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Keratinocyte growth factor signalling: a mathematical model of dermal–epidermal interaction in epidermal wound healing

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Abstract

A wealth of growth factors are known to regulate the various cell functions involved in the repair process. An understanding of their therapeutic value is essential to achieve improved wound healing. Keratinocyte growth factor (KGF) seems to have a unique role as a mediator of mesenchymal–epithelial interactions: it originates from mesenchymal cells, yet acts exclusively on epithelial cells. In this paper, we study KGF's role in epidermal wound healing, since its production is substantially up-regulated after injury. We begin by modelling the dermal–epidermal signalling mechanism of KGF to investigate how this extra production affects the signal range. We then incorporate the effect of KGF on cell proliferation, and using travelling wave analysis we obtain an approximation for the rate of healing. Our modelling shows that the large up-regulation of KGF post-wounding extends the KGF signal range but is above optimal for the rate of wound closure. We predict that other functions of KGF may be more important than its role as a mitogen for the healing process.

Keywords: Keratinocyte growth factor; Wound healing; Dermal–epidermal interaction; Travelling waves

1. Introduction

Injury in mammalian skin initiates a complex but orderly series of events to re-establish a cutaneous cover. These are usually divided into three overlapping phases: inflammation, wound closure and the reformation and remodelling of dermal tissue. In this paper, we are concerned with part of the second stage: epidermal wound healing. Essentially, this involves the migration and proliferation of cells at the wound edge to regenerate an epithelium across the wound. If the

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injury is restricted to the epidermis, this process begins almost immediately and may seal the wound within 24 h. However, when dermal tissue is also damaged, epidermal cell migration and proliferation occur in parallel with wound contraction and matrix remodelling in the dermis. For reviews of wound repair, see [1–3].

The processes that govern cell migration in the epidermis are not fully understood. Two mechanisms of movement have been proposed: one where the cells crawl in a sequential fashion and another where they roll over or 'leap-frog' one another [4]. In general, the rolling mechanism is thought to best describe what occurs in mammalian skin, although firm evidence is lacking. Soon after the onset of migration, mitotic activity increases in cells close to the wound edge, in response to a combination of factors [2]. The 'free-edge' effect, in which the loss of contact inhibition from neighbouring cells at the wound edge signals both mitosis and migration, is thought to play a part; however, the local release of growth factors seems to have an important role as well.

For many years, the key growth factors that were thought to affect epidermal cell mitosis during wound healing were members of the epidermal growth factor family, in particular, epidermal growth factor itself and transforming growth factor-α [3]. These molecules operate in both a juxtacrine and paracrine manner, since they occur in both a membrane-bound and soluble form [5]. There is also evidence that transforming growth factor-α is an autocrine signalling molecule, i.e. it acts on the cells that secrete it [6]. The common feature of all these mechanisms is that the growth factors are either produced in the epidermis itself, or released into the dermis by immune cells; they are not secreted in unwounded dermal tissue. However, experimental work on keratinocyte growth factor (KGF) has shown that inherent dermal–epidermal signalling mechanisms are also important in the regeneration of epidermal tissue following injury.

KGF was first isolated in 1989 by Rubin et al. [7] and was later identified as a member of the fibroblast growth factor family (designated FGF-7) [8]. It seems to have an effect on a variety of epithelial tissues and studies in culture revealed that KGF is more potent a mitogen for human keratinocytes than epidermal growth factor [7,9]. In the skin, KGF expression has only been observed in the dermis [7,10,11], where it is produced by fibroblasts and to a certain extent by $\gamma\delta$T cells [12]. On the other hand, the high affinity receptor to which it binds, keratinocyte growth factor receptor (KGF-R), has only been detected on the surface of epithelial cells [13]. KGF is therefore assumed to act as a paracrine signalling molecule.

Interest in the role of KGF in wound healing was initiated by the work of Werner and colleagues. In 1992, they reported that KGF messenger RNA levels in mice were up-regulated 160-fold within 24 h of injury [10]. Marchese et al. [14] subsequently observed an increase in mRNA levels of 8–10-fold in human skin. In both cases, the levels of mRNA remained elevated for several days after injury. Follow-up work by Werner et al. demonstrated reduced levels of KGF expression during wound healing in healing-impaired animals [15,16]. A series of studies on the effects of exogenous KGF applied to different types of wound support the importance of KGF's role in epithelial regeneration [17,18]. In most cases the rate of wound closure was increased and the healing process resulted in a thicker epithelium.

These results suggest that KGF is essential for the repair process. Paradoxically, genetic knockout studies by Guo et al. [19] observed normal wound healing in the absence of KGF. This could be explained by genetic redundancy [3,20]; although KGF is normally the most important ligand to bind to the KGF receptor, other KGF-R ligands could compensate for a lack of KGF.

Indeed, Beer et al. [21] have recently discovered another member of the fibroblast growth factor family, FGF-10, with similar properties to KGF. What seems to be important is the KGF-R signalling pathway: studies by Werner et al. [22] in which KGF-R signalling was blocked resulted in deficient wound healing. In addition, only incisional (surgical slash) wounds were tested in the KGF knockout studies, whereas the work of Werner et al. [22] was based on excisional wounds, in which there is more tissue damage and thus the extent of re-epithelialisation is much greater. For a recent review of KGF's role in epithelial repair, see [20].

The significant increase in KGF levels upon injury prompted studies into the regulation of KGF production. Independently, two groups [23,24] demonstrated that KGF expression was activated in cultured fibroblasts by other growth factors, in particular by serum growth factors which are released upon haemorrhaging and would account for the initial induction of KGF after wounding [23]. The sustained levels of KGF in the later stages of the repair process may be explained by the presence of leukocytes and macrophages, which infiltrate the wound within 24 h after injury. These cells have been shown to produce various pro-inflammatory cytokines [25,26], especially interleukin-1 which has an important effect on KGF expression [24]. A strong hypothesis is that there is a dermal–epidermal feedback loop operating during the healing process, possibly involving interleukin-1 or other factors known to promote KGF expression which are also produced by keratinocytes, such as transforming growth factor-α [27]. This is supported by the work of Smola et al. [28], who investigated the expression of various growth factors, including KGF, in in vitro experiments where keratinocytes were co-cultured with dermal fibroblasts.

KGF has attracted a great deal of attention, primarily due to its potential role as a mediator of mesenchymal–epithelial interactions. Moreover, KGF appears to have a unique target cell specificity; in the skin, KGF is secreted solely by dermal fibroblasts and yet its activity seems to be restricted to epidermal keratinocytes. This has raised an important question: is the observed level of KGF induction following injury optimal for the speed and/or quality of epidermal wound healing? In this paper we address this question by investigating the importance of the dermal–epidermal signalling of KGF for keratinocyte proliferation during wound closure.

2. Development of a mathematical model

We begin our mathematical modelling by first considering the paracrine signalling mechanism of KGF, before looking at the activity of KGF on re-epithelialisation during wound healing. This allows us to investigate the importance of the dermal–epidermal interaction and in particular to focus on the signalling range of the KGF molecule.

The key features we want to incorporate into the modelling framework are the following:

- The passage of KGF through the basal lamina – the membrane separating the epidermis and the dermis.
- A representation of KGF binding in the epidermis.
- The up-regulation of KGF in the dermis after wounding.

We consider 'one-dimensional' behaviour on either side of the basal membrane away from a wound edge. By 'one-dimensional' we mean a single row of epidermal cells above the membrane and the equivalent depth below in the dermis. This approach is justified by evidence that KGF is produced by dermal fibroblasts close to the basal lamina [10] and that the KGF receptor is found

primarily on the surface of basal keratinocytes in the epidermis [29]. We therefore allow KGF concentration to vary in both the dermis and epidermis, and, taking into account both free and bound receptor numbers in basal epidermal cells, we formulate the model using four variables:

$s(x,t) =$ dermal KGF concentration,

$e(x,t) =$ epidermal KGF concentration,

$r(x,t) =$ number of free receptors per cell,

$b(x,t) =$ number of bound receptors per cell,

where x is the distance from the wound edge (at $x = 0$) and t is time.

