

# Mathematical Modeling of Corneal Epithelial Wound Healing

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## ABSTRACT

We propose a reaction-diffusion model of the mechanisms involved in the healing of corneal surface wounds. The model focuses on the stimulus for increased mitotic and migratory activity, specifically the role of epidermal growth factor. Analysis of the model equations elucidates the interaction and roles of the model parameters in determining the speed of healing and the shape of the traveling wave solutions which correspond to the migration of cells into the wound during the initial phase of healing. We determine an analytic approximation for the speed of traveling wave solutions of the model in terms of the parameters and verify the results numerically. By comparing the predicted speed with experimentally measured healing rates, we conclude that serum-derived factors can alone account for the overall features of the healing process, but that the supply of growth factors by the tear film in the absence of serum-derived factors is not sufficient to give the observed healing rate. Numerical solutions of the model equations also confirm the importance of both migration and mitosis for effective wound healing. By modifying the model we obtain an analytic prediction for the healing rate of corneal surface wounds when epidermal growth factor is applied topically to the wound.

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## 1. INTRODUCTION

The process of corneal epithelial wound healing involves a combination of cell migration, cell-to-substrate adhesion and cell mitosis. Immediately after wounding there is a lag phase, during which the cells adjacent to the wound edge undergo a phenotypic change with the loss of surface microvilli and appearance of lamellipodia indicating their

impending motion. The epithelial cells then migrate into the area of defect and increased mitotic activity is observed at the wound margin. These proliferating cells appear to push the whole epithelium towards the center of the wound, resulting in an epithelial plug, which gradually regresses to give epithelial continuity [3, 7, 16]. In a normal corneal wound the linear healing rate is approximately  $60 \mu\text{m h}^{-1}$  and is largely independent of wound size [9].

We use corneal wound healing as a prototype for the general process of epithelial wound healing because there is a large quantity of specific data readily available. Furthermore, the processes involved in corneal wound healing are of particular clinical significance for the keratectomy operation. This operation involves surgical removal of a fibrovascular growth that is causing abnormality of corneal curvature [14]. A key aspect of this operation is the final curvature of the corneal surface, and this has been investigated in the kinematic model of Kwok [17]. Our approach concentrates on the underlying cell biology, rather than on such detailed geometric considerations.

In this paper, we focus on the stimulus for increased mitotic and migratory activity. Although the absence of contact inhibition and the change in cell shape are both important, we concentrate on the role of the chemical regulator epidermal growth factor (EGF). EGF is a large protein whose receptor is expressed abundantly on epithelial cells. For a general wound topical application of EGF has been shown to stimulate re-epithelialization in a dosage-dependent manner [21]. Moreover, although a number of different growth factors play overlapping and synergetic roles during wound healing, most studies suggest that the EGF family is the main regulator of epithelial repair [19]. For this reason, we focus here on the role of EGF as an agent promoting wound healing. Some aspects of this work have been briefly presented elsewhere [10].

The source of growth factors in healing wounds is an area of recent biological controversy. In the case of the cornea, it is known that EGF is present in the tear film [22], which overlies the epithelium. Here we develop a theoretical model whose solutions suggest that the EGF in the tear film alone is insufficient to account for the experimentally observed healing rate. We go on to consider the possibility that the exposed underlying tissue within the wound acts as an additional source of growth factor.

## 2. MODEL EQUATIONS

We propose a reaction-diffusion model to investigate the relative importance of the stimulatory affects of EGF on migration and cell

division. The governing equations are of the form:

$$\begin{aligned} \text{Rate of Increase of Cell Density} &= \text{Cell Migration} + \text{Mitotic Generation} - \text{Natural Loss} \\ \text{Rate of Increase of EGF Conc.} &= \text{Diffusion} + \text{Production by Cells} - \text{Decay of Active EGF} \end{aligned}$$

We model cell movement by Fickian diffusion to capture cells moving down gradients in cell density due to contact inhibition, and we assume that the cell diffusion coefficient increases linearly with the EGF concentration. We take the EGF diffusion coefficient to be a positive constant  $D_c$ . Following a number of previous authors, we use a logistic growth form for the cell mitotic term [18, 26] and represent the chemical control of mitosis by an increasing function  $s(c)$ , where  $c(r, t)$  is the EGF concentration at position  $r$  and time  $t$ . Sloughing of the outermost epidermal cells is responsible for natural cell loss, and we take this to be a first-order process. Decay of active EGF is due to a combination of natural decay and cellular degradation; we assume the former to be first order in  $c$  and model the latter by a saturating term denoting the rate of internal degradation of bound EGF receptors. The production of chemical by the cells is represented by the function  $f(n)$ , discussed in more detail below, where  $n(r, t)$  is the cell density.

These assumptions imply the following model equations:

$$\frac{\partial n}{\partial t} = \nabla \cdot (D_n(c) \nabla n) + s(c) n \left( \nu - \frac{n}{n_0} \right) - kn \quad (1a)$$

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + f(n) - h(c) n - \delta c, \quad (1b)$$

where  $D_c$ ,  $\nu$ ,  $n_0$ ,  $k$ , and  $\delta$  are all positive constants; the functional forms of  $D_n(c)$ ,  $s(c)$ ,  $f(n)$ , and  $h(c)$  are discussed below.

The thickness of the epidermis is much smaller than the wound length and hence we can treat the epidermis as two dimensional. By considering a "linear" wound geometry, which models a long "strip" wound, we simplify the spatial domain from three dimensions to only one. We solve the equations on a semi-infinite domain  $-\infty \leq x < L$ , with  $0 \leq x < L$  representing half the initial wound domain, where 0 is the boundary of the epithelium and  $L$  is the center of the wound. Since the epithelium does not contract, 0 is a fixed boundary.

