} \) against time.

Smoothed plots for all 13 rabbits are shown in Figure 6. The most frequent pattern showed a gradual, even acceleration through to wound closure, suggesting a quadratic component in this model of epithelial healing. In many animals, plots of the actual \( \frac{\Delta r}{\Delta t} \) data, rather than the smoothed data, suggested the possibility of a circadian rhythm (Fig. 7). However, mathematical modelling for a periodic component based on a 24 hr cycle failed to show a significant circadian rhythm in any animal (data not shown); these results were demonstrated not to be due to statistical colinearities.
To clarify further the nonlinear component(s) of healing, we investigated four rabbits each after 1N or 4N sodium hydroxide burns and concentrated our investigation on the early lag phase and the late preclosure phase. Analysis of hourly photographs in the early phase showed frequent initial instability of the wound edge and somewhat variable onset of epithelial migration around the wound; this precluded satisfactory modelling of the first several hours of epithelial wound healing (after alkali burning). A mean lag phase (±SD) of 3.96 (±0.96) hr and 6.52 (±1.83) hr occurred after 1N and 4N alkali burns, respectively, defined by at least 3 clock-hours of epithelial migration when compared with photographs taken 1 hr earlier. There was a tendency for narrow segmental areas of wound enlargement to occur (within the opaque margins of the corneal burn), even following the onset of wound migration.

In the late plots, the principal linear component was again emphasized (Fig. 8). There was no suggestion of late deceleration of the wound edge when photographs were taken at 2 hr intervals. In this experiment, mean linear healing rates (FOLS) of 86 and 80 μm/hr following 1N and 4N NaOH, respectively, were calculated. By combining the data from both experimental groups, a mean linear healing rate of 76 μm/hr was seen in the 17 rabbits after 1N alkali burning; this was not significantly different from the linear rate seen in four 4N alkali-burned animals (80 μm/hr).

Fig. 4. Temporal relationships of observed episodes of corneal epithelial wound edge attrition (diamonds) in 13 rabbits following 1N NaOH alkali burn.

Discussion

In agreement with Crosson and colleagues\(^1\)1 and Kwok and Madigan,\(^12,13\) we found conclusive evidence that the rate of rabbit corneal epithelial healing, expressed as a point migration along a wound radius, contains a fundamental linear component. By careful analysis, we showed that the linear component of epithelial migration is maintained to wound closure. High coefficients of determination were found for the linear models (mean \(r^2\) of 0.9872 for the two-point slope method, and mean \(r^2\) of 0.9889 for...
A mean epithelial migration rate of 76 μm/hr was calculated for 17 rabbits after a 1N NaOH alkali burn injury. Moreover, essentially linear kinetics were observed in epithelial healing occurring across a substrate subject to marked biochemical changes because of the severe alkali injury (although possibly not yet accompanied by ultrastructural changes in epithelial basement membrane morphology). Although the alkali burn is not a good general model of corneal epithelial healing, closely comparable rates (60–75 μm/hr) have been similarly calculated from published data of wound closure in rabbits after alkali burn, heptanol, or iodine vapor chemical injury, or abrasion injury.

An important observation of this study was the random and sporadic, localized attrition of the migrating epithelial wound edge that was observed in 11 of 13 eyes. Such wound margin instability is presumably a manifestation of the functional immaturity of adhesion mechanisms during active epithelial migration. Late fluorescein leakage into the wound edge may be a physiologic manifestation of this immaturity. The frequent fluorescein staining and handling of the animals for photography possibly may have been factors, although nonpreserved fluorescein has been shown to be nontoxic for the migrating corneal epithelium and overall healing rates were unaffected. It is also not clear whether wound edge attrition may complicate epithelial wound healing after other injuries, but published photographs suggest its occurrence after iodine vapor, heptanol, and keratectomy injuries in the rabbit, mechanical injuries in the rat, and epithelial trauma in man. More vigorous lid function may explain the apparently greater irregularity of healing in man compared with experimental...
animals, and botulinum toxin-induced protective ptosis has been shown to be an effective therapy for many persistent epithelial defects. Frequent observations are required to detect small localized areas of epithelial attrition occurring during the healing process.

The coefficients of determination of the SOLS and TOLS models were superior to those obtained by linear modelling, and improved correlation of fitted curves could be shown to plotted data. There appeared to be a relatively uniform nonlinear component(s) throughout the time course. In general, the addition of quadratic or cubic terms to the linear regressions was about equally effective in explaining this enhancement, but the average extra significance achieved was only at the level of 6 or 7 in 1000. The assumption of second- or third-order functions failed to improve the coefficients of determination obtained with each individually. As marked colinearities were found among the vectors, the nature of the nonlinear component could not be resolved by conventional statistical analysis. (This important observation would have been lost if the data sets had been averaged.) Therefore, more indirect methods of scrutiny were necessary.

