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CORNEAL EPITHELIAL WOUND HEALING

PAUL D. DALE[1], JONATHAN A. SHERRATT[2] and PHILIP K. MAINI[1]

[1] *Centre for Mathematical Biology, Mathematical Institute, 24-29, St Giles', Oxford, OX1 3LB, U.K.*

[2] *Nonlinear Systems Laboratory, Mathematics Institute, University of Warwick, Coventry, CV4 7AL, U.K.*

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. We determine an analytic approximation for the speed of travelling 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.

Keywords: Travelling waves, epidermal growth factor, reaction diffusion, mitosis, migration.

1. Introduction

Cell migration and proliferation are central to the healing of wounds in the corneal epithelium [1]. Biological evidence suggests that both processes are regulated by epidermal growth factor (EGF), which is secreted by the cells themselves. 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 [8], 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. Mathematical Modelling

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

E-mail: pddale@vax.ox.ac.uk

are of the form:

$$\frac{\partial n}{\partial t} = \overbrace{\nabla \cdot (D_n(c)\nabla n)}^{\text{Cell Migration}} + \overbrace{s(c)n\left(\nu - \frac{n}{n_0}\right)}^{\text{Mitotic Generation}} - \overbrace{kn}^{\text{Natural Loss}}, \tag{1a}$$

$$\frac{\partial c}{\partial t} = \underbrace{D_c \nabla^2 c}_{\text{Diffusion}} + \underbrace{f(n)}_{\text{Production by Cells}} - \underbrace{\frac{\mu n c}{(\hat{c}+c)} - \delta c}_{\text{Decay of Active EGF}}, \tag{1b}$$

where $n(\underline{r}, t)$ and $c(\underline{r}, t)$ denote cell density and EGF concentration, respectively, at position $\underline{r}$ and time t, $D_n(c) = \alpha c + \beta$, and D_c, μ, δ, α, β, $\hat{c}$, ν, n_0 and k are all positive constants.

We model cell movement by Fickian diffusion and 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 [6,9] and represent the chemical control of mitosis by an increasing function $s(c)$. 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 incorporating 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.

We consider the problem in one space dimension and investigate the behaviour of travelling wave solutions to these equations which move from a region where the cell density and EGF concentration are at their unwounded levels, $n = c = 1$ (as $x \to -\infty$), into a region of no cell density, $n = 0$, with the EGF concentration at its wounded level, $c = f(0)/\delta$ (as $x \to +\infty$).

Estimating the parameter values is vital to the comparison of model predictions with experimental data. The large quantity of experimental data readily available enables us to establish these parameter values [2]. We thus non-dimensionalise the model to obtain

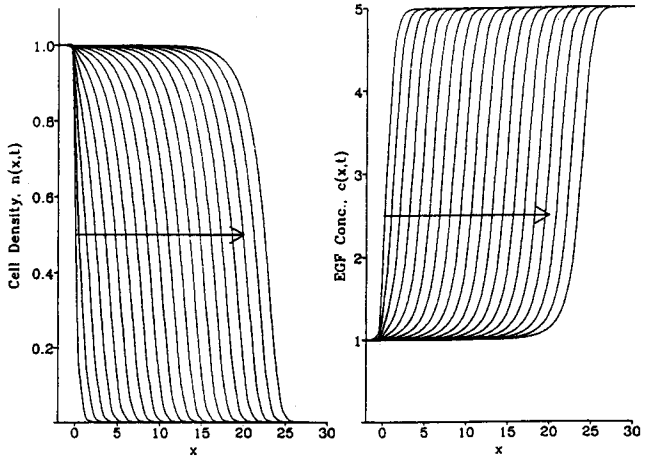
$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x}\left((\alpha c + \beta)\frac{\partial n}{\partial x}\right) + (\alpha_1 c + \beta_1)n(2-n) - n, \tag{2a}$$

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

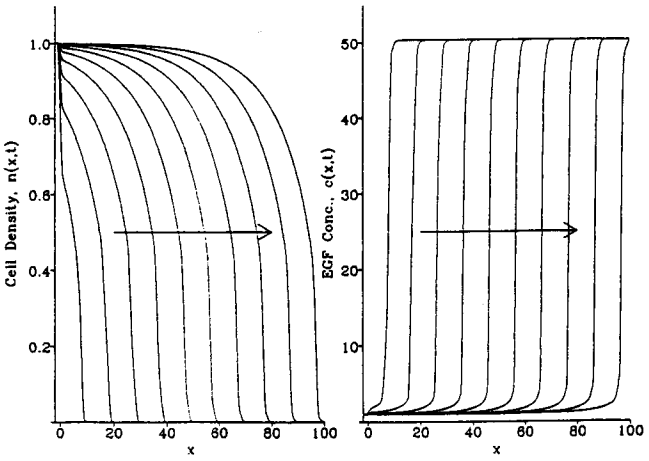
Throughout the paper we use the biologically realistic parameter set given in Fig. 1.