Due to the tightly packed cellular structure of the epidermis, we neglect diffusion of KGF in the epidermis to give a coupled system of one partial differential equation and three ordinary differential equations as follows:

$$\frac{\partial s}{\partial t} = \overbrace{D_s \frac{\partial^2 s}{\partial x^2}}^{\text{diffusion}} + \overbrace{P_s(b)}^{\text{production}} - \overbrace{d_l(s-e)}^{\text{dermal–epidermal interaction}} - \overbrace{k_s s}^{\text{decay}}, \tag{1a}$$

$$\frac{\partial e}{\partial t} = + d_l(s-e) - k_e e - \overbrace{k_a e r}^{\text{binding}} + \overbrace{k_d b}^{\text{dissociation}}, \tag{1b}$$

$$\frac{\partial r}{\partial t} = + P_r(b) - k_r r - k_a e r + k_d b, \tag{1c}$$

$$\frac{\partial b}{\partial t} = - \overbrace{k_i b}^{\text{internalisation}} + k_a e r - k_d b, \tag{1d}$$

where $D_s, d_l, k_s, k_e, k_a, k_d, k_r, k_i$ are all positive constants, and P_s, P_r are increasing functions which will be detailed below.

Diffusion. We assume that the movement of KGF in the dermis can be represented by a Fickian diffusion term with the coefficient D_s.

Production. Furthermore, we assume that production of KGF in the dermis under normal skin conditions is regulated by some positive feedback from the epidermis, as discussed in Section 1. We take this to be a non-linear increasing function of the number of bound receptors. We use a similar functional form for the production of free receptors on the surface of epidermal cells; again non-linear to incorporate a saturation level. Specifically, these are Monod type functions given by

$$P_s(b) = \frac{C_1 b}{C_2 + b}, \tag{2a}$$

$$P_r(b) = C_3 + \frac{C_4 b}{C_5 + b}, \tag{2b}$$

where $C_1, \ldots, C_5$ are positive parameters. Note that $P_r(0)$ is non-zero since there will be a background level of receptor production in the absence of binding. This positive feedback in both ligand and receptor production during binding is familiar from previous mathematical models [30].

Dermal–epidermal interaction. In the absence of any data on the transition of KGF through the basal membrane, we take a simple linear form for the dermal–epidermal interaction term. A similar representation was used by Cruywagen and Murray [31] in their tissue interaction model for skin pattern formation. They introduced such a term to describe the diffusion across the basal lamina of a morphogen produced in one tissue (either the epidermis or dermis) but acting in the other.

Decay. The dermal and epidermal concentrations of KGF and the number of free receptors are all assumed to decay at the rates k_s, k_e and k_r, respectively.

Binding. The binding terms in the three ordinary differential equations can be derived from the laws of mass action under the assumption of the following simple kinetic scheme:

$$\boxed{\text{ligand}} + \boxed{\text{free receptor}} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \boxed{\text{bound receptor complex}} \xrightarrow{k_i} \boxed{\text{internalised receptor complex}}$$

2.1. Parameter values

The rate constants and parameters are estimated from experimental data on KGF or related growth factors, or from steady-state analysis by defining biologically realistic equilibrium levels. Since we are working in numbers of receptors per cell for free and bound receptor quantities we give our spatial measurements in terms of average epidermal cell length, L. For simplicity, we assume that $L = 0.01$ mm.

Diffusion. The diffusion coefficient of KGF in the dermis, D_s, may be estimated theoretically using its apparent molecular weight, M_s, which is around 27 kDa [32]. For large proteins in aqueous solution at a particular temperature and pressure, $D_s \propto M_s^{-1/3}$ is a good approximation from the Stokes–Einstein relation [33]. The constant of proportionality may be estimated from the diffusion coefficient d and molecular weight m of another chemical, so that $D_s \approx d(m/M_s)^{1/3}$. Here we use data for FMLP, whose molecular weight is 60 kDa and diffusion coefficient is 7.3×10^{-6} cm^2 s^{-1}. This gives the estimate 9.5×10^{-6} cm^2 s^{-1} for D_s which converts to $5.7 \times 10^2 L^2$ min^{-1}.

Production. Specifying the forms of the feedback functions, P_s and P_r, is difficult since the data available on production rates of ligand molecules and receptors are extremely limited. However, this can be achieved to some extent because the functions must satisfy a number of conditions that relate them to experimentally measurable quantities:

(i) In the absence of any binding in the epidermis, there will be a background level of receptor expression, say r_0. This is a homogeneous steady state of the model, and so the equation for r (1c) gives

$$P_r(0) = k_r r_0. \tag{3}$$

(ii) Specifying the unwounded equilibrium levels of free and bound receptors, r_{norm} and b_{norm}, defines the normal steady-state levels of free ligand in the epidermis, e_{norm}, and dermis, s_{norm}, implicitly through Eqs. (1d) and (1b), respectively. This gives

$$e_{\text{norm}} = \frac{(k_d + k_i) b_{\text{norm}}}{k_a r_{\text{norm}}}, \quad s_{\text{norm}} = \frac{(d_l + k_e) e_{\text{norm}} + k_i b_{\text{norm}}}{d_l}. \tag{4}$$

The values of the feedback functions at the uniform steady state are similarly specified through the other two equations, so that

$$P_s(b_{\text{norm}}) = k_e e_{\text{norm}} + k_i b_{\text{norm}} + k_s s_{\text{norm}}, \quad \text{and} \quad P_r(b_{\text{norm}}) = k_i b_{\text{norm}} + k_r r_{\text{norm}}. \tag{5}$$

(iii) There will also be a maximum possible level of receptor expression, r_m, say. This can be estimated experimentally by saturating cells with ligand; the value we take, $r_m = 39\,000$, is from a study by Shaoul et al. [34]. Such saturation means that the rate of internalisation of bound receptors must be equal to the rate of free receptor production, giving

$$P_r(r_m) = k_i r_m. \tag{6}$$

Conditions (3), (5) and (6) therefore fix four out of the five parameters in the production functions, leaving one free parameter in P_s, which we take to be C_2 – the number of bound receptors at which the KGF production rate attains half its maximum value. At present we are unaware of any quantitative measurements of normal receptor numbers on the surface of keratinocytes, we therefore make intuitive estimates based on certain biological constraints. Since $0 < r_{\text{norm}} < r_m$ and P_r is a strictly increasing function, then $P_r(0) < P_r(r_{\text{norm}}) < P_r(r_m)$ so that $k_r r_0 < k_i b_{\text{norm}} + k_r r_{\text{norm}} < k_i r_m$ where $k_r \approx k_i$. Also, for there to be endogenous levels of KGF in unwounded skin (i.e. $e_{\text{norm}}, s_{\text{norm}} > 0$) $b_{\text{norm}} > 0$. If we assume that $r_0 = r_{\text{norm}} = b_{\text{norm}} = 3000$, which is less than 10% of the maximum possible level of receptor expression, r_m, given above, then these constraints are always satisfied. However, we will discuss the implications of varying the parameters r_0, r_{norm} and b_{norm} later.

Dermal–epidermal interaction. The rate constant d_l, controlling the diffusion of KGF through the basal membrane, is unknown but could be estimated if the unwounded level of dermal KGF, s_{norm}, was specified. However, we are not aware of any quantitative measurements for KGF concentrations in normal skin. We therefore take the parameter d_l to vary in our investigations, which implies that both s_{norm} and the production parameter specified by condition (5) will vary accordingly.

Decay. The half-life of KGF RNA was determined by Chedid et al. [24] to be 140 min, which gives a decay rate of $(\ln 2)/140 = 0.005\ \text{min}^{-1}$. This is consistent with data for the turnover of transforming growth factor-α molecules whose decay rate is $0.006\ \text{min}^{-1}$. Since we are unaware of data on the decay rate of KGF-R, we approximate this value by the turnover of epidermal growth factor receptors. This seems reasonable because both types of receptors are part of paracrine signalling mechanisms and located on the surface of epidermal keratinocytes. We also assume that KGF molecules decay at the same rate in both the epidermis and dermis, to give $k_e = k_s = 0.005\ \text{min}^{-1}$ and $k_r = 0.03\ \text{min}^{-1}$.

