Epidermal Wound Healing: A Theoretical Approach

The mechanisms responsible for cell migration across the surface of an epidermal wound are the subject of much biological debate, and modelling the processes mathematically presents many challenges for theoreticians. We focus on biochemical auto-regulation of mitosis, and develop a cell-reaction-diffusion model. The solutions of the model compare well with experimental data, suggesting that such biochemical regulation is a fundamental aspect of the healing process. Analytical investigation of the solutions as travelling waves clarifies the roles of the various model parameters in the wave form and the speed of healing, and provides further corroborative evidence for the model mechanism proposed. We then use the model to perform 'mathematical experiments' on the variation of healing time with wound shape.

Key Words: *wound healing, mathematical models, epidermal migration*

BIOLOGICAL BACKGROUND

When mammalian skin is injured, two processes of wound closure occur simultaneously. The dermal wound tissue contracts, pulling the wound edges together, and epidermal cells spread across the top of this tissue. The epidermis is a relatively simple structure, consisting almost entirely of a single cell type arranged in layers,[1,2] and epidermal wounds enable the process of 'epidermal migration' to be studied independently of the much more complicated healing processes in the dermis. Epidermal cells in un-

wounded skin are non-motile, but in the neighbourhood of the wound they undergo a marked phenotype alteration ('mobilization') that gives them the ability to move via lamellipodia.[3] The main factor controlling cell movement seems to be contact inhibition,[4,5] although chemotaxis and contact guidance may also be involved.[6] Remnants of glands and hair follicles can act as sources of migrating cells, in addition to the wound edges.[7,8]

Two mechanisms have been proposed for the movement of the cell sheet. In the 'rolling mechanism', the leading cells are successively implanted as new basal cells, and other cells roll over these.[9-11] Conversely, in the 'sliding mechanism', cells in the interior of the sheet respond passively to the pull of the marginal cells, although all of the migrating cells do have the potential to be motile, since if a gap opens up in the migrating sheet, cells at the boundary of the gap develop lamellipodia and move inwards to close it.[12] Though the morphological data of mammalian epidermal wound healing are convincingly explained by the rolling mechanism,[13] unequivocal evidence is lacking, whereas the sliding mechanism is well documented in simpler systems such as amphibian epidermal wound closure.[14]

Soon after the onset of epidermal migration, mitotic activity increases near the wound edge, providing an additional population of cells.[5,15-18] The greatest proliferation rate is actually at the wound edge, where it can be as much as 15 times the rate in normal epidermis,[7,17] and cell division decreases rapidly going away from the wound. The stimulus for this increase in mitotic activity is uncertain. Two factors that are certainly involved are the absence of contact inhibition, which applies to mitosis as well as to cell motion,[6] and change in cell shape: as the cells spread out they become flatter, which tends to increase their rate of division.[19,20] There is also experimental evidence for biochemical auto-regulation of epidermal mitosis, suggesting that in response to wounding, epidermal cells produce either more of a mitosis-activating chemical or less of an inhibitory chemical.

Inhibitors of cell proliferation that are produced by the cell types on which they are known as 'chalones'. Although the term itself is somewhat out of vogue,[21] the evidence for such inhibitory growth regulators is now considerable in a wide range of

cell types.[21,22] In particular, epidermal chalones are well documented in a number of mammalian species.[23-30] There are few direct experimental studies of the role of chalones in wound healing, although Yamaguchi et al.[31] used epidermal wounds to investigate chalone inhibition of mitosis in mice.

Evidence for auto-activation of epidermal mitosis comes from two types of experiment. First, epidermal cell extracts and exudates have been found to increase proliferation and healing rates in epidermal wounds, without identification of a particular active ingredient.[32-34] Also, specific reagents which are known to be produced by epidermal cells have been found to increase the mitotic rate of these cells, including type alpha transforming growth factor[35] and fibroblast growth factor.[36,37]

THEORETICAL APPROACHES

While the literature on epidermal wound healing raises many interesting and important questions, two stand out: whether the cell sheet moves via the 'rolling' or 'sliding' mechanism, and how important a role mitotic auto-regulation plays in the healing process. In what follows, we focus on the second question, and construct a mathematical model which suggests that this auto-regulation could be a crucial aspect of epidermal wound healing. Our model is not intended to incorporate all the factors contributing to the healing process: such a model would necessarily involve very large numbers of equations and unknown parameters, to an extent that would render it of little use. On the contrary, we focus on a single factor involved in epidermal migration, and use a relatively simple model to investigate it.