Biologically relevant initial and boundary conditions are

$$\begin{aligned} (1) \quad n(x, 0) &= c(x, 0) = 0 \quad \text{for } 0 \leq x < L \\ n(x, 0) &= n_0, \quad c(x, 0) = c_0 \quad \text{for } -\infty < x < 0 \end{aligned}$$

$$(2) \quad n(-\infty, t) = n_0, \quad c(-\infty, t) = c_0 \quad \forall t \geq 0 \\ n_x(L, t) = c_x(L, t) = 0 \text{ by symmetry } \forall t \geq 0,$$

where  $n_0$  and  $c_0$  represent the unwounded levels of cell density and EGF concentration, respectively.

Estimating the parameter values is vital to the comparison of model predictions with experimental data. Here we briefly discuss our estimation techniques.

(1) The cell cycle time is approximately 6.6 days, giving  $k = (\log 2 / 6.6 \times 24) \text{ h}^{-1} = 6.31 \times 10^{-3} \text{ h}^{-1}$ .

(2) The concentration of EGF in reflex tears is 4 ng ml<sup>-1</sup> [22] and the molecular weight of EGF is 6145 [5], so the unwounded level of chemical concentration  $c_0$  is about  $6.6 \times 10^{-10} \text{ M}$ .

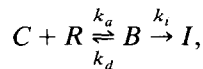
(3) The corneal epithelial cell length is approximately 10  $\mu\text{m}$  [14] and hence the unwounded level of cell density,  $n_0 \approx (1/10^{-5})^3 \text{ m}^3 = 10^{12} \text{ liter}^{-1}$ .

(4) In the absence of cellular degradation EGF has a half-life of approximately 1 h in vivo [6], so take  $\delta = \log 2 \text{ h}^{-1}$ .

(5) To estimate  $\nu$ , we follow Sherratt and Murray [28] and assume that when the chemical concentration  $c$  is at its unwounded level  $c_0$ , the net reaction term in the cell conservation equation is of logistic growth form  $kn(1 - n/n_0)$ . The rate of cell loss is  $-kn$ , so that  $s(c_0)n(\nu - n/n_0) - kn = kn(1 - n/n_0)$ . Therefore  $\nu = 2$  and  $s(c_0) = k$ .

(6) Previous modeling experience suggests that the order of magnitude of a dimensional cell diffusion coefficient, in vivo, is about  $10^{-9} \text{ cm}^2 \text{ s}^{-1}$  [20, 26]. As a first approximation we assume that the cellular diffusion coefficient increases linearly with EGF concentration and consider the functional form  $D_n(c) = \alpha c + \beta$ . We estimate the values of  $\alpha$  and  $\beta$  by matching model solutions with experimental data, within the constraint that  $\alpha c_0 + \beta \approx 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ .

(7) To determine the functional form for the cellular degradation term  $h(c)$  we begin by considering the interaction between the chemical  $C$ , the free receptors on the cell surface  $R$ , the bound receptors  $B$  and the internalized receptor-chemical complex  $I$ , which can be represented by



where  $k_a$ ,  $k_d$ , and  $k_i$  are positive rate constants. We use the law of mass action to derive three ordinary differential equations for  $c$ ,  $r$ , and  $b$  (the concentrations of  $C$ ,  $R$ , and  $B$ , respectively) and to circumvent the

complexities of receptor recycling we follow Sherratt et al. [29] and assume that the total number of receptors is a function of the number of bound receptors, specifically  $R + B = \rho + \gamma B$ , where  $\rho$  and  $\gamma$  are positive constants to be determined. Comparison of the time scales of these processes with cell movement and chemical diffusion shows that to a good approximation, the bound receptor density can be assumed to be at its steady-state level [24]. This leads us to look for a function of the form  $h(c) = \mu c / (\hat{c} + c)$ , where  $\mu$  and  $\hat{c}$  are positive constants. Using data for the variation in the number of bound and free receptors with bound receptor density [30] we can estimate  $\gamma$  and  $\rho$  which, in turn, gives  $\hat{c} \approx 2 \times 10^{-9} M$  and  $\mu \approx 5.75 \times 10^{-20} \text{ mol cell}^{-1} \text{ h}^{-1}$ .

(8) Any functional form for  $s(c)$  is subject to the nondimensional constraint  $s(c_0) = 1$ . As a first approximation, we assume that  $s(c)$  is linear and use data for the mitotic rate in the absence of any additional EGF [6] to estimate  $s(0) = 6 \times 10^{-4}$ . Later we show that  $s(c)$  must saturate for large concentrations of EGF to agree with other experimental data.

(9) The overlying tear film supplies a constant source of EGF, independent of cell number, and in the simplest model we take  $f(n) = A$ , where  $A$  is a constant, evaluated using the condition that  $n = n_0$ ,  $c = c_0$  is a steady state. We consider a more complex form for  $f(n)$  in a later refinement of the model.

Having established these parameter values, we nondimensionalize the model by defining the following dimensionless quantities:

$$\begin{aligned} n^* &= n / n_0, & c^* &= c / c_0, & x^* &= x / L \\ t^* &= kt, & \hat{c}^* &= \hat{c} / c_0, & s^*(c_0 c^*) &= s(c) / k \\ D_c^* &= D_c / kL^2, & f^*(n_0 n^*) &= f(n) / kc_0, & \mu^* &= \mu n_0 / kc_0 \\ \delta^* &= \delta / k, & D_n^*(c_0 c^*) &= D_n(c) / kL^2. \end{aligned}$$

Dropping the asterisks for algebraic convenience, we obtain the dimensionless model

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} \left( (\alpha c + \beta) \frac{\partial n}{\partial x} \right) + (\alpha_1 c + \beta_1) n(2 - n) - n \quad (2a)$$

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + f(n) - \frac{\mu n c}{(\hat{c} + c)} - \delta c. \quad (2b)$$

Throughout this paper we assume that the wound length  $2L$  is 4 mm, which gives the following dimensionless parameters:  $\alpha = 0.01$ ,  $\beta = 0.1$ ,  $D_c = 25$ ,  $\mu = 1.37 \times 10^4$ ,  $\hat{c} = 3.02$ ,  $\delta = 110$ ,  $\alpha_1 = 0.9$ , and  $\beta_1 = 0.1$ .