Binding. Here we make use of data for KGF binding in tandem with estimates for related growth factors. The average binding affinity constant, i.e. the ratio $k_a : k_d$, has been determined as 1.1 nM for KGF to KGF-R in [35]. Although data are lacking for the individual binding constants of fibroblast growth factors, we can use this ratio along with the dissociation constant for epidermal growth factor binding to epidermal growth factor receptor, $k_d = 0.12\ \text{min}^{-1}$, to calculate an estimate for the association rate, k_a. After converting from moles into molecules, this gives $k_a = 1.8 \times 10^{-4}\ \text{molecule}^{-1} L^3\ \text{min}^{-1}$. At present, there are little quantitative data on the internalisation of the KGF receptor. Our estimate for the internalisation constant,

$k_i = 0.043$ min^{-1}, is taken from a binding study of another member of the fibroblast growth factor family, basic fibroblast growth factor [36], which binds to many FGF receptors, including KGF-R. This is justified by a recent study which found that the rate of internalisation for bound KGF-R complexes is in fact very similar to other members of the FGF family, with specific reference to basic fibroblast growth factor [37].

3. Numerical simulations

In this section, we present the results of our numerical simulations. We are specifically concerned with the signalling range of KGF and its dependence on model parameters. By using finite differences to discretise our spatial variable, we convert the system of equations (1a)–(1d) to four ordinary differential equations which we then solve with a stiff ode solver. We take our domain to

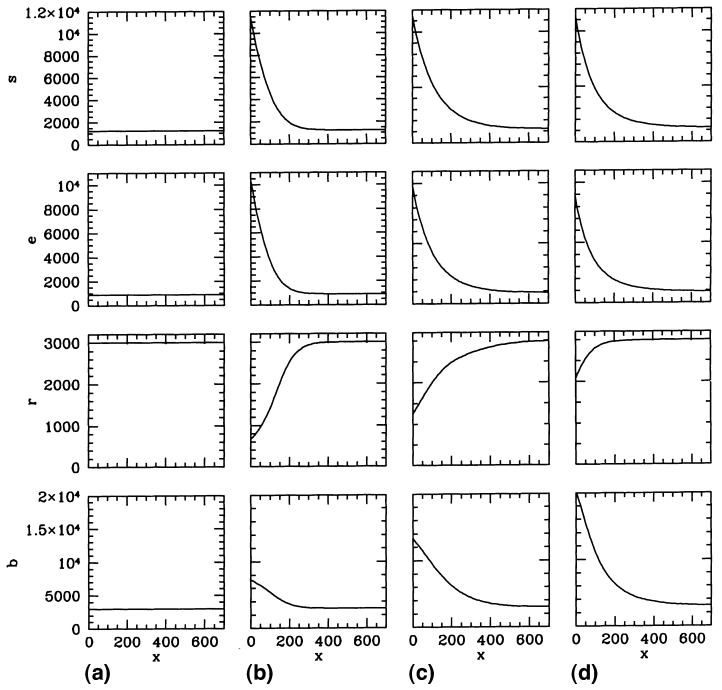

Fig. 1. Typical simulation of the model equations (1a)–(1d): at (a) $t = 0$ h, (b) $t = 0.5$ h, (c) $t = 5$ h and (d) $t = 10$ h, where t is time in hours. In (b), we observe an increase in epidermal KGF concentration at the wound edge in response to the induction of KGF in the dermis. This leads directly to the down-regulation of free receptors and up-regulation of bound receptors close to the boundary. After 5 h, KGF has diffused further into the unwounded tissue, increasing the number of bound receptors in up to 500 cells from the wound edge. In (d) the system has reached a heterogeneous steady state: elevated concentrations of KGF and high numbers of bound receptors at the wound boundary decrease towards normal steady-state levels over 400–500 cell lengths, whereas free receptor numbers increase to the unwounded level after only 200 cells from the wound edge. The parameter values are $\alpha = 10$, $d_l = 0.4$ min^{-1}, $C_2 = 8000$, $d_s = 570L^2$ min^{-1}, $k_a = 1.8 \times 10^{-4}$ molecule^{-1} min^{-1}, $k_d = 0.12$ min^{-1}, $k_i = 0.043$ min^{-1}, $k_e = k_s = 0.005$ min^{-1}, $k_r = 0.03$ min^{-1}, $r_0 = 3000$, $r_{\text{norm}} = 3000$, $b_{\text{norm}} = 3000$, $r_m = 39\,000$. The spatial variable x is in units of cell length $L = 0.01$ mm.

be the equivalent of 2000 epidermal cell lengths, about 20 mm: at one boundary is the wound edge, where $s = \alpha s_{\text{norm}}$ corresponds to the up-regulation of KGF following injury, and at the other, where the tissue is undamaged, we impose symmetric boundary conditions. The parameter α is varied to simulate different responses in KGF production at the wound edge; we use the form αs_{norm} because the experimental data available are related to the 'normal' level of KGF present in the dermis and we define this as s_{norm} in our model parameters. Initial conditions are such that the levels of ligand concentration and receptor numbers are at the unwounded homogeneous steady state, i.e. $s = s_{\text{norm}}$, $e = e_{\text{norm}}$, $r = r_{\text{norm}}$, $b = b_{\text{norm}}$ at $t = 0$, except that $s(x = 0, t = 0) = \alpha s_{\text{norm}}$ so that boundary and initial conditions are compatible.

A typical simulation is given in Fig. 1. This illustrates the temporal evolution in the solution of the model equations (1a)–(1d) for each of the four variables. At the wound boundary, we immediately observe an increase in KGF concentration in both tissues which leads to the down-regulation of free receptors and up-regulation of bound receptors in the epidermis. As time progresses, KGF diffuses further into the dermis and therefore the epidermis, affecting points up to 500 cell lengths from the wound edge. After 10 h, the system has reached a heterogeneous steady state: high numbers of bound receptors are present near the boundary, decreasing towards the normal steady-state level over 400–500 cells. This is similar for the KGF concentrations,

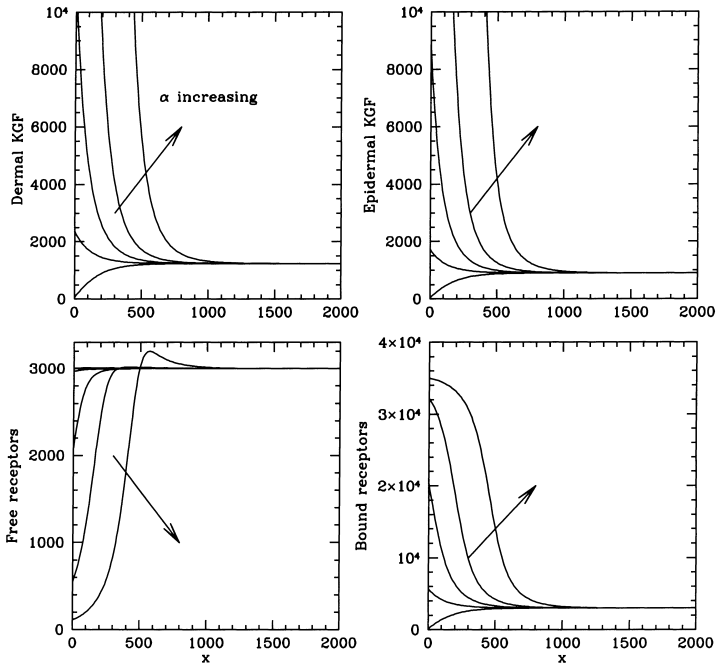

Fig. 2. Solution of the model (1a)–(1d) after 10 h for varying levels of KGF at the wound edge. Imposing 10 times the normal level of KGF at the wound boundary ($\alpha = 10$) generates a signal that significantly affects the number of bound receptors in up to 400 cells from the wound edge. There is not a substantial extension in the paracrine signal until we up-regulate KGF to levels where receptors are saturated at the boundary. The values of α, where $s = \alpha s_{\text{norm}}$ at the left of the domain, are 0, 2, 10, 50 and 250. The other parameters are as in Fig. 1.

whereas the number of free receptors is almost at the unwounded steady state after 200 cells from the wound edge.