The general form of the governing equations we use is

Rate of increase = Cell   + Mitotic   – Natural
of cell density   migration   generation loss

Rate of increase of   = Diffusion + Production – Decay of
chemical concen-   by cells   active
tration   chemical

We treat the epidermis as two-dimensional since its thickness is about 10^{-2} cm,[38] while the wounds we consider have linear dimensions of about 1 cm. We consider two cases, one in which the chemical activates mitosis, and the other in which it inhibits it.

As explained above, we do not address here detailed questions concerning the motility of the cell sheet, and instead use the somewhat generic model of linear diffusion for the cell migration term.[39] We take chemical decay and natural cell loss as first order processes, with rate constants λ and k respectively. The rate of cell loss is due to the sloughing of the outermost layer of epidermal cells, and is a key aspect of the dynamics of unwounded epidermis: the cells that are lost are replaced by frequent mitosis in the basal cell layer.[1,2] To reflect this dynamic regulation, we assume that when the chemical concentration $c(\underline{r}, t)$ at position $\underline{r}$ and time t is at its unwounded level c_0, the net reaction term in the cell conservation equation is of logistic growth form, $kn(1 - n/n_0)$; here $n(\underline{r}, t)$ is the cell density and n_0 is its unwounded level. The logistic form is a commonly used metaphor for simple growth in population biology models. Since we are taking the rate of cell loss as $-kn$, this condition suggests that during wound healing, the rate of cell division is given by $s(c)n(2 - n/n_0)$, where $s(c)$ reflects the chemical control of mitosis; we then have $s(c_0)n(2 - n/n_0) - kn = kn(1 - n/n_0)$ provided $s(c_0) = k$. The qualitative form required for $s(c)$ is as shown in Figure 1. In the case of a chemical activator, a decrease of $s(c)$ to $s(0)$ for large c is included because it is found experimentally *in vitro*;[32] however, one prediction of the model is that this phenomenon has little effect *in vivo*. In both cases we require $0 < s(\infty) < s_{max} = hk$, say, where h is a constant, and we take $s(\infty) = k/2$. We take simple functional forms satisfying these criteria, namely

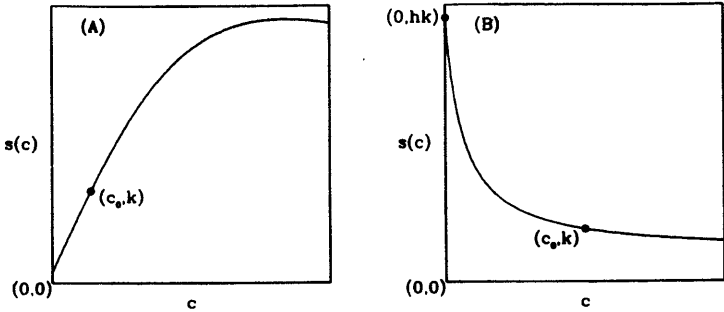$$s(c) = k \cdot \frac{(h - 1)c + hc_0}{2(h - 1)c + c_0}$$

for the inhibitor, and

FIGURE 1 The qualitative form of the function $s(c)$, which reflects the chemical control of mitosis. (A) Biochemical activation of mitosis. (B) Biochemical inhibition of mitosis. The constant c_0 represents the chemical concentration in the unwounded steady state, and k is a parameter equal to the reciprocal of the cell cycle time.

$$s(c) = k \cdot \left\{ \frac{2c_m(h - \beta)c}{c_m^2 + c^2} + \beta \right\} \quad \text{where} \quad \beta = \frac{c_m^2 - 2hc_0c_m + c_0^2}{(c_m - c_0)^2}$$

for the activator, where c_m ($> c_0$) is a constant parameter which relates to the maximum level of chemical activation of mitosis. We take $c_m = 4hc_0$: the condition $c_m/c_0 > (2h - 1) + \sqrt{[(2h - 1)^2 - 1]}$ is implied by the biological requirement that the wounded steady state is unstable to small perturbations while the unwounded state is stable. The model solutions are not at all sensitive to variations in the value of c_m.

