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Commentary

EPIDERMAL WOUND HEALING: THE CLINICAL IMPLICATIONS OF A SIMPLE MATHEMATICAL MODEL

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☐ **Abstract – The role of biochemical regulation in the healing of epidermal wounds remains the subject of much biological debate. We have previously developed a mathematical model which focusses on the role of mitogenic autoregulation in reepithelialization (23–25). Here, we discuss some predictions of our model and their clinical relevance. We investigate both the effects of adding mitotic regulators to healing wounds and the dependence of healing time on wound shape. The latter study suggests a possible mechanism for the control of changes in wound shape during healing. The predictions we make are amenable to experimental verification, and suggest new ideas for experimental research.**

☐ **Keywords – Epidermal migration; Mitotic regulation; Wound healing; Mathematical models.**

INTRODUCTION

Reepithelialization is the dominant mode of healing in partial thickness human skin wounds, and an understanding of the basic biology underlying the process is, therefore, of major clinical importance. Normal epidermal cells are nonmotile, but in the neighbourhood of the wound they undergo marked phenotype alteration, giving them the ability to move via lamellipodia (4,12,31,32). This movement is controlled in part by contact inhibition (1,15), but is undoubtedly regulated by the growth factor profile, probably via a combination of chemotactic, chemokinetic, and mitogenic effects (3). The available experimental evidence suggests that of these, mitogenic autoregulation is the dominant control mechanism. Epidermal autoinhibitors (chalones) are well documented in a number of mammalian species (2,9,11,14,16,19,22,26), and type α transforming growth factor (5) and basic fibroblast growth factor (13,21) have been shown to be autoactivators of epidermal profileration. In unwounded epidermis, homeostasis seems to be due to an interplay between growth activators and inhibitors (29,30), and the role of such regulators in reepithelialization has been demonstrated both in vitro and in vivo (7,8,18,33).

In previous publications (23–25) we have developed a mathematical model for epidermal wound healing which focusses on the role of biochemical autoregulation of cell proliferation. The technique of mathematical modelling enables selected key aspects of the underlying biology to be investigated individually, and has been successfully applied to a wide range of biological systems [see (20) for review]. Having tested a model against existing experimental data, one can go on to make predictions which may suggest new ideas and directions for experimental studies. In this paper, we discuss such predictions arising from our model for reepithelialization. We begin by outlining the model, although we omit the mathematical details, which have been described in detail elsewhere (24).

We consider the simplest possible case of epidermal movement controlled by a single mitotic regulator, which can be either an activator or an inhibitor. Our model consists of two coupled equations, which express local conservation of cells and chemical. The equations have the following general form:

$$\begin{aligned}\text{Rate of increase of cell density} &= \text{Cell migration} \\ &+ \text{Mitotic generation (regulated by chemical)} \\ &- \text{Natural cell loss (sloughing)}\end{aligned}$$

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Rate of increase of chemical concentration = Chemical diffusion + Production of chemical by cells − Decay of active chemical.

Contact inhibition causes cells to move down gradients in cell density and, thus, we model cell migration by linear diffusion. This representation is sufficiently general to avoid the controversy over whether mammalian epidermal cell sheets in vivo move by a 'rolling' or 'sliding' mechanism (27), and enables us to focus entirely on the role of mitotic autoregulation. Such regulation is reflected in our model by taking the rate of cell division to be dependent on the extracellular chemical concentration. For a mitotic activator, this division rate increases with chemical concentration, while it decreases in the inhibitor case. We also include a cell carrying capacity, to reflect the physical constraints on cell proliferation. The production rate of chemical is a function of cell density, and we take different forms for this functional dependence in the activator and inhibitor cases. We assume that a reduction in cell density will tend to increase the production of a mitotic activator in an attempt to stabilize the cell population; however, there will clearly be an upper limit on the production rate per cell and, thus, as the cell density is reduced, the total chemical production rate will eventually start to decrease, until the production rate and cell density are both zero. In the inhibitor case, we assume that the chemical production rate is simply an increasing function of cell density. Finally, we take both natural cell loss and chemical decay to be first-order processes; the rate constants are such that the cell density and chemical concentration in unwounded epidermis are a stable equilibrium state of the model.