We begin our investigation into the signalling range of KGF by varying the quantity of KGF at the boundary, corresponding to a change in the amount present at the wound edge after injury. Fig. 2 shows solutions of the model (1a)–(1d) after 10 h for different amounts of KGF imposed at the wound boundary – all other parameters remain fixed. Increasing KGF at the wound edge to 10 times the steady-state level does not have a great effect on the signal range compared to twice the normal level: both affect between 400 and 500 cells from the wound edge. It is only on elevating KGF to levels where receptors are saturated at the boundary that the signalling range is significantly extended. This will be discussed in more detail later. As mentioned in Section 1, experimental results show that levels of KGF mRNA during wound healing in human skin peak at around 10-fold the normal levels. Therefore the higher levels of KGF imposed at the boundary are only relevant as a representation of the addition of exogenous KGF to the wound bed.

If we then fix the boundary condition, and study the variation of d_l – the parameter controlling the dermal–epidermal interaction – the alteration in the signalling range is more pronounced. This

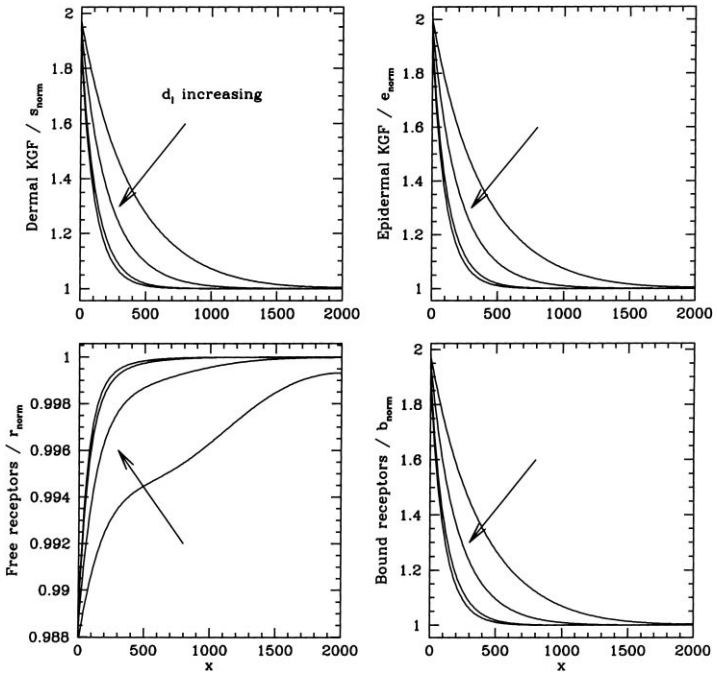

Fig. 3. Solution of the model (1a)–(1d) after 10 h for increasing values of d_l, the parameter that controls the rate of the dermal–epidermal interaction. For the purpose of displaying all the simulations on the same graph, the variables have been rescaled by their homogeneous steady-state levels. As d_l decreases, the effect of the KGF up-regulation at the wound edge extends over more cells. However, to ensure that s_{norm} remains the unwounded equilibrium level of KGF in each simulation, if we reduce d_l then we must increase C_1, the limit of the production function $P_s(b)$. Therefore independent investigation of d_l and the feedback parameters is needed to see whether a slow interaction across the basal membrane and/or strong feedback from the epidermis account for the longest ranges shown above. The parameter $\alpha = 2$ and d_l takes the values 0.004, 0.04, 0.4, and 4 min^{-1}. The other parameters are as in Fig. 1.

is illustrated in Fig. 3: as d_l increases, we observe that the spatial decay rate of the KGF signal increases. A large value of d_l corresponds to rapid interaction between the two tissues, so that the induction of KGF at the wound edge is quickly transferred to the epidermis before it can diffuse further into the dermis.

In Fig. 3, s_{norm} and C_1 have been changed in parallel with d_l so that s_{norm} remains the unwounded equilibrium level of KGF. Recall that s_{norm} is the 'normal' steady-state level of dermal KGF concentration and C_1 is the maximum production rate of KGF via feedback from the epidermis. In Fig. 4, we show the effects of varying each of the parameters independently. Consequently, s_{norm} is no longer an equilibrium of the model equations (1a)–(1d) except for a single value of each parameter. Fig. 4 demonstrates how the parameters α, d_l, C_1 and C_2 influence the number of bound receptors in a cell 5 mm from the wound edge, 10 h after the creation of the

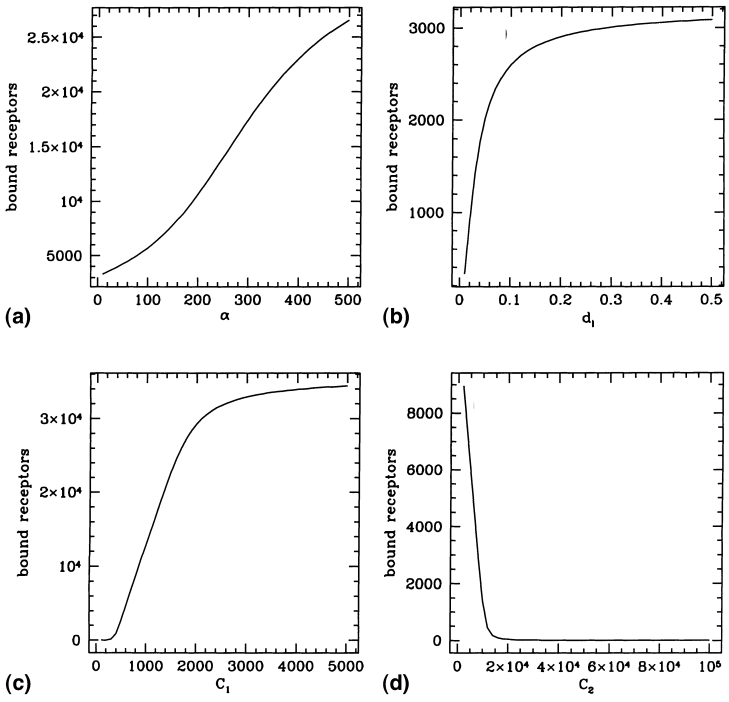

Fig. 4. The influence of four parameters on the number of bound receptors in a cell 5 mm from the wound edge, at $t = 10$ h. In (a) we vary α, the parameter which controls the induction of KGF at the wound boundary. We observe that the number of bound receptors increases as α increases but this is greatest for $150 < \alpha < 350$. As d_l increases in (b), the number of bound receptors also increases but not significantly above the unwounded level of 3000. We note that d_l alone cannot account for the increases in bound receptor numbers seen in the previous simulations of Fig. 3, where both d_l and C_1 were varied. This can be explained by (c): increasing C_1 obtains the most dramatic rise in the number of bound receptors. For example, doubling C_1 from 1000 to 2000, triples the number of bound receptors 500 cell lengths from the wound boundary. Small values of C_2 also induce an increase in bound receptor numbers compared to the 'normal' level of 3000, but ultimately this depends on C_1, since for $C_2 = 0$, $P_s(b) = C_1$. Thus, the limit of the feedback function, C_1 has the greatest effect on the signal range of KGF. In each simulation, $s_{\text{norm}} = 1200$ molecules L^{-3} and only one of the four parameters α, d_l, C_1 and C_2 is allowed to vary. When these parameters are fixed they take the following values: $\alpha = 2$, $d_l = 0.4\ \text{min}^{-1}$, $C_1 = 520\ \text{min}^{-1}$, $C_2 = 8000$. The other parameters are as in Fig. 1.

boundary. Increasing the value of α, the parameter that controls the induction of KGF at the wound boundary, increases the number of bound receptors and as suggested by Fig. 2, this is greatest for $150 < \alpha < 350$. As we increase d_l, the number of bound receptors again increases, but not significantly above the unwounded level of 3000. Therefore, d_l alone cannot account for the increases in bound receptor numbers seen in the previous simulations of Fig. 3, where d_l and C_1 were varied simultaneously. This can be explained by Fig. 4(c): the most dramatic rise in the number of bound receptors is obtained by increasing C_1. For example, doubling C_1 from 1000 to 2000, triples the number of bound receptors 500 cell lengths from the wound boundary. Small values of C_2 also induce an increase in bound receptor numbers compared to the 'normal' level of 3000, but the scale of this ultimately depends on C_1, since for $C_2 = 0$, $P_s(b) = C_1$. Recall that C_2 is the number of bound receptors at which the production rate attains half its maximum value.