The mitosis regulating chemical is produced by the epidermal cells themselves, and we assume that the rate of production occurs as a direct response to changes in cell density. When $n = 0$, there are no cells and thus no chemical can be produced, and when $n = n_0$ the rate of production must be λc_0, so that the unwounded state is a steady state. Further, the chemical production function, $f(n)$ say, must reflect an appropriate cellular response to injury depending on whether the chemical activates or inhibits mitosis. The qualitative form of $f(n)$ in the two cases is as shown in Figure 2.
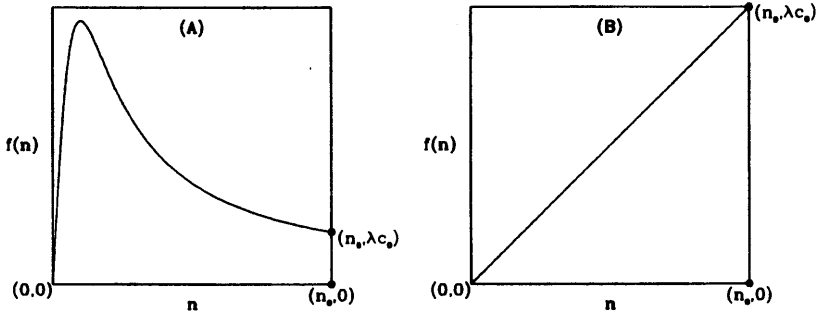
FIGURE 2   The qualitative form of the function $f(n)$, which reflects the rate of chemical production by epidermal cells. (A) Biochemical activation of mitosis. (B) Biochemical inhibition of mitosis. The constants n_0 and c_0 represent the unwounded cell density and chemical concentration, respectively; λ is the positive rate constant for the decay of active chemical.

Again we take simple functional forms that conform to these requirements, namely

$$f(n) = \lambda c_0 \cdot \frac{n}{n_0} \cdot \left(\frac{n_0^2 + \alpha^2}{n^2 + \alpha^2} \right) \quad \text{for the activator}$$

and

$$f(n) = \frac{\lambda c_0}{n_0} \cdot n \quad \text{for the inhibitor,}$$

where α is a positive constant which relates to the maximum rate of chemical production; we take $\alpha = n_0/10$ to give an appropriate qualitative form.

Thus the model is represented by two coupled nonlinear reaction-diffusion equations

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$$\frac{\partial n}{\partial t} = D\nabla^2 n + s(c) \cdot n \cdot \left(2 - \frac{n}{n_0}\right) - kn \qquad (1.a)$$

$$\frac{\partial c}{\partial t} = D_c\nabla^2 c + f(n) - \lambda c \qquad (1.b)$$

where D and D_c are the diffusion coefficients of cell density and chemical concentration respectively. Biologically relevant end conditions are $n = c = 0$ at $t = 0$ inside the wound domain, and $n = n_0$ and $c = c_0$ on the wound boundary at all times t.

In this presentation of the model, we have not addressed the issue of whether a model simply of the form of (1.a), with $s(c) \equiv k$, can capture the essential aspects of epidermal migration, without biochemical regulation of cell mitosis. However, in a previous publication[40] we considered such a model, as well as including a density-dependent diffusion term in the equation, and found that these even simpler models are unable to capture crucial aspects of the healing process.