### 3. NUMERICAL SOLUTIONS

The biological observation of a front of cells moving into the wound at constant speed suggests that the model solutions should have a traveling wave form. However, numerical solutions of the model equations with  $f(n) = A$ , corresponding to no sources of EGF other than the tear film, fail to show definite wave fronts as in Figure 1a. Moreover, the "model wound" is far from healed in the experimentally observed time, which is 37 h for a 2-mm wound. We suspected that the absence of wave fronts was due to a long transient time for the evolution of a wave profile [2]. To give a clearer perspective on the model solutions, we thus solve the equations on  $-\infty < x < \infty$ , subject to initial conditions  $n = c = 0$  for  $x \geq 0$  and  $n = c = 1$  for  $x < 0$ , with boundary conditions  $n(-\infty, t) = c(-\infty, t) = 1$  and  $n_x = c_x = 0$  as  $x \rightarrow \infty$ . These new end conditions are not directly relevant to the biological problem; rather, they enable us to obtain a clearer understanding of mathematical aspects of our model. Numerical solutions show that with these amended end conditions, the system evolves to traveling waves of constant speed and shape in  $n$  and  $c$  as in Figure 2a, but the speed of these traveling waves ( $\approx 20.45 \mu\text{m h}^{-1}$ ) is much slower than the healing rate of actual corneal wounds ( $\approx 60 \mu\text{m h}^{-1}$ ). This result strongly suggests that there is another source of EGF in the normal healing process, in addition to the tear film.

We thus consider the possibility that the exposed underlying tissue within the wound releases EGF, which is rapidly degraded by cells at the wound edge. In this scheme, it is the rapidity of the degradation that results in the experimentally observed increase in cell proliferation and motility in a band of cells at the wound edge; the cells in this band degrade the additional chemical, preventing it reaching cells further from the wound edge. The possibility of the underlying tissue acting as a source of EGF is suggested by the experiments performed on other cell types by Dunn and Ireland [12] and Barrandon and Green [1]. In Barrandon and Green's experiments, the growth of large keratinocyte colonies was observed in vitro and was found to be dependent on the outward migration of the rapidly proliferating cells located in a thin rim close to the colony perimeter. Dunn and Ireland [12] examined the growth of 3T3 cells in rotating culture dishes and showed that in the presence of serum, new growth occurs at the margin of a wound in a cell sheet. They found that increased cell division occurs only down-

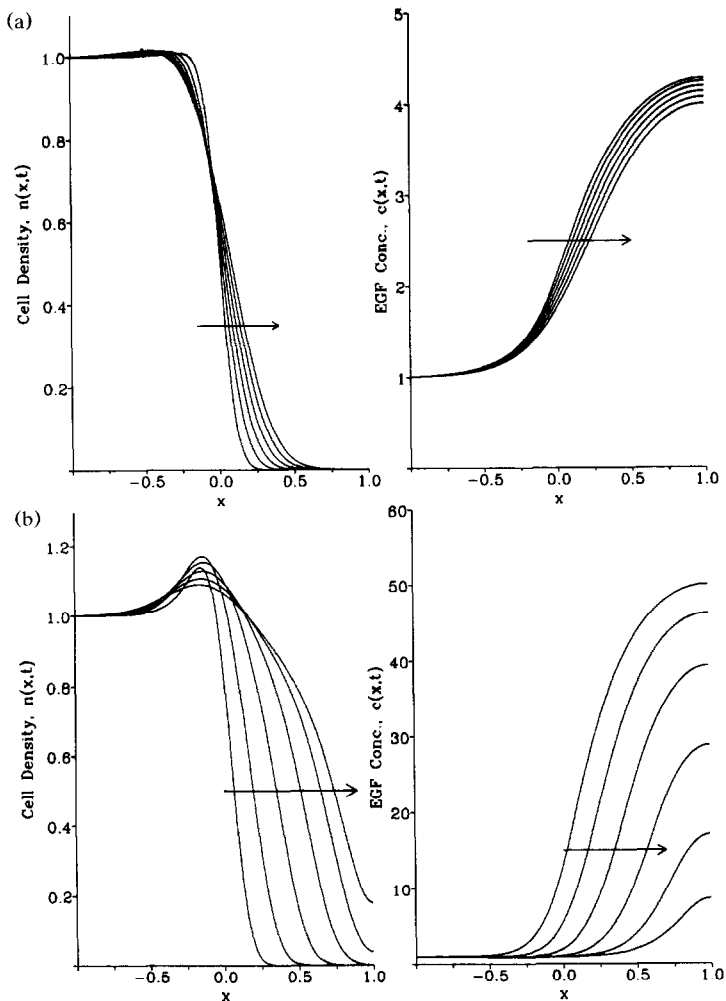


FIG. 1. Numerical solutions of the model equations showing cell density and EGF concentration as functions of space at equal time intervals up to 37 h for a wound length of 4 mm. In (a) we take  $f(n) = A$ , corresponding to no sources of EGF other than the tear film. The solutions fail to show a definite wave front and the levels of cell density and EGF concentration are well below those experimentally predicted after 37 h. In (b) we consider an additional source of chemical and take  $f(n) = A + B(n)$  (see text for details). The solutions then have the form of a front of cells moving into the wound with an associated wave of EGF. The parameter values are  $\alpha = 0.01$ ,  $\beta = 0.1$ ,  $D_c = 25$ ,  $\mu = 1.37 \times 10^4$ ,  $\hat{c} = 3.02$ ,  $\delta = 110$ ,  $\alpha_1 = 0.9$ ,  $\beta_1 = 0.1$ , and  $\sigma = 4000$ , and the initial conditions are  $n = c = 0$  for  $0 < x < 1$  and  $n = c = 1$  for  $x < 0$ . Here, and for the numerical solutions in the other figures, the equations were solved numerically using the method of lines and Gear's Method.