Thus, the KGF signal has the greatest effect when there is strong feedback from the epidermis, independent of the induction of KGF at the wound edge and the passage of KGF through the basal lamina. In particular, increasing the rate of diffusion through the basement membrane has little effect on extending the range of the paracrine signal. From our simulations, we conclude that a large induction of KGF at the wound bed (above endogenous production levels) and/or strong feedback from the epidermis are the important factors regulating KGF signal range.

4. Modelling re-epithelialisation

To simulate wound closure in the epidermis, we now introduce a variable for the density of the basal keratinocyte population, $c(x,t)$. We represent the rate of change in cell density by the following reaction–diffusion equation:

$$\frac{\partial c}{\partial t} = \overbrace{D_c \frac{\partial^2 c}{\partial x^2}}^{\text{migration}} + \overbrace{cf(b)\left(2 - \frac{c}{c_0}\right)}^{\text{mitosis}} - \overbrace{k_c c}^{\text{loss}}. \tag{7}$$

Cell migration. As in previous models of epidermal wound healing, we use a diffusion term to model cell migration controlled by contact inhibition [38]. Diffusion has been similarly used in models for tumour growth, see for example [39–41]. There is some evidence that KGF stimulates the migration of keratinocytes in vitro, possibly by increasing plasminogen activator activity. This effect is, however, less significant than KGF's stimulation of cell growth [42]. Moreover, Zeigler et al. [43] suggest that cell motility is not substantially affected by KGF in comparison with epidermal growth factor and hepatocyte growth factor. In the absence of further data suggesting either a chemotactic or chemokinetic role for KGF, we therefore assume that cell movement is independent of KGF concentration in our model.

Cell mitosis and loss. The loss in basal cells due to differentiation and vertical movement into the suprabasal layer is part of the continual process of epidermal renewal. We assume that at the unwounded state $b = b_{\text{norm}}$, the rate of cell division and loss is of logistic growth form, $k_c c(1 - c/c_0)$. We therefore model cell proliferation by the term $cf(b)(2 - c/c_0)$, where $f(b)$ reflects the effect of KGF on cell mitosis, with $f(b_{\text{norm}}) = k_c$. The qualitative form of $f(b)$ is taken from in vitro experiments which show that keratinocytes proliferate in the absence of KGF but

that there is an optimal dose of KGF which induces up to a 6-fold increase in the cell growth rate [9]. We represent this production rate by the simple functional form

$$f(b) = A_0 + \frac{A_1 b}{A_2^2 + b^2}, \tag{8}$$

where $f(0) = A_0 > k_c/2$ to allow proliferation in the absence of KGF and A_2 is the number of bound receptors corresponding to the maximum proliferation rate in vitro. The data available give the KGF concentration that stimulates the greatest growth rate, so to convert this to the number of bound receptors and obtain A_2, we solve the steady-state equations of r and b, (1c) and (1d), for the particular value of e. If we choose $k_c/2 < A_0 < k_c$, then A_1 is uniquely determined by the condition $f(b_{\text{norm}}) = k_c$; we take $A_0 = 3k_c/4$ arbitrarily.

We estimate the coefficient of cell migration by considering the conservation equation (7) when $b = 0$ and so $f(0) = 3k_c/4$. The differential equation representing change in cell density is then just the Fisher equation and for travelling wave solutions, the minimum speed where $c = 0$ ahead of the wave is given by $\sqrt{2D_c k_c}$ [44]. From experimental data, a human skin wound of diameter 4 mm takes approximately 15 days to re-epithelialise without the addition of exogenous factors [14]. This is consistent with data from animal models, for example see [17]. Assuming that cells migrate from both sides of the wound at the same rate, this gives an average speed of 0.13 mm day^{-1} or in the dimensions of our system 9.3×10^{-3} L min^{-1}. Moreover, the parameter k_c is just the reciprocal of the epidermal cell cycle which is approximately 100 h [45], thus $k_c = 1.7 \times 10^{-4}$ min^{-1}. Together these estimates imply that the coefficient of cell movement $D_c = 0.26$ L^2 min^{-1}.

The model (1a)–(1d) now becomes the following system of conservation equations:

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + c f(b)\left(2 - \frac{c}{c_0}\right) - k_c c, \tag{9a}$$

$$\frac{\partial s}{\partial t} = D_s \frac{\partial^2 s}{\partial x^2} + c P_s(b) + K(c_0 - c) - d_l(s - e) - k_s s, \tag{9b}$$

$$\frac{\partial e}{\partial t} = d_l(s - e) - k_e e - k_a e r c + k_d b c, \tag{9c}$$

$$\frac{\partial r}{\partial t} = P_r(b) - k_r r - k_a e r + k_d b, \tag{9d}$$

$$\frac{\partial b}{\partial t} = -k_i b + k_a e r - k_d b. \tag{9e}$$

The binding terms in Eq. (9c) have been modified since the variable e is an extracellular concentration whereas r and b are the number of receptors per cell. Thus the binding and dissociation of KGF molecules to receptors on the cell surface depend on cell density; in the previous model (1a)–(1d) we did not consider a change in cell density. Production of KGF in the dermis is still regulated by the feedback function from the epidermis, $P_s(b)$; this is the production rate per cell, and is thus multiplied by the keratinocyte density c in Eq. (9b). The additional term $K(c_0 - c)$ represents the up-regulation of KGF after injury, which was previously incorporated in the wound boundary condition.

The extended model (9a)–(9e) has homogeneous equilibria which represent wounded and unwounded states. 'Normal' skin is assumed to be the steady state where $c = c_0$, $s = s_{\text{norm}}$, $e = e_{\text{norm}}$, $r = r_{\text{norm}}$ and $b = b_{\text{norm}}$. The unwounded cell density c_0 is taken to be 1 cell L^{-3}, since the kera-

tinocytes are tightly packed in the epidermis and our dimension L is approximately the length of one epidermal cell. For comparison, this corresponds to the unwounded equilibrium of the previous model (1a)–(1d). After injury, when the basal layer of epidermal tissue has been removed, the wounded steady state is given by $c = 0$, $s = s_w$, $e = e_w$, $r = r_w$ and $b = b_w$. Note that $c = 0$, $s = s_w$, $e = r = b = 0$ is not a steady state of our model since r is always non-zero. Thus there will be positive amounts of free and bound receptors in the absence of keratinocytes; this is because the variables r and b are not measures of density but rather are the number of receptors on the surface of any cell present in the epidermal extracellular concentration of KGF, e.

We determine the parameter K by specifying the value of s_w, since at the homogeneous steady state when $c = 0$, Eqs. (9b) and (9c) give

$$K = \frac{s_w}{c_0}\left(k_s + \frac{d_l k_e}{d_l + k_e}\right). \tag{10}$$

As in our previous numerical simulations, the wounded level of dermal KGF may be considered as a function of the unwounded level, s_{norm}. Thus, we write $s_w = \hat{\alpha} s_{norm}$ where $\hat{\alpha}$ is a parameter that