To assess the relative importance of the various parameters, we nondimensionalize the equations. We use a length scale L, a typical linear dimension of the wound, and a time scale $1/k$: the cell cycle time seems the most relevant time scale. We define the following dimensionless quantities:

$$n* = n/n_0 \quad c* = c/c_0 \quad \underline{r}* = \underline{r}/L \quad t* = kt$$

$$f*(n*) = f(n)/\lambda c_0 \quad s*(c*) = s(c)/k \quad D* = D/(kL^2)$$

$$D_c^* = D_c/(kL^2) \quad \lambda* = \lambda/k \quad c_m^* = c_m/c_0 \quad \alpha* = \alpha/n_0.$$

For the circular wounds considered below, we take L to be the initial wound radius. With these definitions, the dimensionless model equations are, dropping the asterisks for notational simplicity,

$$\frac{\partial n}{\partial t} = D\nabla^2 n + s(c) \cdot n \cdot (2 - n) - n \qquad (2.a)$$

$$\frac{\partial c}{\partial t} = D_c\nabla^2 c + \lambda f(n) - \lambda c \qquad (2.b)$$

with initial conditions $n = c = 0$ at $t = 0$ inside the wound domain, and boundary conditions $n = c = 1$ on the wound boundary at all times t. Here, for the activator

$$f(n) = \frac{n(1 + \alpha^2)}{n^2 + \alpha^2}, \quad s(c) = \frac{2c_m(h - \beta)c}{c_m^2 + c^2} + \beta \quad \text{where} \quad \beta = \frac{c_m^2 - 2hc_m + 1}{(c_m - 1)^2}$$

and for the inhibitor

$$f(n) = n, \quad s(c) = \frac{(h - 1)c + h}{2(h - 1)c + 1},$$

where we assume $h > 1$ and $c_m > 1$.

There is a debate in the biological literature as to whether mitosis drives cell migration or vice versa.[41,42] The biologically reasonable results given by our model and discussed below are based on the assumption that, in fact, both processes are dependent on the local cell density.

PARAMETER VALUES AND COMPARISON WITH DATA

The decay of active chalones has been investigated in a number of experimental studies.[22] In particular, Brugal and Pelmont[43] found that after injection with epithelial extract, the proliferation rate in intestinal epithelium decreased for about 12 hours. Also Hennings et al.[44] were able to maintain suppression of epidermal DNA synthesis by repeated injection of epidermal extract at 12 hour intervals. Based on these studies, we take the half-life of chemical decay as 12 hours. The chemical decay term in equation (1.b) cor-

responds to exponential decay with a dimensional half-life of ($\log 2)/\lambda^{dim}$, where λ^{dim} is the dimensional parameter corresponding to λ, and we therefore take $\lambda^{dim} = 0.05$ ($\approx 1/12 \log 2$) h^{-1}.

In the case of auto-activation of mitosis, there is little direct experimental data on the rate of chemical decay. However, comparison of the work of Eisinger et al.[32,33] on chemical activators in wound healing and the clinical studies of chalone effects by Rytömaa and Kiviniemi[45,46] suggests a longer time scale for the chalone activity, by a factor of about 6, so we take $\lambda^{dim} = 0.3$ h^{-1} for the activator.

The parameter h is the maximum factor by which the mitotic rate can increase as a result of chemical interaction. We take $h = 10$, since this factor is suggested both by a study of epidermal wound healing in pigs[7] and by experiments on corneal wound healing.[17] The value of k is simply the reciprocal of the epidermal cell cycle time. This varies from species to species, but is typically about 100 hours,[47] so we take $k = 0.01$ h^{-1}. The values of the diffusion coefficients D and D_c were estimated by comparing the model solutions with experimental data on wound healing, as discussed below, since there is at present no direct experimental data from which they can be determined.