The mathematical formulation of these equations involves a number of parameters, and using a combination of in vitro and in vivo data, we have been able to quantitatively estimate the values of these parameters (23,24). The model solutions, then, compare well with data from quantitative experimental studies of reepithelialization, as illustrated in Fig. 1.

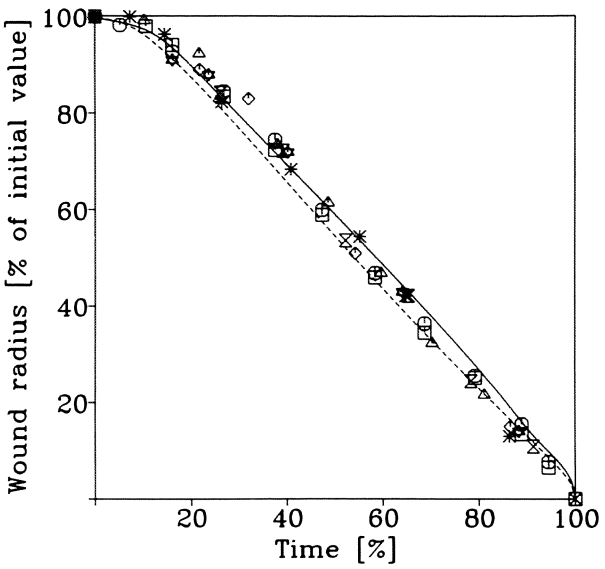

Fig. 1. The decrease in wound radius with time for the normal healing of a circular wound, with time expressed as a percentage of total healing time. The results of our model are denoted by —— (activator mechanism) and ----- (inhibitor mechanism), and we compare these to data from experiments involving a range of species and wounding location, but all involving wounds of diameter about 1 cm, which is the value used in the model solutions. The sources of the data are: □, ○. Van den Brenk (28); △ Crosson et al. (6); ◇, ✡ Zieske et al. (34); ⧖ Lindquist (17); ✳ Frantz et al. (10). In Lindquist's (17) experiments there is some dermal contraction, and we have extrapolated to the case of no contraction. The parameter values used in the model solutions are those discussed in our previous publications (23–25). For both types of mitotic regulation, the model solutions compare well with experimental data.

TOPICAL APPLICATION OF MITOTIC REGULATORS

Our representation of epidermal migration focusses on chemical autoregulation of cell division, and we begin by investigating the effect of applying additional quantities of the mitosis-regulating chemical onto the wound surface. At first we considered the effects of a single, 'one off' addition of chemical at various points during healing, but this failed to produce a significant effect, even when the added chemical concentration was so high as to be experimentally unfeasible. This was caused by the combination of diffusive spread of the added chemical away from the wound, and exponential decay of active chemical. These effects suggested a different approach: the gradual release of regulatory chemical into the wound. This can be achieved experimentally by using a dressing soaked either in a solution of isolated chemical or in an epidermal cell extract or exudate, a technique used in vivo by Eisinger et al. (7) and Madden et al. (18). When such a chemical release is incorporated into the model, the effects on the healing profile are significant, as illustrated in Fig. 2. A given rate of chemical release has