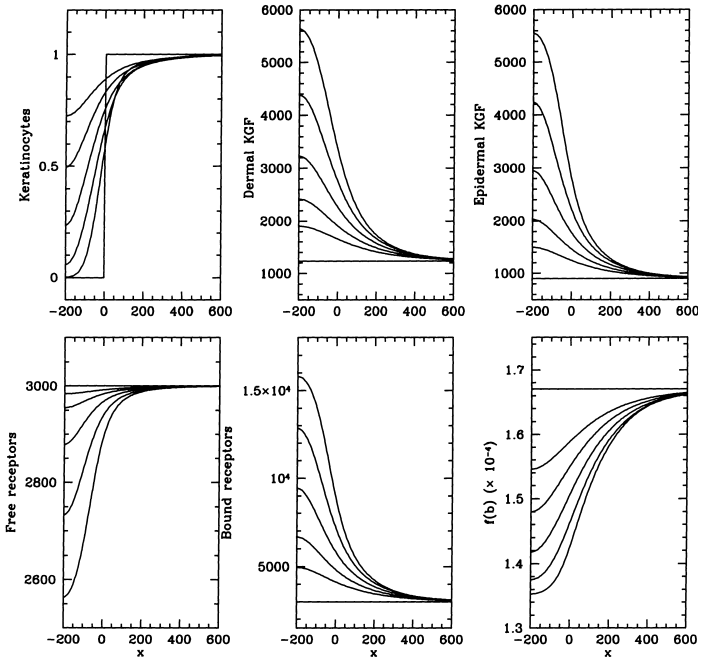

Fig. 5. Temporal evolution of a solution of the extended model (9a)–(9e). The profiles shown are for $t = 0$, 5, 10, 15, 20 and 25 days. The wound region, $x \leqslant 0$, is taken as 2 mm in radius. The migration of cells from the wound edge and KGF-regulated mitosis induce the increase in keratinocyte density in the wound region. After 20 days the density of the cells in the centre of the wound bed is already half the unwounded equilibrium level, ($c_0 = 1$). Since the parameter $\hat{\alpha} > 1$, the wounded steady state of dermal KGF is greater than the unwounded level and therefore the profile of the variables s, e and b increases towards the wound centre. A graph of $f(b)$, the function regulating the proliferation of basal keratinocytes, is displayed to illustrate that for this value of $\hat{\alpha}$, $f(b)$ is greatest in cells furthest from the wound bed but never attains its maximum since $f(b) \leqslant k_c$. The parameter values are $\hat{\alpha} = 10$, $d_c = 0.26\ \mathrm{L}^2\ \mathrm{min}^{-1}$, $k_c = 1.67 \times 10^{-4}\ \mathrm{min}^{-1}$, $A_0 = 3k_c/4$, $A_2 = 1600$. The other parameters are as in Fig. 1. The spatial variable x is in units of cell length $L = 0.01$ mm.

we allow to vary. Recall that after injury, KGF mRNA levels in the dermis reach up to 10 times the levels found in unwounded human skin [14].

4.1. Numerical solutions of the extended model

Fig. 5 shows a simulation of the extended model for the same parameter set as in Fig. 1. The domain now incorporates the wound region: the left boundary represents the centre of the wound, $x = 0$ is the position of the wound edge, and to the right of this is the region where the basal cells were left intact when the wound was created. The wound region in Fig. 5 has a radius of 2 mm. The profiles of each variable are displayed at 5-day intervals over a period of 25 days, including the value of the function $f(b)$ which regulates the proliferation rate of the cell population. As time evolves, the keratinocytes migrate and proliferate to regenerate a basal layer over the wound region and thus provide a primary cutaneous cover. Indeed, after 20 days the density of the cells in the centre of the wound bed is already half the unwounded equilibrium level. The dermal

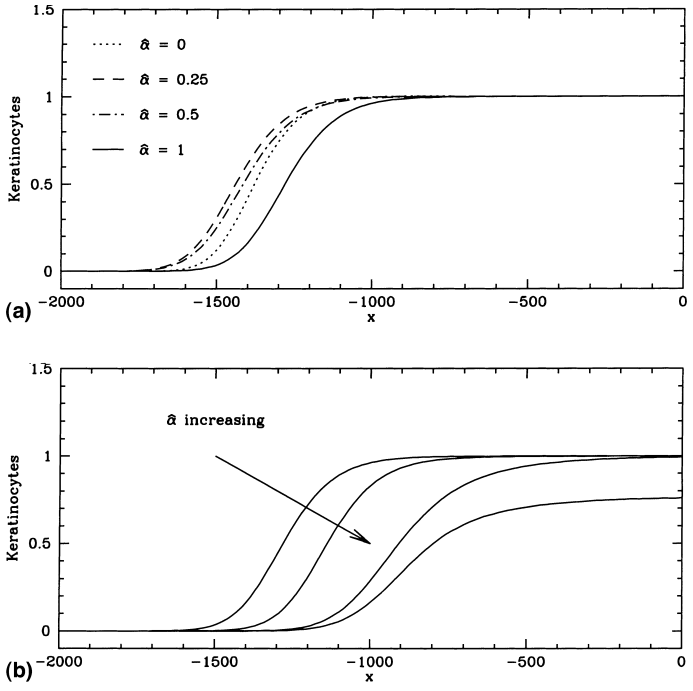

Fig. 6. The effects of different levels of KGF production after injury on the speed and quality of wound healing. In (a) we consider values of $\hat{\alpha}$ in the interval [0,1], simulations for $\hat{\alpha} \geqslant 1$ are presented in (b). The solid line in (a) is the keratinocyte profile when $\hat{\alpha} = 1$, or when production of KGF in the wound is such that the unwounded and wounded levels of KGF are the same. We observe that the optimal dose of KGF for the quickest rate of healing corresponds to $\hat{\alpha} \approx 0.25$. Graph (b) shows the advance of the keratinocyte population for $\hat{\alpha} = 1, 2, 10, 100$. As $\hat{\alpha}$ increases above 1 the speed of healing decreases, and for some $\hat{\alpha}$ above 10, the unwounded density of basal cells also falls; steady-state analysis shows that there is a bifurcation at $\hat{\alpha} \approx 20$. This implies that both the rate and quality of re-epithelialisation are impaired if too much KGF is produced or added after injury. The profiles are displayed at $t = 2000$ h. The other parameters are as in Fig. 5.

concentration of KGF increases towards the centre of the wound in this case, since $\widehat{\alpha} > 1$ so that $s_w > s_{norm}$. The same profile emerges for epidermal KGF concentration and the number of bound receptors per cell. Thus for these parameters, $b \geqslant b_{norm} > A_2$, implying that $f(b)$ never attains its maximum, which suggests that the level of KGF induced after wounding in this simulation is far from optimal.

We wish to investigate how the level of KGF produced after injury affects the speed and quality of epidermal wound healing. This can be achieved in our simulations by changing $\widehat{\alpha}$, the parameter which regulates the wounded level of KGF in the dermis. We begin by increasing the length of the wound domain so that we are able to observe fully developed travelling wave solutions of the keratinocyte density. This facilitates a comparison of the solutions for different values of the parameter $\widehat{\alpha}$, as illustrated in Fig. 6. We observe that a value of $\widehat{\alpha}$ between 0 and 0.5 gives the quickest rate of healing. This implies that the level of KGF induced on injury should be less than the level normally found in dermal tissue in order to maximise the healing rate. Moreover, if the amount of KGF produced or added after injury exceeds a certain value – our results suggest between 10 and 100 times the normal level – then the regenerated epidermis will be

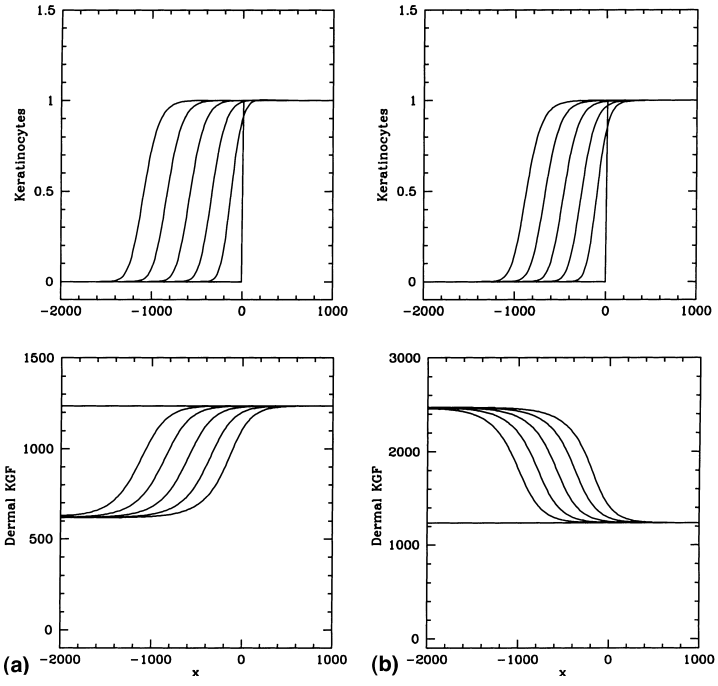

Fig. 7. Travelling wave profiles of keratinocyte density and dermal KGF concentration for two different values of $\widehat{\alpha}$, the parameter that controls the wounded level of KGF in the dermis. In (a) $\widehat{\alpha} = 0.5$, so that the unwounded steady state is twice the wounded steady state, illustrating that for $\widehat{\alpha} < 1$, the wave of dermal KGF concentration has a negative decay rate. This rate is positive when $\widehat{\alpha} > 1$, as shown in (b) for $\widehat{\alpha} = 2$. We observe that in both cases the leading tail of cell density decays more rapidly than that of the KGF concentration. Numerical investigation shows that despite this difference both profiles decay exponentially and move with the same speed. The profiles are displayed at regular time intervals from $t = 0$. The other parameters are as in Fig. 5.

less dense and therefore of poorer quality than the original tissue. Indeed steady-state analysis shows that there is a bifurcation at $\widehat{\alpha} \approx 20$ with the appearance of another stable equilibrium where cell density is less than the normal level, c_0.