There are a number of quantitative studies of epidermal wound healing, almost all of which involve circular wounds. The speed of healing differs widely between species and wounding location, almost by an order of magnitude. However, when time is expressed as a fraction of total healing time, the agreement between different sets of data is remarkable (Figure 3). The key aspect of this data is the biphasic nature of the healing process: a lag phase followed by a linear phase. Given that we are basing the values of parameters in the reaction terms on experiments spanning a range of species and cell locations, we felt it inappropriate to select one set of data and then choose the diffusion coefficients D and D_c by fitting the model solution to both the form and precise time course of this data. Rather, we fit the model solution to the data as illustrated in Figure 3, with time expressed as a fraction of total healing time. Of course we look for the model to predict wound speeds of the observed order of magnitude, but more exact quantitative prediction of both the form and speed of healing will require data from the
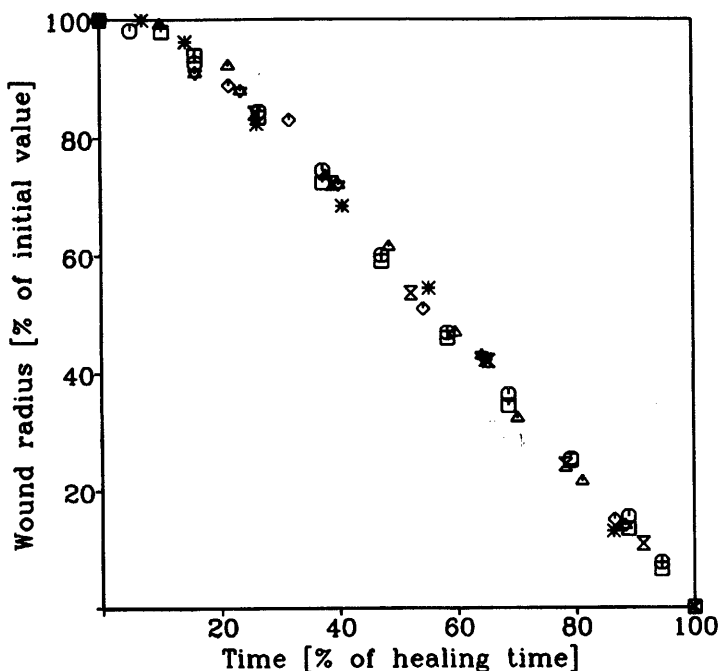
FIGURE 3 Comparison of data from a number of quantitative studies on the healing of circular epidermal wounds. The sources of the data are: □, ○ Van den Brenk[48]; △ Crossen et al.[49]; ◇, ☆ Zieske et al.[50]; ⅄ Lindquist[51]; * Frantz et al.[52]. In Lindquist's[51] experiments there is some dermal contraction, and we have extrapolated to the case of no contraction. These studies involve a range of species and wounding locations, and the speed of healing varies by almost an order of magnitude. However, when time is expressed as a percentage of total healing time, as here, the agreement between the different data sets is remarkable. As discussed in the text, this agreement does depend crucially on all the wounds having approximately the same initial size.

particular species and wound location, from which the parameters in the reaction terms can be estimated.

The results of this approach are illustrated in Figure 4, which shows the numerically calculated decrease in wound radius with time. To capture the concept of 'wound radius' from our model, we take the wound as 'healed' when the cell density reaches 80%
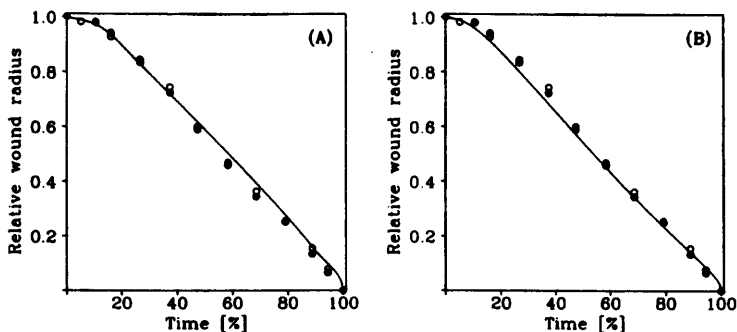
FIGURE 4 The model prediction of the decrease in wound radius with time as compared to data, denoted by ○ and ● [48]. Time is expressed as a percentage of total healing time, as discussed in the text. (A) Biochemical activation of mitosis with dimensionless parameter values $D = 5 \times 10^{-4}$, $D_c = 0.45$, $\lambda = 30$, $h = 10$, $\alpha = 0.1$, $c_m = 40$. (B) Biochemical inhibition of mitosis with dimensionless parameter values $D = 10^{-4}$, $D_c = 0.85$, $\lambda = 5$, $h = 10$.