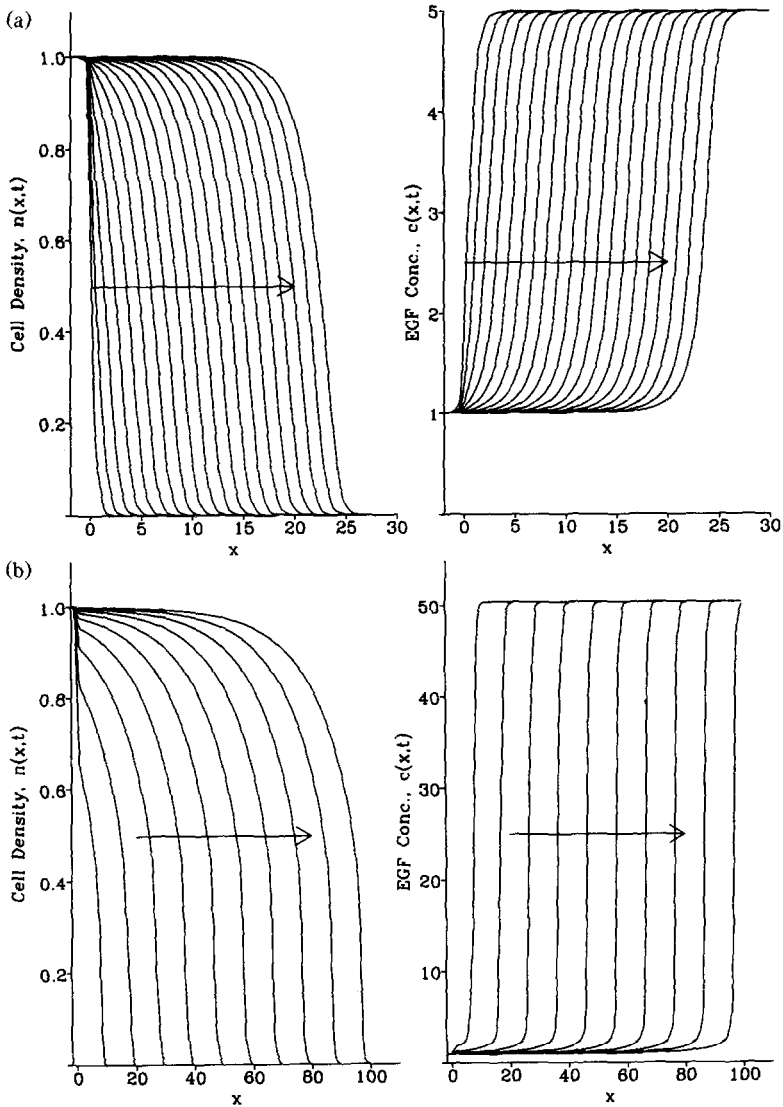


FIG. 2. Numerical solutions of the model equations over an infinite domain, showing cell density and EGF concentration as functions of space at equal time intervals of 47 h. (a) Corresponds to the tear film model whereas (b) shows simulations for the improved model with the same time interval. The solutions for both models evolve to traveling waves of constant speed and shape in  $n$  and  $c$  but the speed for the tear film model is much slower than the healing rate of actual corneal wounds. The parameter values are as in Figure 1, and the initial conditions are  $n = c = 0$  for  $x \geq 0$  and  $n = c = 1$  for  $x < 0$ .



stream from the wound. Since a serum-derived factor would be carried with the flow, this supports the possibility that a serum-derived factor is responsible for the elevated rate of cell division after wounding.

For our amended form of  $f(n)$ , we look for a function which is constant for low cell densities and decreases linearly to zero for larger cell densities, motivated by the above experimental results. We thus take  $f(n) = A + B(n)$ , where

$$B(n) = \begin{cases} \sigma & \text{if } n < 0.2 \\ \sigma(2 - 5n) & \text{if } 0.2 \leq n \leq 0.4 \\ 0 & \text{if } n > 0.4 \end{cases}$$

and  $\sigma$  is a positive constant. Consistent results are obtained provided  $B(n)$  is nonzero only for  $n$  less than about 0.5. We chose the range  $0.2 < n < 0.4$  to be specific, but the results are insensitive to any small change in these threshold values. We use the same parameters as before and fit the model solutions to estimate the source coefficient  $\sigma$ . Numerical simulations show that we need  $\sigma$  to be greater than about 1000 to obtain traveling waves and to match the experimentally observed healing rate we must take  $\sigma = 4000$ . Smaller values of  $\sigma$  give reduced mitotic activity, reduced wave speed, and loss of "traveling wave" form.

The solutions of this amended model have the experimentally expected form of a front of cells moving into the wound, with an associated wave of EGF as in Figure 1b. Moreover, a plot of the mitotic generation term against space shows a wave moving with the cell front and peaking at approximately 14 times the unwounded level, which agrees favorably with the experimentally observed increase in mitotic rate [10] as in Figure 3. The results are also qualitatively very similar to those obtained by Winter [31] for the mitotic rate as a function of distance from the wound edge in the healing of epidermal wounds in domestic pigs. Figure 1b shows that the wound length is reduced significantly by the experimentally predicted time, but that healing is still incomplete. However, by extending our domain size, as discussed previously, long-term simulations evolve to fronts of constant shape, moving with a speed ( $\approx 64 \mu\text{m h}^{-1}$ ) that compares favorably with the experimentally predicted healing rate (Figure 2b). Qualitatively similar solutions are obtained when radially symmetric circular geometry is used rather than linear geometry. The radially symmetric case is of course more relevant to applications, but the advantage of the linear geometry is that the solutions can be studied relatively easily as travel-

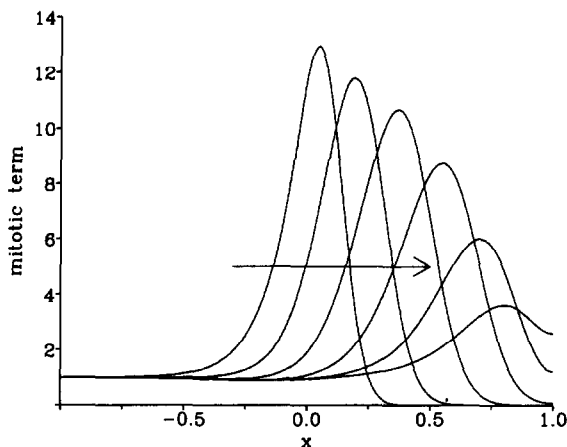


FIG. 3. Numerical solutions of the model equations over a semi-infinite domain, showing the mitotic generation term  $s(c)n(2-n)$  as a function of space at equal time intervals up to 37 h with  $f(n) = A + B(n)$ . The mitotic rate peaks at approximately 14 times the unwounded level, in agreement with experimental data.