The inferiority of the coefficients of determination with the first-order difference equation showed that it was impossible to reconcile the nonlinearity stochastically, so that wound edge instability could not adequately explain the nonlinearity. A sensitive analysis was provided by plotting the incremental wound edge velocities against time, $\frac{\Delta \text{vt}}{\Delta t}$ vs. $t$; the frequent data points were particularly valuable. There was no real evidence in the smoothed plots for an inherent cubic component to epithelial healing. Most plots indicated a quadratic component with a gradual, even acceleration of differing magnitude through to wound closure.

Kwok suggested that epithelial healing occurs with a combination of linear, quadratic and cubic terms, involving an initial increasing acceleration of the wound edge which then decelerates progressively before closure. Supportive evidence is adduced from the literature. However, most of these models are based on a small number of data points, incorporate the epithelial migration lag phase (which is insufficiently documented), and are dependent on the assumed normality of initial wound healing post-injury, and the vulnerability of the data to bias caused by any wound edge instability, particularly in the terminal healing phase.

We further investigated the epithelial healing kinetics of the early lag phase and the late preclosure phase in two groups of animals after 1N or 4N alkali burning. Modelling the early healing kinetics in these alkali burn models was confounded by an irregular onset of epithelial migration around the wound and by significant wound edge instability; a working definition had to be adopted for the end of the lag phase. To determine whether residual corneal alkalinity is responsible for these findings, it will be necessary to study early wound healing in other models. Our initial experiment generally failed to show the late decrease in wound edge velocity that has commonly been reported, and this was confirmed by the late 2-hourly data points in these eight animals. In total, our evidence suggests that the nonlinear component in these rabbit models of epithelial healing is best described by a quadratic (second-order) function.

Although an approximation, linear modelling remains, with caution, a reasonable, practical solution for therapeutic and toxicologic studies. Linear models are relatively simple and straightforward for predictive purposes and can be analyzed by methods in the mainstream of established statistical procedures; nonlinear models present greater analytical problems. However, it is important for the purpose of such studies to carefully characterize each model individually, and a priori assumptions between models should not be made. Frequent observations and side-by-side comparison of photographic prints give richer data than infrequent slide photography. The possibility of circadian rhythms in epithelial healing has not been excluded by this study because of the greater number of time points required to satisfactorily model a periodic component, the unknowing influence of the severe corneal injury, and potential interruption of the frequent examinations.

The analysis of all data sets by both linear and nonlinear techniques provide important measures of the biology of epithelial healing, and also information as to the reproducibility and quality control of such experiments. The methods we have used can be implemented in any computer capable of handling standard multiple regression problems. The fact that the resistant coefficient of determination, $r^2$, which is less sensitive to outliers, was similar in value to the conventional $r^2$ is important evidence in this work that our data measurement was reliable. Parametric methods are based on the statistical assumption of normally distributed observations as well as the absence of errors in time measurement. Because errors in least-squares analyses are squared, resulting regression estimates are attracted toward outlying observations. The nonparametric two-point slope method is more robust to errors and allows greater flexibility in data interpretation. Nonparametric and least-squares analyses are therefore complementary,
and we recommend that they both be used when measuring epithelial healing.

Besides simple errors of measurement, other methodologic problems can be identified that may artifactually introduce nonlinear components. Corneal wounds are never perfectly spherical, and wound radii are estimated from averaged circles. Other sources of nonlinear error include the assumption of corneal sphericity (although the corneal periphery is increasingly nonspheric), inaccuracies in burn centration and photographic angulation and focusing variability. Although some nonlinearities may be artificial, we are satisfied that the potential errors in this study are unlikely to lead to bias in systematic interpretation.

The almost identical kinetic behavior of the 1N and 4N NaOH burn wounds and the manifest comparability of the linear epithelial healing rates calculated following these injuries with those from different injuries13 argue that, other than affecting the lag phase, the epithelium healed "normally" over the first 2–3 days following alkali injury. Time restriction of the data sets, to exclude the period of somewhat irregular healing immediately following alkali burning, did not improve the coefficients of determination of the linear or nonlinear models, except for the NONPAR(2PS) method.

In conclusion, our analysis of epithelial healing in the rabbit cornea following a severe alkali burn suggests the existence of an intrinsic and principally linear component in wound closure. However, there also is a quadratic nonlinear component above and beyond linearity. The instability of cellular adhesion during epithelial migration leading to wound edge attrition and several methodologic errors are important considerations. Further investigation is necessary to determine whether epithelial wound edge attrition is an inherent feature of corneal wound healing. For predictive pharmacologic and toxicologic purposes,12,13,26–29,31–35,37,39 our data further suggest that it is practical and reasonably accurate to resolve the kinetics of this model in favor of linear dynamics unless the detection of small changes is required. However, the kinetics of each model should be individually established. We have attempted to partly relax the assumption of overall linearity by replacing it in 2-point slope nonparametric modelling with the somewhat weaker model of stochastic linearity only between two immediate points, and the $R^2$ provides additional robustness to the method.

**Key words:** alkali burn, corneal epithelium, mathematical modelling, statistics, wound healing

**References**