of its unwounded level, that is when $n = 0.8$ for the dimensionless equations. The choice of this critical level as 80% is somewhat arbitrary, but does not significantly affect the results since the solutions for n and c have travelling wave form, as discussed below. For clarity, in Figure 4 we have compared this predicted variation in wound radius to the data of only one of the authors discussed above. The dimensional diffusion coefficients giving this healing profile are $D = 4 \times 10^{-10}$ cm^{2}s^{-1}, $D_c = 3 \times 10^{-7}$ cm^{2}s^{-1} for the activator, and $D = 7 \times 10^{-11}$ cm^{2}s^{-1}, $D_c = 6 \times 10^{-6}$ cm^{2}s^{-1} for the inhibitor. These are biologically reasonable for cells and biochemicals of relatively low molecular weight, respectively. In Figure 5 we plot the cell density n and chemical concentration c as a function of the radius r at a selection of equally spaced times. As expected intuitively, the form of the solutions is of a front of epidermal cells moving into the wound, with an associated wave of chemical.

We should stress that the agreement between data sets illustrated in Figure 3 does depend crucially on all the wounds having roughly the same initial size. It is clear intuitively that for a large
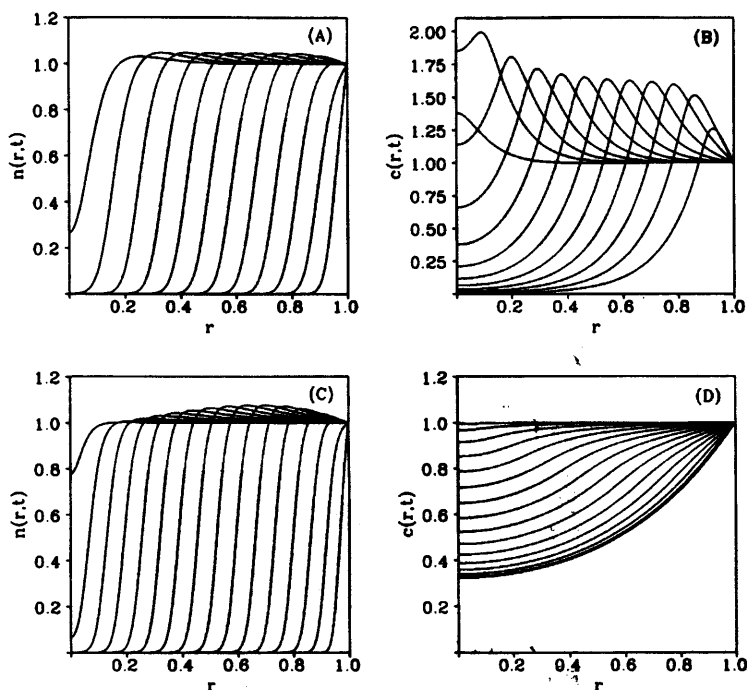
FIGURE 5 Cell density n and chemical concentration c as a function of radius r at a selection of equally spaced times, as predicted by the model. (A,B) Biochemical activation of mitosis with dimensionless parameter values $D = 5 \times 10^{-4}$, $D_c = 0.45$, $\lambda = 30$, $h = 10$, $\alpha = 0.1$, $c_m = 40$. (C,D) Biochemical inhibition of mitosis with dimensionless parameter values $D = 10^{-4}$, $D_c = 0.85$, $\lambda = 5$, $h = 10$.

wound, the lag phase will represent a smaller fraction of the total healing time, and vice versa. This phenomenon is reflected in the model solutions, since as the wound radius R increases, the dimensionless diffusion coefficients decrease as $1/R^2$.

Although numerical solutions enable us to compare the model with experimental data, an understanding of the roles of the various model parameters in these solutions requires analytical investigation. For both types of chemical, the qualitative form of the

solutions illustrated in Figure 5 is, in the linear phase of healing, a wave front moving with constant shape and speed. Such a solution is amenable to analysis if we consider a one-dimensional geometry, rather than the two-dimensional radially symmetric geometry considered above. This is biologically relevant for large wounds of any shape, since to a good approximation these are one-dimensional during much of the healing process. Numerical solutions of the model equations in this new geometry are not significantly different from those illustrated in Figure 5. Thus we look for travelling wave solutions in this one-dimensional geometry. In a previous publication[53] we have presented a detailed study of the resulting ordinary differential equations. Briefly, we showed that the approximation $D = 0$ retained all the essential features of the wave form, and within this approximation we obtained an asymptotic representation of the shape of the travelling wave and an upper bound on the wave speed in the activator case.