ing waves. The discrepancy between model solutions and experiments on the finite domain may be explained by noting that experimentalists only monitor the healing time until the wound radius is reduced by about 75% and then extrapolate to obtain the “total” healing time. However, our model is invalid for the final stages of healing, since the processes involved become more complex, with the re-establishment of cell-cell contacts and other remodeling processes as the opposing wound fronts meet [7]. Therefore, it is the rate of healing, rather than the exact healing time, that we require our model solutions to capture. A detailed study of the final stages of the healing process could be a valuable area for both experimental and theoretical research. In the next section we focus on the improved model and verify analytically the agreement between healing rates in the model and in experiments.

An important biological question is the relative importance of mitosis versus migration in the healing process. The equations in (2a) are strongly coupled by terms accounting for these processes. We investigate this by first reducing  $\alpha$  and  $\beta$  in (2a) by two orders of magnitude, which corresponds to a healing process dominated by mitosis. In this case, the solutions evolve to traveling waves of greater slope as in Figure 4a, moving across the wound at a greatly reduced speed of about  $45 \mu\text{m h}^{-1}$  for the given parameter values. Similarly, when the mitotic

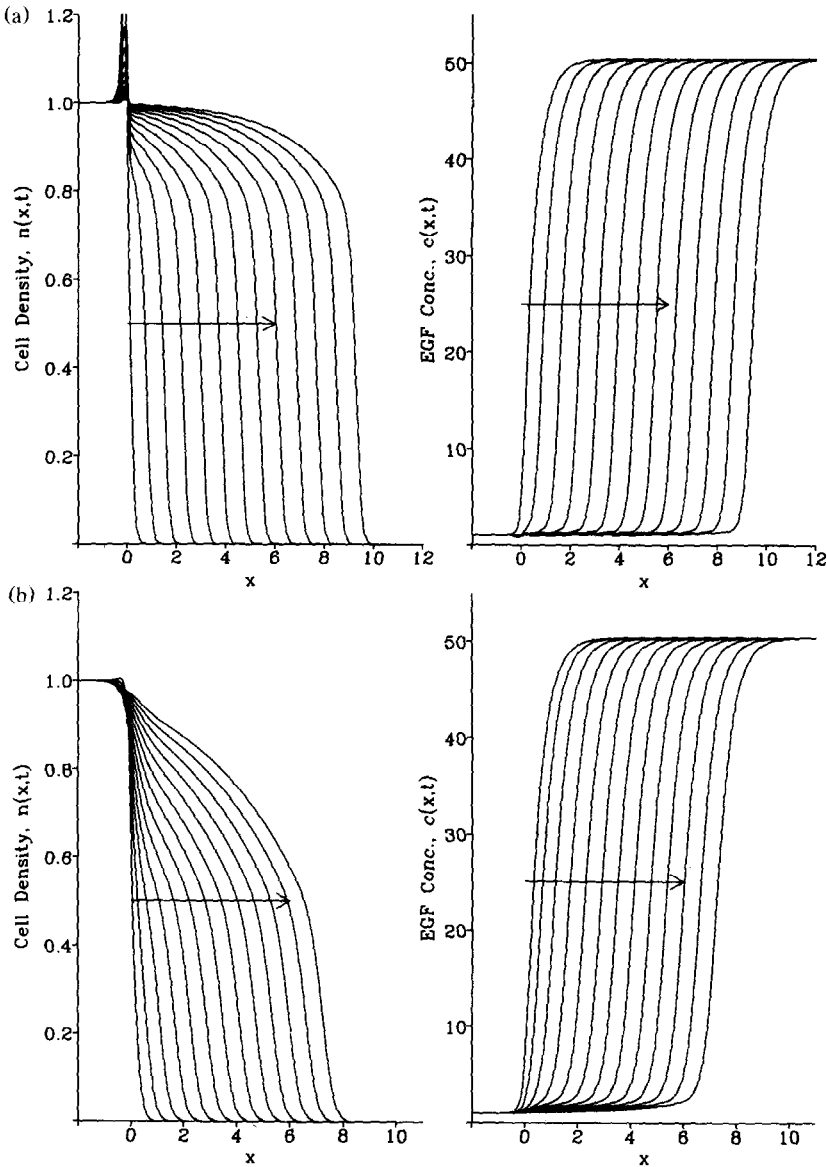


FIG. 4. Numerical solutions of the model equations, over an infinite domain, showing cell density and EGF concentration as functions of space at equal time intervals. In (a) we take  $\alpha = \beta = 0.005$ , which corresponds to healing dominated by mitosis and in (b) we take  $s(c) = 0.01c + 0.99$ , which corresponds to healing dominated by migration. In both cases, the other parameters are as in Figure 1. Wave fronts are still observed but the rate of healing is greatly reduced.

generation term is reduced by one order of magnitude, healing occurs at a slower rate of about  $35 \mu\text{m h}^{-1}$  (Figure 4b). Hence, although our model predicts that wounds can heal in the virtual absence of either mitosis or migration, both processes are important for effective wound healing.

A separate but equally important question concerns the contribution of EGF to migration and mitosis. In the derivation of the functional form  $B(n)$ , we evaluated  $\sigma$  by matching the increase in mitotic rate with experimental data. Hence, EGF has been shown to have an enhancing effect on mitosis. The effect on migration can be investigated by varying the parameters  $\alpha$  and  $\beta$ , with the sum  $\alpha c_0 + \beta$  maintained at a biologically realistic value of about  $10^{-9} \text{ cm}^2 \text{ s}^{-1}$ . Numerical solutions indicate loss of traveling wave fronts if  $\beta$  is very small, resulting in a “fill up” mechanism. However, if  $\alpha$  is very small fronts of the same constant speed and shape are still observed. This suggests that the role of EGF as a regulator of cell mobility is relatively unimportant; however, EGF is crucial to healing as a mitotic regulator. Hence, to simplify the following analysis, we shall take  $\alpha = 0$ , corresponding to constant cellular diffusion.