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 travelling wave form. However, numerical solutions of the model equations with $f(n) = A$, corresponding to



(a)



(b)

Fig. 1. Numerical solutions of the model equations showing cell density and EGF concentration as functions of space at equal time intervals of 47 hours. Figure (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 travelling 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 $\alpha = 0.01$, $\beta = 0.1$, $D_c = 25$, $\mu = 1.37 \times 10^4$, $\hat{c} = 3.02$, $\delta = 110$, $\alpha_1 = 0.9$, $\beta_1 = 0.1$ and $\sigma = 4000$, and the initial conditions are $n = c = 0$ for $x \geq 0$ and $n = c = 1$ for $x < 0$. The equations were solved numerically using the method of lines and Gear's Method.

no sources of EGF other than the tear film, fail to show definite wave fronts. We suspect that the absence of wave fronts was due to a long transient time for the evolution of a wave profile. 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 $x \to \infty$. These new end conditions are not directly relevant to the biological problem; rather, they enable us to obtain a clearer understanding of the mathematical aspects of our model. Numerical solutions show that with these amended end conditions, the system evolves to travelling waves of constant speed and shape in n and c (Fig. 1(a)), but the speed of these travelling waves ($\approx 20.45\ \mu\text{mh}^{-1}$) is much slower than the healing rate of actual corneal wounds ($\approx 60\ \mu\text{mh}^{-1}$). 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. 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. This is a simple way to model stimulation of EGF production at the wound margin. 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 A and σ are positive constants [3]. Consistent results are obtained provided $B(n)$ is non-zero 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. Numerical solutions of the model (Fig. 1(b)) suggest that the system evolves to travelling waves of constant speed and shape in n and c. The speed of these travelling waves ($\approx 64\ \mu\text{mh}^{-1}$) compares very favourably with the healing rate of actual corneal wounds ($\approx 60\ \mu\text{mh}^{-1}$) for sufficiently large values of σ [2] and is robust to any small change in parameters. 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 favourably with the experimentally observed increase in mitotic rate [4]. Qualitatively similar solutions are obtained when a 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 travelling waves.

An important biological question is the relative importance of mitosis versus migration in the healing process. First we reduced α and β in (2a) by two orders of magnitude, which corresponds to a healing process dominated by mitosis, and observed healing at a greatly reduced speed. Similarly, when the mitotic generation term is reduced by one order of magnitude, healing occurs at a slower rate. 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.

4. The Speed of Healing

In the single Fisher reaction-diffusion equation [5], travelling 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. 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 travelling waves which result from initial conditions with compact support (see [6] for review). The solutions to the partial differential equations 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 eigenvalues of the fourth order system of travelling wave ordinary differential equations at the wounded and unwounded steady states. 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 fixed as discussed previously, the eigenvalues at the wounded steady state change character only once. Specifically, one pair of eigenvalues change from complex to real at a dimensional wave speed of about 68.4 μmh^{-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 $\frac{d(\log n)}{dx}$ for large x, we can evaluate numerically the rate at which the wave of the partial differential equation system approaches the wounded steady state. This verifies that the trajectory corresponding to the travelling wave solution does indeed approach the equilibrium point along the eigenvector corresponding to the eigenvalue which bifurcates at $a = 68.4\ \mu\text{mh}^{-1}$ (Fig. 2). 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 travelling wave speeds by linearisation about the leading edge give rise to eigenvalue equations that factorise into two quadratic equations [6]; the eigenvalues are thus determined easily. In our system, the eigenvalue equation does not factorise in an obvious way. However, for biologically realistic parameter values, it is possible to determine an approximate solution of the quartic equation for the eigenvalues, λ, and hence derive an analytical expression for the wave speed. Crucially, for the parameter values we have derived from experimental data, the coefficients of λ^3 and λ are small compared with the coefficients of λ^4 and λ^2,

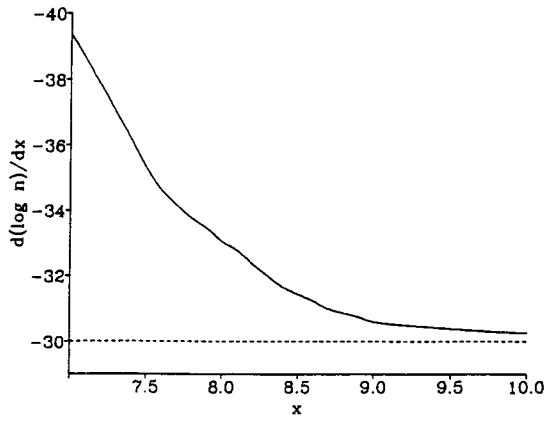


Fig. 2. The change in $\frac{d(\log n)}{dx}$ with x for the waves illustrated in Fig. 1(b). Under the assumption that $n \sim n_0 e^{\lambda x}$ as $x \to \infty$, this function should tend to λ. Here $\lambda = -30$, which is the real part of the eigenvalue that changes character at the critical wave speed, $a = 68.4\ \mu\text{mh}^{-1}$. 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 are as in Fig. 1.