EFFECTS OF WOUND GEOMETRY ON HEALING

Having established a model, with parameter values based largely on biological data, and tested it against experimental results, we can now make theoretical predictions, by doing 'mathematical experiments'. We were particularly interested in the effects of wound geometry on healing time, and to this end we solved the dimensionless model equations (2) numerically for a range of initial wound shapes. The solutions can be illustrated most instructively by plotting the location of the wound edge at a selection of equally spaced times. As before, we take the 'wound edge' as the contour of points at which the cell density is 80% of its unwounded level. In Figure 6 we show these contours for several initial wound shapes, as predicted by either the activator or inhibitor kinetics.

To quantify the concept of wound geometry, we considered one-parameter families of wound shapes. One such family is illus-
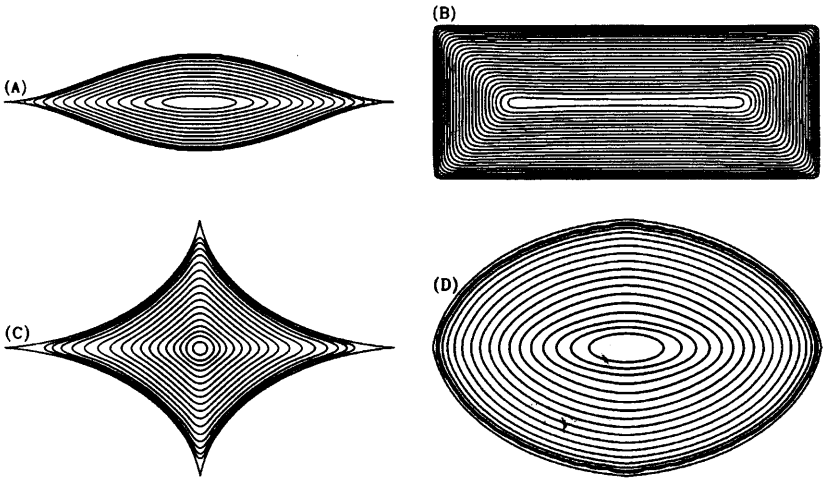
FIGURE 6   The wound edge at a selection of equally spaced times for a selection of initial wound shapes. (A) The healing of an 'eye-shaped' wound, as predicted by the inhibitor kinetics. (B) the healing of a rectangular wound, as predicted by the inhibitor kinetics. (C) The healing of a cusped wound, defined as in the text with α = -0.8, for the activator kinetics. (D) The healing of an ovate wound, defined as in the text with α = +0.8, for the activator kinetics. In each case the wounds have an initial dimensionless area of 1, and the dimensionless model parameter values are as in Figures 4 and 5.