#### 4. THE SPEED OF HEALING

In the single Fisher reaction-diffusion equation [15], traveling wave solutions can be investigated by introducing the coordinate  $z = x - at$ , where  $a$  is the speed in the positive  $x$  direction. This transforms the parabolic partial differential equation to a second-order system of ordinary differential equations. In contrast to excitable systems such as the Fitzhugh-Naguma (FHN) equations, typical cell population models such as the Fisher equation, whose kinetics have only one stable equilibrium, do not determine a particular speed for which traveling wave fronts exist. Rather there is a range of possible wave speeds, and determination of the speed induced by particular initial conditions is of vital importance in applications. In the case of the Fisher equation, it is well known that the value of the parameter  $a$  at which the eigenvalues at the trivial steady state change from complex (stable spiral) to real (stable node) determines the speed of the traveling waves which result from initial conditions with compact support (see [20] for review). The partial differential equation solutions discussed above appear to evolve rapidly to waves moving with constant speed and shape. Therefore, by analogy, we look for solutions of the form  $n(x, t) = N(z)$  and  $c(x, t) = C(z)$  and investigate the dependence of the wave speed on the model parameters by considering the resulting *fourth*-order system of ordinary

differential equations

$$\frac{dN}{dz} = V \quad (3a)$$

$$\frac{dC}{dz} = W \quad (3b)$$

$$\frac{dV}{dz} = \frac{1}{\beta} (-aV - s(C)N(2-N) + N) \quad (3c)$$

$$\frac{dW}{dz} = \frac{1}{D_c} \left( \frac{\mu NC}{\hat{C} + C} + \delta C - A - B(N) - aW \right). \quad (3d)$$

This system has two steady states, wounded  $(0, (A + B(0))/\delta, 0, 0)$  and unwounded  $(1, 1, 0, 0)$ , the stability of which are determined by the roots (eigenvalues) of the dispersion relation. The eigenvalues at the wounded steady state satisfy the quartic equation

$$\lambda^4 + P\lambda^3 + Q\lambda^2 + R\lambda + S = 0, \quad (4)$$

where

$$P = a \left( \frac{1}{D_c} + \frac{1}{\beta} \right) \quad (5a)$$

$$Q = \frac{1}{\beta D_c} (a^2 - \beta\delta - D_c + 2D_c s(A/\delta)) \quad (5b)$$

$$R = \frac{1}{\beta D_c \delta^2} \left( 2s \left( \frac{A + B(0)}{\delta} \right) - 1 - \delta \right) \quad (5c)$$

$$S = \frac{\delta}{\beta D_c} \left( 1 - 2s \left( \frac{A + B(0)}{\delta} \right) \right). \quad (5d)$$

For general parameter values, it is unfeasible to determine the eigenvalues analytically. However, it is straightforward to calculate the eigenvalues and eigenvectors of the Jacobian matrix numerically. This shows that as the parameter  $a$  is varied, with the other parameters as discussed previously the eigenvalues at the wounded steady state changes character only once; namely a change from complex to real occurs in one pair of eigenvalues at a dimensional wave speed of about  $68.4 \mu\text{m h}^{-1}$ , which is very close to the wave speed observed in numerical solutions of the partial differential equations. This suggests that this change may determine the observed wave speed.

To confirm this we use the partial differential equation solutions to investigate the form of the conjectured heteroclinic connection near the

wounded steady state. We are looking for solutions of the form  $n \sim n_0 e^{\lambda x}$  and hence by estimating  $d(\log n)/dx$  for large  $x$ , we can evaluate numerically the rate at which the wave of the partial differential equation approaches the wounded steady state. This verifies that the trajectory corresponding to the traveling wave solutions does indeed approach the equilibrium point along the eigenvector corresponding to the eigenvalue which bifurcates at  $a = 68.4 \mu\text{m h}^{-1}$  as in Figure 5. At this point, there is a change from oscillatory (complex eigenvalues) to monotone (real) convergence, and the former would result in negative cell densities which is biologically implausible. Although the figures illustrate results for only a single set of parameter values, the method correctly predicts the wave speed for a wide range of parameter domains.

Typical analyses of traveling wave speeds by linearization about the leading edge give rise to eigenvalue equations that factorize into two quadratic equations [20, 24]; the eigenvalues are thus determined easily. In our system, the eigenvalue equation (4) does not factorize in an obvious way. However, for biologically realistic parameter values it is

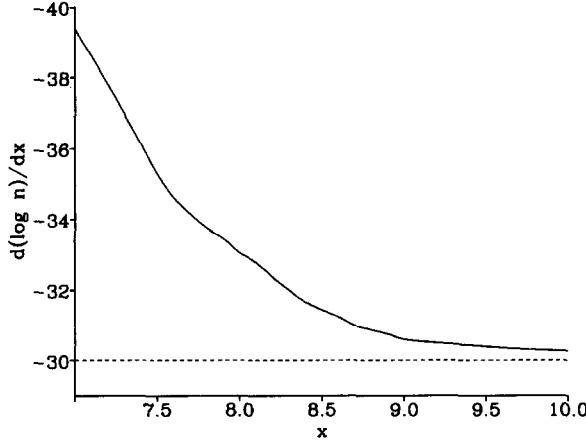


FIG. 5. The change in  $d(\log n)/dx$  with  $x$  for the wave illustrated in Figure 2b. Under the assumption that  $n \sim n_0 e^{\lambda x}$  as  $x \rightarrow \infty$ , this function should tend to  $\lambda$ . Here  $\lambda = -30$ , which is the real part of the eigenvalue that changes character at the critical wave speed  $a = 68.4 \mu\text{m h}^{-1}$ . This verifies our conjecture that the solution trajectory approaches the wounded steady state along the eigenvector whose eigenvalue changes from complex to real at the critical wave speed. The irregular appearance of the curve is a true reflection of the approach and does not indicate any numerical inaccuracy. The parameter values and method of numerical solutions are as in Figure 1.