and setting these coefficients to zero gives a quadratic equation in λ^2, whose roots are independent of the wave speed. Substituting these roots into the quartic and looking for a change of the non-constant roots from complex to real, we determine a critical wave speed

$$a_{\text{crit}} \approx \frac{2}{\beta + D_c}\sqrt{\beta D_c\left[2D_c s\left(\frac{A+\sigma}{\delta}\right) - D_c - \delta\beta\right]}. \tag{3}$$

This expression gives wave speeds of 22.4 μmh^{-1} and 68.3 μmh^{-1} for the tear film and improved models, respectively, which compare very well with the numerically evaluated wave speeds of 23 μmh^{-1} and 68.4 μmh^{-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 $\mu\text{gml}^{-1}\text{h}^{-1}$ [7]. 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 (3) 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 $s(c) = \frac{(1+m)c}{m+c}$, 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

$$s(c) = \frac{135c}{134 + c}. \tag{4}$$

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 representing the topical application of EGF. Numerical simulations again evolve to travelling 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 3 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.

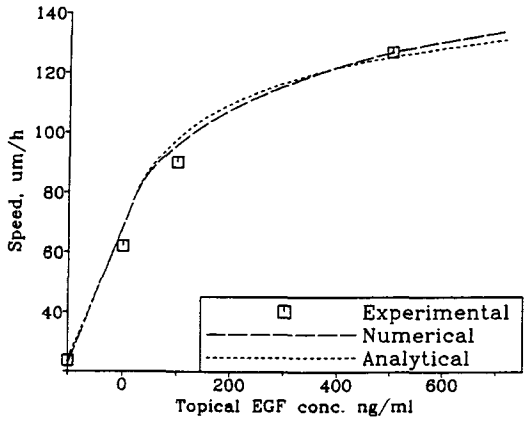


Fig. 3. The change in the rate of healing of 2 mm corneal surface wounds with topical application of EGF. The rate saturates as we increase the concentration of EGF, in agreement with experimental results. Numerically simulated and analytically derived wave speeds compare favourably.

5. Discussion

Previous models have been proposed for general epidermal wound healing [9–13]. Here we have presented a model for the specific case of corneal epithelial repair, focussing 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 centre of the wound. This source 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 [5], 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 travelling wave solutions. The predicted wave speeds compare favourably 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 alone can 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.

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References

[1] Clark R. A. F., Wound repair, *Curr. Op. Cell Biol.* **1** (1989) pp. 1000–1008.

[2] Dale P. D., Maini, P. K. and Sherratt J. A., Mathematical modelling of corneal epithelial wound healing, *Math. Biosci.* **124** (1994) pp. 127–141.

[3] Dale P. D., Sherratt J. A. and Maini P. K., The speed of corneal epithelial wound healing, *Appl. Math. Lett.* **7** (1994) pp. 11–14.

[4] Danjo S., Friend J. and Thoft R. A., Conjunctival epithelium in healing of corneal epithelial wounds, *Invest. Ophthalmol. Vis. Sci.* **27** (1986) pp. 464–473.

[5] Kolmogoroff A., Petrovsky I. and Piscounoff N., Etude de l'equation de la diffusion avec croissance de la quantité de matière et son application à un problème biologique, *Moscow Univ. Bull. Math* **1** (1937) pp. 1–25.

[6] Murray J. D., *Mathematical Biology* (Springer-Verlag, Berlin, 1989).

[7] Nishida T., Nakamura M., Mishima H. and Otori T., Differential modes of action of fibronectin and epidermal growth factor on rabbit corneal epithelial migration, *J. Cell. Physiol.* **145** (1990) pp. 549–554.

[8] Ohashi Y., Morokura M., Kinoshita Y., Mano T., Watanabe H., Kinoshita S., Manabe R., Oshiden K. and Yanaihara C., Presence of epidermal growth factor in human tears, *Invest. Ophthalmol. Vis. Sci.* **30** (1989) pp. 1879–1882.

[9] Sherratt J. A. and Murray J. D., Models of epidermal wound healing, *Proc. R. Soc. Lond.* **B241** (1990) pp. 29–36.

[10] Sherratt J. A. and Murray J. D., Mathematical analysis of a basic model for epidermal wound healing, *J. Math. Biol.* **29** (1991) pp. 389–404.

[11] Sherratt J. A. and Murray J. D., Epidemal wound healing: The clinical implications of a simple mathematical model, *Cell Transplantation* **1** (1992) pp. 365–371.

[12] Sherratt J. A., Sage E. H. and Murray J. D., Chemical control of eukaryotic cell movement: a new model, *J. Theor. Biol.* **162** (1993) pp. 23–40.
[13] Sherratt J. A., Cellular growth control and travelling waves of cancer, *SIAM J. Appl. Math.* **53** (1993) pp. 1713–1730.