4.2. On the speed of re-epithelialisation

In this section, we make some brief observations on how the concentration of KGF secreted or added after injury affects the speed of healing in the epidermis. We previously demonstrated in Fig. 6 that the rate of re-epithelialisation can be increased by adjusting the KGF concentration in the wound bed. Here, we discuss an analytic approximation for the minimum speed of healing and its dependence on model parameters, as compared to the speed obtained from numerical simulations.

In the extended model, the travelling wave profiles of the variables e, r and b are very similar to the form of s. We therefore consider only the profiles of cell density, c, and dermal KGF concentration, s, in more detail. Fig. 7 shows numerical solutions of the model equations (9a)–(9e) for two different values of $\widehat{\alpha}$, the parameter controlling the level of KGF in the wound region. The profiles illustrated are for cell density and dermal KGF concentration. If $\widehat{\alpha} < 1$, so that the unwounded steady state, s_{norm}, is greater than the wounded steady state, s_{w}, then the decay rate of the variable s is positive. Conversely in Fig. 7(b), in which $\widehat{\alpha} > 1$, the decay rate is negative. We also observe that in both cases the travelling wave of keratinocyte density decays more rapidly than that of the dermal KGF concentration.

This prompted us to investigate the speed and the decay rate of the travelling waves numerically. We found that, despite the difference between the decay rates, the waves of cell density and KGF concentration are moving at the same speed. It was then natural, before conducting any analysis, to examine the type of decay in the leading tails; numerical calculations confirmed that, in both cases, the decay is exponential rather than algebraic, but with different decay rates for the two variables. Based on these numerical observations, we apply a travelling wave transformation of the form $z = x + \omega t$, where ω is the speed in the negative x direction, to the model equations (9a)–(9d) so that they become a system of ordinary differential equations in z. If we then linearise about the equilibrium ahead of the wave, i.e. the wounded steady state $c = 0$, $s = s_{\text{w}}$, $e = e_{\text{w}}$, $r = r_{\text{w}}$, $b = b_{\text{w}}$, we arrive at the following system of differential equations governing the linearised variables, denoted by $\tilde{c}$ etc.:

$$\omega \frac{\mathrm{d}\tilde{c}}{\mathrm{d}z} = D_{\text{c}} \frac{\mathrm{d}^2\tilde{c}}{\mathrm{d}z^2} + (2f(b_{\text{w}}) - k_c)\tilde{c}, \tag{11a}$$

$$\omega \frac{\mathrm{d}\tilde{s}}{\mathrm{d}z} = D_s \frac{\mathrm{d}^2\tilde{s}}{\mathrm{d}z^2} + (P_s(b_{\text{w}}) - K)\tilde{c} - (d_l + k_s)\tilde{s} + d_l\tilde{e}, \tag{11b}$$

$$\omega \frac{\mathrm{d}\tilde{e}}{\mathrm{d}z} = (k_d b_{\text{w}} - k_a e_{\text{w}} r_{\text{w}})\tilde{c} + d_l\tilde{s} - (d_l + k_e)\tilde{e}, \tag{11c}$$

$$\omega \frac{\mathrm{d}\tilde{r}}{\mathrm{d}z} = -k_a r_{\text{w}}\tilde{e} - (k_a e_{\text{w}} + k_r)\tilde{r} + (P_r'(b_{\text{w}}) + k_d)\tilde{b}, \tag{11d}$$

$$\omega \frac{\mathrm{d}\tilde{b}}{\mathrm{d}z} = k_a r_{\text{w}}\tilde{e} + k_a e_{\text{w}}\tilde{r} - (k_d + k_i)\tilde{b}. \tag{11e}$$

The equation for $\tilde{c}$ is decoupled from the rest of the system; b_w may be found by solving the equations of the full model (9c)–(9e) at the steady state, given $c = 0$ and $s = s_w$. By analogy with the Fisher equation, a condition on the minimum wave speed may be obtained: if we substitute the solution form $\tilde{c} \propto e^{\lambda_c z}$ into the linearised ordinary differential equation for c (11a) we derive a relationship between the decay rate of keratinocyte density, λ_c, and the wave speed, ω

$$D_c\lambda_c^2 - \omega\lambda_c + (2f(b_w) - k_c) = 0. \tag{12}$$

In order to have real eigenvalues, λ_c, the following inequality must therefore hold:

$$\omega \geqslant 2\sqrt{D_c(2f(b_w) - k_c)}. \tag{13}$$

Any further conditions on the travelling wave speed may be obtained by considering equations (11b) and (11c) in more detail. These are in fact decoupled from the linearised equations for r and b. By substituting the solution form, $\tilde{c} = \bar{c}e^{\lambda_c z}$, where $\bar{c}$ is a constant and λ_c satisfies (12), into (11b) and (11c) and eliminating $\tilde{e}$, we therefore obtain a third-order ordinary differential equation for $\tilde{s}$. Since this is linear, we can write down the explicit solution of $\tilde{s}$ in terms of its complementary function and particular integral, where the particular integral is proportional to $\tilde{c}$. For our set of parameters, the decay rate of c is much greater than s, so we expect the complementary function to dominate the solution of $\tilde{s}$ as $z \to -\infty$. The eigenvalues, λ_s, of the complementary function must satisfy the following cubic polynomial, relating them to the wave speed, ω:

$$D_s\omega\lambda_s^3 + (D_s(d_l + k_e) - \omega^2)\lambda_s^2 - (2d_l + k_e + k_s)\omega\lambda_s - k_s(d_l + k_e) - d_l k_e = 0. \tag{14}$$

For the range of speeds that we observe numerically, $\omega \in (0, 0.02)$, Eq. (14) has one positive and two negative real roots. The decay rates that we observe in the numerical simulations for $\widehat{\alpha} < 1$ and $\widehat{\alpha} > 1$ correspond to the positive and smallest negative roots, respectively. These can be approximated by looking for solutions for λ_s as a power series in ω; giving

$$\lambda_s = \pm\sqrt{\frac{1}{D_s}\left(k_s + \frac{d_l k_e}{d_l + k_e}\right)} + \frac{1}{2D_s}\left(1 + \frac{d_l^2}{(d_l + k_e)^2}\right)\omega + \mathrm{O}(\omega^2). \tag{15}$$

For the parameter values used in our numerical simulations (see Fig. 1) the coefficients are evaluated below

$$\lambda_s = \pm 0.0042 + 0.0017\omega + \cdots.$$

The first coefficient agrees very well with the decay rate observed in the numerical simulations. Thus, the decay rate of s imposes no further conditions on the wave speed, and based on experience with the Fisher equation we anticipate that the waves will travel at the minimum speed allowed by the inequality (13).

In Fig. 8 we plot the minimum speed given by (13) for $0 \leqslant \widehat{\alpha} \leqslant 5$, since b_w depends on $s_w = \widehat{\alpha}s_{\mathrm{norm}}$, along with values obtained numerically. Using the analytical estimate, the value of $\widehat{\alpha}$ that optimises the speed of healing is 0.389, and from numerical investigation this seems to be close to the actual optimum. This result implies that the wounded level of dermal KGF should be approximately 40% of the unwounded levels that we have assumed. Overall, we can see that the minimum speed is in good agreement with the numerical results. The numerical values that lie below the analytical estimate for large $\widehat{\alpha}$ can be explained as transients in the calculations, where

the speeds measured in the numerical simulations have not yet converged sufficiently close to their limit. However, the discrepancies for small $\widehat{\alpha}$, for which the approximation underestimates the speed observed in the numerical simulations, cannot be attributed to transients and require further discussion.