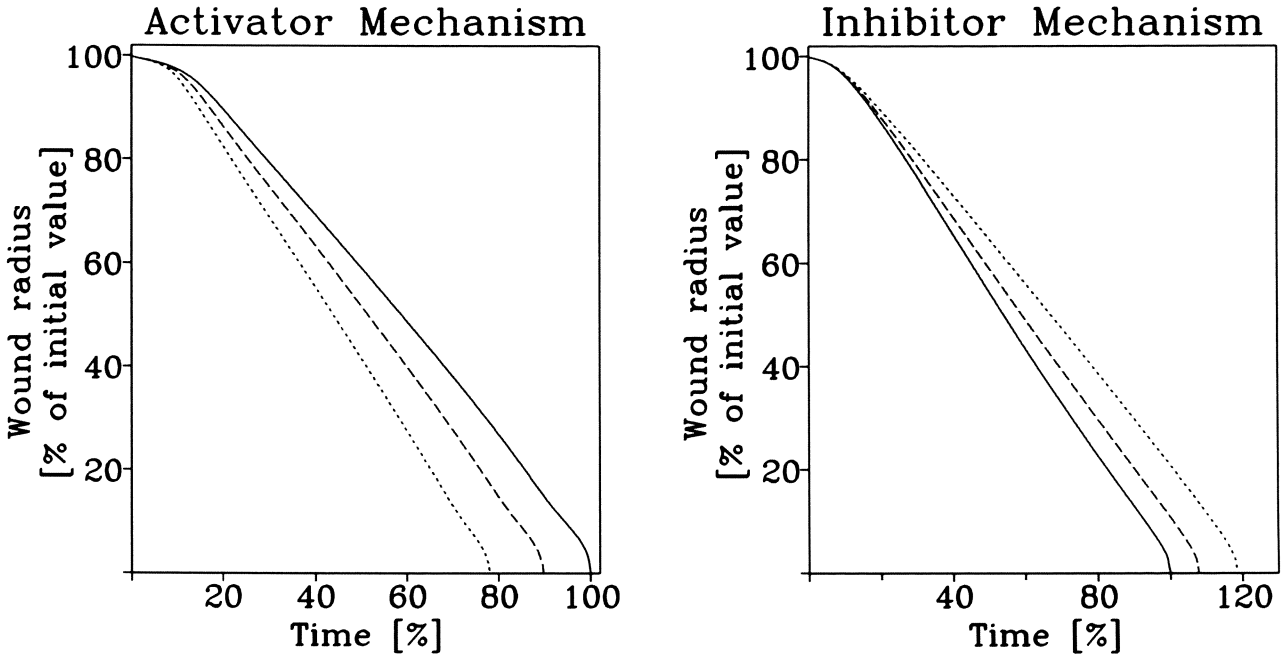

Fig. 2. The model prediction of the effects of a constant gradual release of mitosis-regulating chemical onto a healing epidermal wound. The solid curves denote the healing profile for a control wound with either autoactivation or autoinhibition of mitosis, and time is expressed as a percentage of the total healing time in these cases. The dashed curves denote the healing profile when chemical is added to the wounded area throughout healing. In each case, we show the results for two different rates of chemical release, which are $c_0/5$ (---) and $c_0/2$ (----) per hour in the activator case, and $c_0/50$ (---) and $c_0/20$ (----) per hour in the inhibitor case. Here c_0 is the concentration of regulatory chemical in unwounded epidermis.

a greater effect in the inhibitor case than in the activator case, because experimental data suggests that the rate of chemical decay is significantly higher in the activator case (23).

The experiments of Eisinger et al. (7) and Madden et al. (18) are, unfortunately, only qualitative. However, the predictions illustrated in Fig. 2 could be tested against data from similar quantitative studies, if such data were available. Moreover, the mathematical formulation depends only on the ratio of the amount of chemical release per hour and the concentration present in unwounded skin. Therefore, experimental measurements of the rate of chemical release required to produce a given change in the total healing time would enable the model solutions to quantitatively predict the concentrations of mitotic regulators that are present in unwounded epidermis in vivo.

VARYING WOUND GEOMETRY

For simplicity, we considered only circular wounds in the previous section, but our model can easily be solved for any initial wound shape; the solutions for four different shapes are illustrated in Fig. 3. Such solutions enable us to predict, from our model, the variation in healing time with various aspects of initial wound geometry. One simple study of this kind is to consider the effect on healing time of the length to width ratio of rectangular wounds of given initial area. The model prediction of this variation is illustrated in Fig. 4 for both chemical activation and inhibition of mitosis. As expected, the healing time decreases as the side-length ratio increases; moreover, there is no significant difference between the results for the two types of chemical control. Again, these results are amenable to testing against experimental data.