trated in Figure 7. The initial wound edge is defined by four-fold symmetry, with the wound edge in the lower right-hand quadrant being an arc of a circle with centre $(1/2 - 1/2\alpha, 1/2 + 1/2\alpha)$ and radius $\sqrt{(1 + 1/\alpha^2)}/2$; the parameter α is restricted to $-1 < \alpha < 1$. Here we are taking the origin of coordinates as the lower left-hand corner of the bounding rectangle. Thus as the parameter α increases from -1, the wound changes from a highly cusped shape, through a diamond shape at $\alpha = 0$, to an ovate shape, and finally to an ellipse at $\alpha = +1$. For clarity we have illustrated all these shapes within the same bounding rectangle in Figure 7, but in our simulations we choose the midline lengths x_{dim} and y_{dim}, in a specified ratio, so that the initial dimensionless wound area is 1.
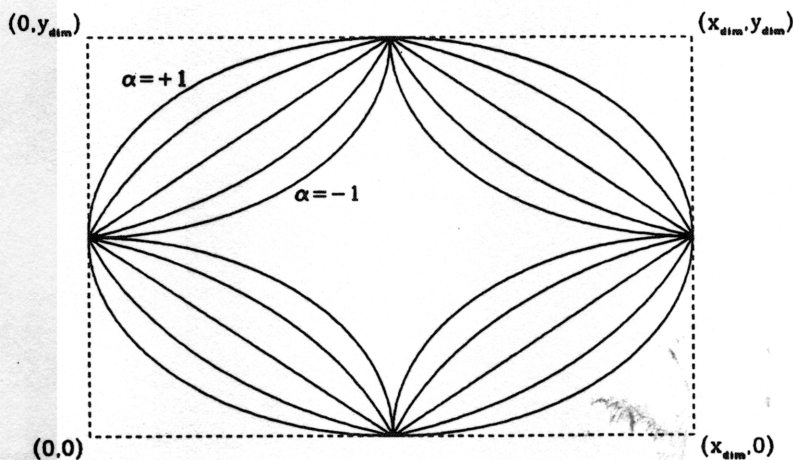
FIGURE 7 A one-parameter family of wound shapes composed of arcs of circles, as defined in the text. For clarity, all the shapes are illustrated within the same bounding rectangle, but in the numerical simulations, the dimensions of this rectangle are chosen, in a specified ratio, so that in each case the initial wound area has a dimensionless value of 1.

The variation in healing time with the parameter α, as predicted by the activator and inhibitor mechanisms, is shown in Figure 8. These results suggest that the variation in healing time with wound shape is very similar for wounds that are concave when viewed from the centre, but quite different for convex wound shapes. This trend is borne out by other families of wound shapes we have considered. Thus, while chemical auto-activation and auto-inhibition are both able to account for data on the normal healing of circular epidermal wounds, it may be possible to distinguish these mechanisms experimentally by examining the variation in healing time with wound shape.

CONCLUSION

Epidermal wound healing presents a major challenge to theoretical biologists, with a variety of quite diverse problems. We have
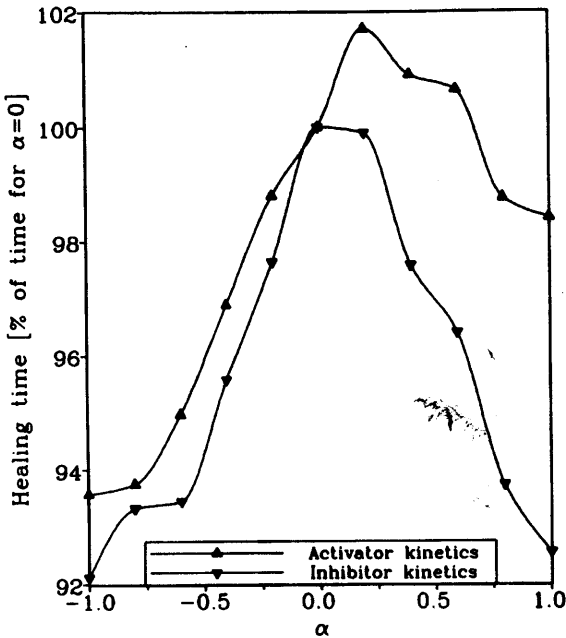
FIGURE 8   The variation in healing time with the parameter α of the family of wound shapes illustrated in Figure 7, as predicted by the model with both biochemical activation (▲) and inhibition (▼) of mitosis. In each case the side lengths of the bounding rectangle are in the ratio 3:2. Healing time is shown as a percentage of that for a diamond-shaped wound. The dimensionless model parameter values are as in Figures 4 and 5.

focussed here on the crucial issue of biochemical auto-regulation of cell mitosis, and have developed a cell-reaction-diffusion model to investigate this phenomenon. The parameter values are based as far as possible on experimental fact, and solutions of the model either with chemical activation or inhibition of mitosis compare well with experimental data on the normal healing of circular wounds, supporting the view that biochemical regulation of mitosis is fundamental to the healing process. Analytical investigation of the solutions as travelling waves has clarified the

roles of the various model parameters in the wave form and the speed of healing. Further, we have been able to use the model to predict trends in the variation of healing time with wound shape, and this has enabled us to suggest a possible approach for distinguishing experimentally between the activator and inhibitor mechanisms.

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