A notable feature of the minimum wave speed is that it does not depend on D_s, the diffusion coefficient of the dermal KGF concentration. We know from the work by Hosono on travelling waves for a diffusive Lotka–Volterra competition model that as the ratio of the diffusion coefficients increases in that system, solutions begin to move at speeds greater than the minimum possible value [46]. The ratio of $D_c : D_s$ in our model is indeed very high, of the order of 1000:1. Thus, we hypothesise that the same phenomenon is occurring in our case, with waves moving above the minimum speed because of the large difference in diffusion coefficients. Moreover, numerical investigation has shown us that as we reduce D_s the wave speeds observed numerically approach the curve of the minimum speed in Fig. 8.

As an aside, steady state analysis shows that there is a bifurcation at $\widehat{\alpha} = 0$ (i.e. $K = 0$). Instead of there being just two steady states, corresponding to the wounded and unwounded equilibria, there is a third where $c = 2/3c_0$, $s = e = b = 0$ and $r = r_0$. It is therefore possible for there to be

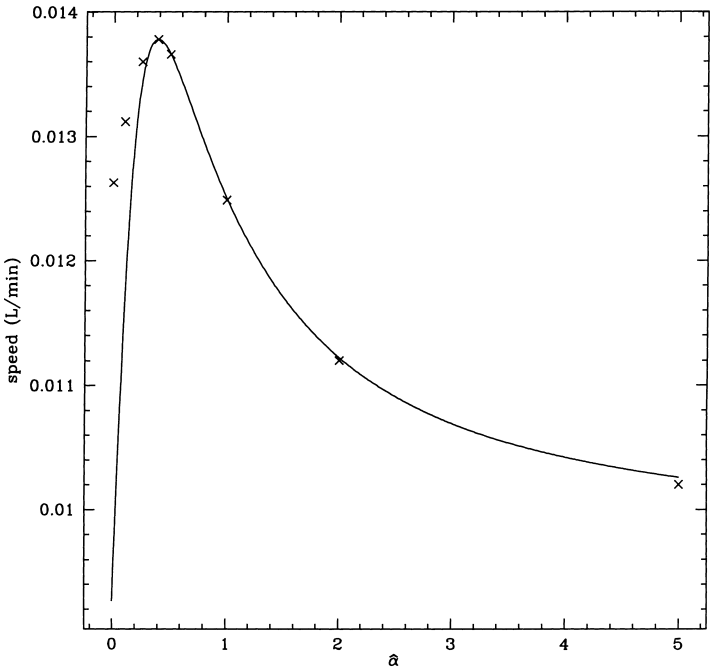

Fig. 8. Graph showing an analytical estimate (13) of the wave speed for $\widehat{\alpha} \in [0, 5]$ (—) compared to numerically obtained values (×). For most values of $\widehat{\alpha}$ the estimate is in good agreement with numerical results. The numerical values that lie below the minimum speed can be explained as transients in the calculations, where the speeds measured in the numerical simulations have not yet converged sufficiently close to their limit. However, for $\widehat{\alpha}$ close to zero, the speed observed numerically is much greater than that given by the estimate, and further investigation shows that this may be attributed to the large ratio of the diffusion coefficients, $D_c : D_s$. For the numerical results, the values of $\widehat{\alpha}$ plotted are 0, 0.1, 0.25, 0.389, 0.5, 1, 2, and 5 and the other parameters are as in Fig. 5.

two different travelling waves of cell density that both decay to the unwounded steady state: the one that we observe in the numerical simulations and the other that we would obtain if there was no KGF present in normal skin. For the latter, the variables s, e, r and b remain constant and thus play no role in determining the speed of the wave. Therefore the analytical approximation given above by (13) predicts the speed of this wave but not the speed of the wave corresponding to $K = 0$ in our numerical simulations.

5. Discussion

There is a great deal of interest in the benefits of applying exogenous growth factors to skin wounds to stimulate cell proliferation and improve the repair process. KGF is a potent mitogen for epithelial cells in vitro, and coupled with its unique signalling pathway in vivo it is a candidate for therapeutic application. In this paper we have assumed a model framework with which to study KGF signalling in human skin and considered how KGF's unique properties may play a role in the dermal–epidermal interaction during wound healing. Our first model demonstrates that by adding KGF to the wound bed we can extend the signalling range of KGF. Moreover, we have shown that the amplification of this signal is regulated by the production of KGF in normal dermal tissue via positive feedback from the epidermis. This last point is significant since it distinguishes KGF from other growth factors which are only released into the dermis post-wounding. We then extended the model to consider how production of KGF after injury regulates keratinocyte proliferation during wound closure. The results of this modelling show that the effect of additional KGF on cell mitosis in the epidermis is, surprisingly, suboptimal for the speed of re-epithelialisation. Our main conclusion is thus the large up-regulation of KGF that is observed experimentally in the wound bed extends the signalling range of the growth factor but reduces the rate of epidermal regeneration.

The outcome of our modelling is based on biological data and some assumptions that require further discussion. Indeed, there are three different sources of information for the optimal concentration of KGF for epidermal cell proliferation and all of these are in vitro studies: one on mouse [7] and two on human cultured keratinocytes [9,47]. We have taken the middle value of the three [9], but they all show that KGF has a significant effect on cell proliferation at low concentrations, even at 0.1 nM. The other important parameters that we have assumed are the endogenous levels of receptors on the surface of basal keratinocytes. We are unaware of any quantitative data for these levels but varying the parameters r_0 (the background level of free receptors), r_{norm} and b_{norm} (the unwounded steady-state levels of free and bound receptors) in the first model gives qualitatively the same solution as that in Fig. 1. In the second model, where we included cell density, the optimal level of KGF in the wound bed is less than the level of KGF in unwounded skin for a large range of the parameters r_{norm} and b_{norm}, and does not depend directly on r_0. Indeed, b_{norm} must be an order of magnitude smaller than r_{norm} before up-regulation of KGF in the wound bed would be beneficial to re-epithelialisation in our model.

There are animal studies that have reported improved wound healing after the application of exogenous KGF. Pierce et al. [18] observed an increased rate of re-epithelialisation and increased thickness of new epithelium in KGF-treated partial thickness wounds in the rabbit ear. In a study by Staiano-Coico et al. [17], KGF was also shown to enhance re-epithelialisation in porcine

partial thickness wounds. However, when the experiments were performed on full thickness wounds the indirect effects of KGF on collagen deposition in the dermis and the strengthening of the basement membrane junction were more marked than an increase in the speed of healing. Moreover, in the case of severe tissue damage in porcine skin burns, the rate of re-epithelialisation was not induced by a clinically significant level [48]. Recent research has also identified that KGF exhibits a protective effect on various cells of epithelial origin. In particular, studies on injury induced by chemotherapy and radiotherapy in the mouse intestine have demonstrated the benefits of pretreatment with KGF [49,50]. Furthermore, Frank et al. [51] have identified an enzyme that is up-regulated by KGF, and which may play a part in protecting keratinocytes from the oxidative burst that is generated by leukocytes after injury. Therefore, it would be necessary to investigate whether there is a trade-off between KGF's established function as a growth factor and its protective role in wound healing.

The key prediction that arises from our modelling is that if normal levels of KGF are above the optimal level for cell proliferation, and thus any induction upon injury is also suboptimal, then perhaps other properties of KGF are more important to the skin during wound healing; in particular, the signalling range of KGF is extended by up-regulation of the growth factor in the wound bed. The model we have presented in this paper focuses on what are thought to be the main properties of KGF signalling: its dermal–epidermal interaction and mitogenic role. Of course, there are extensions that could be made to the model to incorporate a more realistic situation. Specifically, we have neglected KGF's effect on cell differentiation to concentrate on the simple one-dimensional situation of basal keratinocyte cells. However, there are also proliferating cells in the suprabasal stratum and KGF's effect on differentiation is known to distinguish it from epidermal growth factor [9]. In addition, a model incorporating the interplay between other growth factors would lead to a better understanding of the stimulation of epidermal cell proliferation during wound healing. These extensions would enable investigation of the relative importance of KGF's effects on the overall repair process.

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