A more interesting investigation, however, is to vary the actual shape of the initial wound. To do this in a quantifiable way, we considered a single parameter family of wound shapes, as illustrated in Fig. 5. For clarity, all the wound shapes in this figure are shown with the same midline lengths, but in our solutions, the lengths of the midlines are chosen, in a given ratio, so that the initial wound area is the same in each case. As the parameter α increases from -1 to $+1$, the initial wound geometry changes from a cusped shape, through a diamond at $\alpha = 0$, to an ovate shape, and finally to an ellipse at $\alpha = +1$. By solving the model

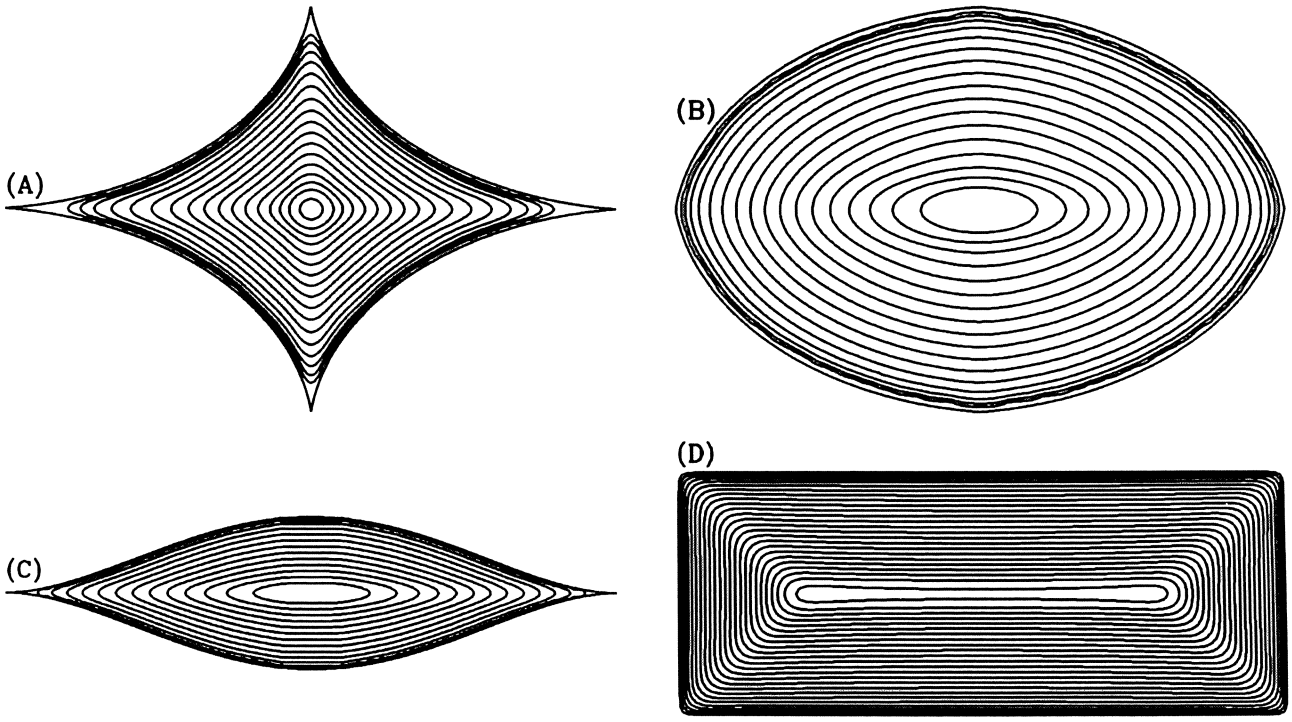

Fig. 3. The wound edge at a selection of equally spaced times for different initial wound shapes. (A) The healing of a cusped wound from the family illustrated in Fig. 5 (with $\alpha = -0.8$) for the activator mechanism. (B) The healing of an ovate wound from the family illustrated in Fig. 5 (with $\alpha = +0.8$) for the activator mechanism. (C) The healing of an 'eye-shaped' wound, as predicted by the inhibitor mechanism. (D) The healing of a rectangular wound, as predicted by the inhibitor mechanism. In terms of the dimensionless parameter rescalings discussed in our previous publications (23–25), the initial dimensionless wound area is 1 in each case, and the dimensionless parameters are the same as used in Fig. 1.