possible to determine an approximate solution of the quartic equation for the eigenvalues  $\lambda$  and hence derive an analytical expression for the wave speed. Crucially, for the parameter values we have derived from experimental data, the coefficients  $P$  and  $R$  are small compared with  $Q$  and  $S$ . This holds for all biologically realistic values of  $D_c$  and  $D_n(c)$ . Setting the coefficients  $P$  and  $R$  to zero gives a quadratic equation in  $\lambda^2$ , whose roots are independent of the wave speed  $a$ . Substituting these roots back into the quartic and looking for a change of the other two roots (which do not depend on  $a$  to leading order) from complex to real gives the following expression for the critical wave speed

$$a_{\text{crit}} \approx \frac{2}{\beta + D_c} \sqrt{\beta D_c \left[ 2 D_c s \left( \frac{A + \sigma}{\delta} \right) - D_c - \delta \beta \right]}. \quad (6)$$

The expression gives wave speeds of 22.4 and 68.3  $\mu\text{m h}^{-1}$  for the tear film and improved models, respectively, which compare very well with the numerically evaluated wave speeds of 23 and 68.4  $\mu\text{m h}^{-1}$ . An important biological implication of this result is that the rate of healing of corneal epithelial wounds can be increased by increasing either the cell diffusion coefficient or the secretion rate of EGF. However, increasing the chemical diffusion coefficient does not have a significant effect.

A few experiments have been carried out to determine the increase in the speed of healing when EGF is applied topically to the wound. The limited data indicates that the healing rate saturates at about 45% greater than normal, at an EGF concentration of about 100  $\mu\text{g ml}^{-1}$   $\text{h}^{-1}$  [21]. We now modify the analytical expression for the wave speed to predict the details of this increase in healing rate. Lack of experimental information led us to initially choose an unbounded, linearly increasing mitotic generation function,  $s(c)$ , which contradicts these experimental results. Our expression (6) for the speed of healing enables us to use experimental data to derive a more realistic form for  $s(c)$ . We require  $s(c)$  to be approximately linear for small  $c$ , to saturate for large  $c$ , and to satisfy the condition  $s(1) = 1$ . A simple example of such a function is  $s(c) = (1 + m)c / (m + c)$ , where  $m$  is a constant. In vivo experiments in which EGF was applied externally to a corneal surface wound indicate a dimensionless saturating level of 135 and hence we take  $m = 134$ , giving

$$s(c) = \frac{135c}{134 + c}. \quad (7)$$

To model the topical application experiments, we amend our model by taking  $f(n) = A + B(n) + q$ , where  $q$  is an external source term repre-

senting the topical application of EGF. Numerical simulations again evolve to traveling waves, the speed of which increases as we increase the external source term. The analytical expression for the wave speed is modified accordingly, with  $A + \sigma$  replaced by  $A + \sigma + q$ . Figure 6 shows the very good agreement between the numerically simulated and analytically derived speeds. Hence, we can predict the speed of healing of corneal surface wounds with topical application of any concentration of EGF. This result could easily be tested by further experiments.

## 5. THE WAVE FORM

The production and decay of EGF occur on a considerably faster time scale than chemical diffusion. This suggests that we may be able to derive an analytical approximation for the functional form of the wave fronts by assuming that the derivative terms in the EGF equation are negligible compared to the reaction terms. This leads to a reduced model consisting of a single partial differential equation

$$\frac{\partial n}{\partial t} = \beta \frac{\partial^2 n}{\partial x^2} + g(n), \quad (8)$$

where

$$g(n) = \frac{(1-b)}{2\delta} \left[ (A - \mu n - \delta \hat{c}) + \sqrt{(\mu n + \delta \hat{c} - A)^2 + 4A\delta \hat{c}} \right] \times n(2-n) - n. \quad (9)$$

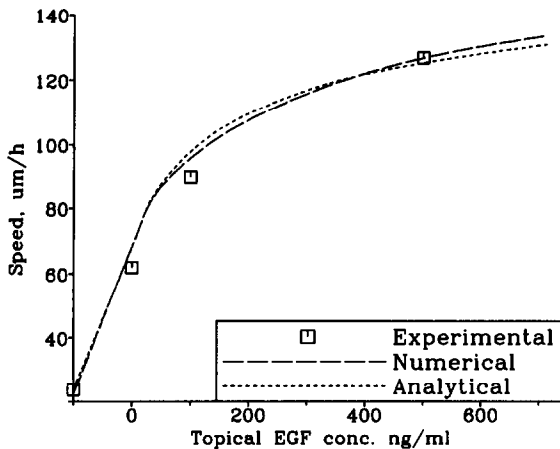


FIG. 6. The change in the rate of healing of 2-mm corneal surface wounds with topical application of EGF. The rate saturates as we increase the concentration of EGF, in agreement with experimental results. Numerically simulated and analytically derived wave speeds compare favorably. The parameter values are as in Figure 1.



By analogy with the analysis for the Fisher equation (reviewed in [20]), we rescale the independent variable  $\zeta = z/a$  giving a boundary value problem for the traveling wave

$$\epsilon N'' + N' + g(N) = 0 \quad (10)$$

subject to  $N(-\infty) = 1$  and  $N(\infty) = 0$ . Here  $g(N)$  is defined by (9),  $\epsilon = \beta/a^2$  and prime denotes  $d/d\zeta$ . For our model parameters,  $\epsilon = 3.83 \times 10^{-3}$  and we can thus solve the equations analytically as a regular perturbation problem in the small parameter  $\epsilon$ , by looking for a solution of the form

$$N(\zeta; \epsilon) = N_0(\zeta) + \epsilon N_1(\zeta) + \epsilon^2 N_2(\zeta) + \dots$$

Substituting this into (10) and equating coefficients of powers of  $\epsilon$  gives

$$N'_0 = -g(N_0) \quad (11)$$

subject to  $N_0(-\infty) = 1$ ,  $N_0(\infty) = 0$ , and  $N_i(-\infty) = N_i(0) = N_i(\infty) = 0$  for all  $i \geq 1$ ; to determine the origin of  $\zeta$  we must also specify  $N_0(0)$ , as  $\frac{1}{2}$  say. Equation (11) can then be integrated numerically, giving a first-order approximation for the wave of cell density that compares well with the numerically simulated solution.