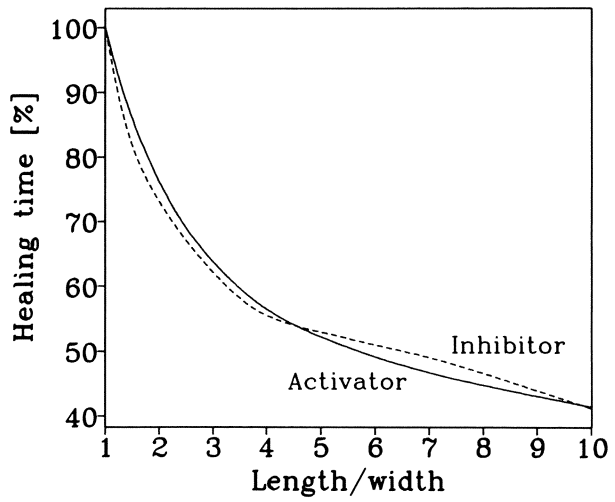

Fig. 4. The variation in healing time with the length to width ratio of rectangular wounds, as predicted by our model with both autoactivation (——) and autoinhibition (----) of cell proliferation. Healing time is expressed as percentage of that for a square wound. In terms of the dimensionless parameter rescalings discussed in our previous publications (23–25), the initial dimensionless wound area is 1 in each case, and the dimensionless parameters are as used in Fig. 1.

equations for a range of values of α, we can, therefore, predict the dependence of healing time on the initial wound geometry. Our results are illustrated in Fig. 6, and suggest that autoregulation of mitosis via a chemical activator and inhibitor imply similar variations in healing time with wound shape when that shape is cusped ($\alpha < 0$), but quite different variations for ovate wound shapes ($\alpha > 0$). This difference is borne out by other families of wound shapes we have investigated, and suggests a possible experimental approach for distinguishing between healing controlled by autoactivation and autoinhibition of mitosis.

An explanation for this difference between the two mechanisms is suggested by changes in the wound shape during healing. As illustrated schematically in Fig. 7, when the wound has an initially cusped shape, the wound edge rounds up during healing, in both the activator and inhibitor cases. In contrast, for ovate wound shapes, the wound tends to flatten during healing, and this occurs to a much greater extent for the inhibitor mechanism than for the activator mechanism. More pronounced flattening results in a larger wound perimeter and, thus, faster healing.

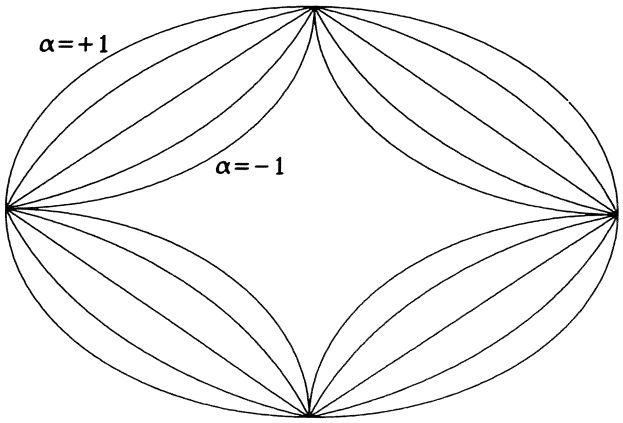

Fig. 5. A family of wound shapes, parameterized by a single parameter α lying between -1 and $+1$. The case $\alpha = 0$ corresponds to a diamond shaped wound. The mathematical definition of the wound shapes is as described previously (25). In the figure and in our model solutions, we take the ratio of the wound midline lengths to be 3:2.

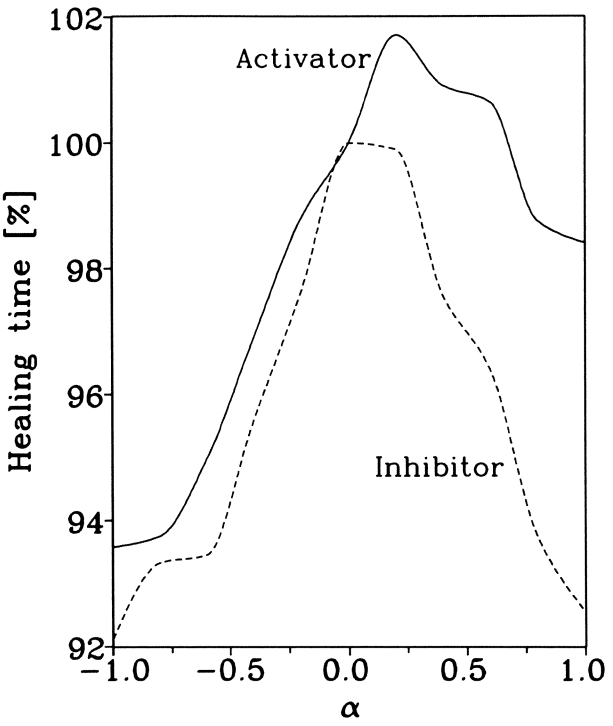

Fig. 6. The variation in healing time with wound shape for the family of shapes illustrated in Fig. 5 and parameterized by α. The model predictions are shown both for the activator mechanism (——) and for the inhibitor mechanism (-----), and healing time is expressed as a percentage of that for a diamond shaped wound, given by $\alpha = 0$. In terms of the dimensionless parameter rescalings discussed in our previous publications (23–25), the initial dimensionless wound area is 1 in each case, and the dimensionless parameters are as in Fig. 1.

This explanation raises an important question: what are the key factors controlling the extent to which an ovate wound flattens during healing? To answer this we considered a very simple equation which mathematically caricatures our full model, with the form:

$$\begin{matrix}\text{Rate of increase} \\ \text{of cell density}\end{matrix} = \begin{matrix}\text{Cell} \\ \text{migration}\end{matrix} + \begin{matrix}\text{Mitotic} \\ \text{generation}.\end{matrix}$$

An equation of this form ignores all biochemical effects and, thus, cannot be expected to represent the healing process quantitatively. However, the mathematical formulation of this caricature, which is discussed elsewhere (23), is particularly simple, and involves a single dimensionless parameter, Γ say, which reflects the relative contributions of cell mitosis and cell migration to the healing process. We can, therefore, use this simple caricature to investigate the dependence of change in wound shape on these two basic aspects of the healing process. Our results are illustrated in Fig. 8: as Γ increases, corresponding to an increase in the role played by mitosis relative to migration, the extent to which an initially ovate wound flattens during healing also increases. This suggests that shape change during healing is a competitive process, with cell migration tending to cause the wound to round up, while cell division tends to cause the wound to flatten. This simple prediction could be tested experimentally by biochemically altering the relative importance of one of these two processes.

DISCUSSION

A number of biochemicals have recently been identified as possible regulators of epidermal wound healing in vivo, but the way in which these biochemicals might conspire to produce healing remains poorly understood. In this paper, we have used a mathematical model to investigate the case of epidermal migration controlled by a single mitotic regulator, which can be either an activator or an inhibitor of cell division. This is undoubtedly a gross simplification of in vivo reepithelialization, but it is a useful first approximation which has enabled us to make quantitative predictions on the effects of adding mitotic regulators to healing wounds, and on the variation of healing time with wound shape. This latter investigation has led to an understanding of a possible mechanism for the control of changes in wound shape during healing. All our predictions are amenable to experimental verification, and all suggest new experiments that would improve the current understanding of the mechanisms responsible for epidermal wound healing.