We can use this first approximation to the solution to investigate the dependence of the slope of the wave front on the model parameter values. Since the gradient is everywhere negative, a measure of the steepness  $\hat{s}$  is the magnitude of the maximum of gradient  $U'(z)$  at the point of inflexion of the wave front. Using (11), the gradient at  $z = 0$  gives  $\hat{s} = -g(k)/a$ , which implies that the faster the wave moves, the less steep the wave front. Using (9), we find that the steepness depends crucially on the source term. In Figure 7 we plot the change in slope with increasing concentration of EGF; there is a good correspondence between the steepness of the numerically simulated wave front and the analytically determined slope.

## 6. DISCUSSION

Previous models have been proposed for general epidermal wound healing [4, 25–29]. Here we have presented a model for the specific case of corneal epithelial repair, focusing on the effect of EGF on mitotic and migratory activity. Numerical simulations indicate that there is insufficient EGF in the tear film to heal the wound in the experimentally observed time and hence we amend our model to include an additional source of chemical at the center of the wound. This source

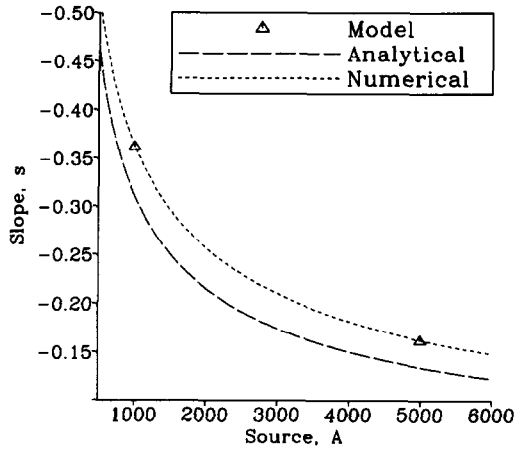


FIG. 7. The change in steepness of slope as we increase the source term. The wave front becomes less steep with increasing EGF concentration (corresponding to increasing wave speed). The discrepancy between the analytically determined wave slope and the steepness of the numerically simulated waves is due to our approximation that the derivative terms in the EGF equation are negligible compared to the reaction terms.

term decays as the cell density increases in the wound and hence further amendments are necessary to model the final stages of healing. Based on experience with scalar reaction diffusion equations [15], we derive an analytical approximation to the healing rate by looking for a change in the type of eigenvalue at the leading steady state in the ordinary differential equations governing traveling wave solutions. The predicted wave speeds compare favorably with experimental data. An important biological implication of this result is that the rate of healing of corneal epithelial wounds can be increased by increasing the cell diffusion coefficient or the secretion rate of the EGF but the chemical diffusion coefficient does not have a significant effect. The results of our simple model suggest that serum-derived factors can alone account for the overall features of the healing process and that both migration and mitosis are necessary for effective healing. Furthermore, modification of the analytical expression for the wave speed, to include a saturating mitotic generation term, has enabled us to make clinically testable predictions of the healing rate when EGF is applied topically to the wound.

In this paper, we have concentrated on the chemical EGF, which has been established as an important regulator of epithelial repair. We now briefly discuss a number of the other growth factors which play overlapping and synergetic roles during the general wound healing process.

Type  $\alpha$  transforming growth factor (TGF- $\alpha$ ) is a very similar protein to EGF, which binds and activates the EGF receptor, but, although topical application of TGF- $\alpha$  has been shown to increase healing rates in the burn wounds [23], its presence in the corneal epithelium is biologically unproven. Fibroblast growth factor (FGF) has both basic and acidic forms and topical application of a cocktail of FGFs has been shown to increase mitotic rates and hence speed up healing of corneal epithelial lesions [13]. However, there is again no evidence for its presence in the tear film or its secretion by epithelial cells in response to injury. The effects of platelet derived growth factor and TGF- $\beta$  are restricted to the healing of full depth wounds (reviewed in [19]).

In the presence of serum-derived growth factors, rapid cell division occurs at the margin of a wound in an epithelial cell sheet. Numerous attempts have been made to explain this band of increased proliferation. Coffey et al. [8] suggested an auto-induction process, for a general epithelial wound, showing that wounding by abrasion increases the detectable quantities of TGF- $\alpha$  by freeing bound TGF- $\alpha$  from the epidermis of the normal adult breast skin and activating a process which releases bound TGF- $\alpha$  from the cells. These EGF-like peptides bind to EGF receptors and include an enhanced TGF- $\alpha$  synthesis in epithelial cells to increase their rate of proliferation. This autocrine feedback process is the basis of numerous models for epithelial repair [28], and, although there is evidence to support it in some epithelium, the process remains biologically unproven. Ohashi et al. [22] determined the average concentration of EGF released by damaged cells and found it to be negligible compared with the tear film concentration. Hence we have chosen to neglect any autocrine mechanism in the specific case of the cornea. Absence of contact inhibition and change in cell shape also increase the proliferation of the basal cells at the wound edge. Our model does reflect these effects, although it does not focus on them. However, a very simple model of these processes involving a single reaction diffusion equation for the cell density has been studied previously and predicts greatly reduced speeds of healing [28].

In this paper, we have considered a quite different explanation for the elevated mitotic rate at the wound edge, based upon the previously discussed ideas of Barrandon and Green [1] and Dunn and Ireland [12]. Although these experiments were performed on different cell types, we speculate similar results for EGF and assume that damage to the underlying layers of the cornea cause these to release EGF, near the center of the wound. The EGF released by this additional source is then "mopped up" by the leading row of cells at the wound edge because of their very high affinity for EGF, resulting in a preferential increase in the mitotic rate and an inward migration to heal the wound.

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