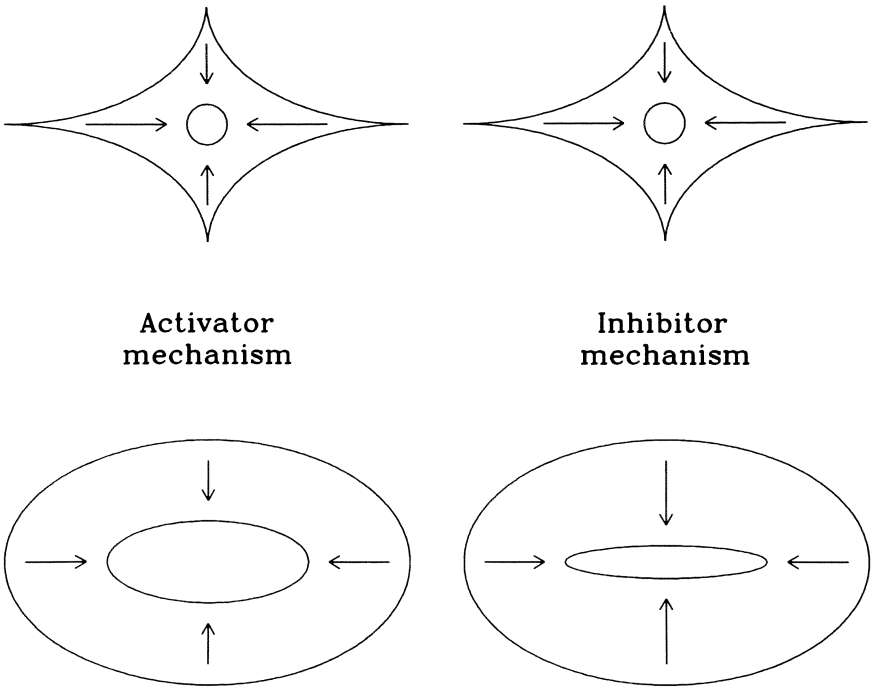

Fig. 7. A schematic illustration of the changes in wound shape during healing. Our model predicts that a cusped wound rounds up during healing, in both the activtor and inhibitor cases, but that an ovate wound flattens during healing. Moreover, this flattening occurs to a much greater extent for the inhibitor mechanism than for the activator mechanism.

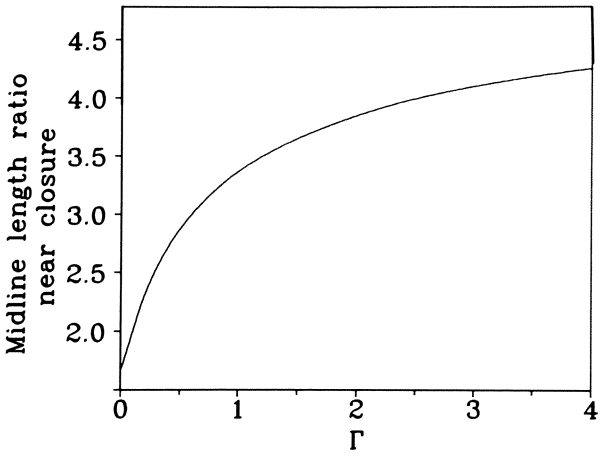

Fig. 8. An illustration of the dependence of change in wound shape during healing on the relative importance of cell division and cell migration. We show the midline length ratio of a wound near closure, when its area reaches 10% of its initial value, as predicted by our simple caricature of the full mathematical model, for a range of values of Γ. As Γ increases, cell division becomes more important in the healing process, relative to the role of cell migration. In each case, the wound is initially elliptical with midline length ratio 2. In terms of the mathematical formulation and dimensionless parameter rescalings discussed previously (23), the initial dimensionless wound area is 1, and $\Gamma = 5 \times 10^{-4}/D^*$.

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