Multi-scale modeling of a wound-healing cell migration assay

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Abstract

A continuum model and a discrete model are developed to capture the population-scale and cell-scale behavior in a wound-healing cell migration assay created from a scrape wound in a confluent cell monolayer. During wound closure, the cell population forms a sustained traveling wave, with close contact between cells behind the wavefront. Cells exhibit contact inhibition of migration and contact-limited proliferation. The continuum model includes the two dominant mechanisms and characteristics of cell migration and proliferation, using a cell diffusivity function that decreases with cell density and a logistic proliferative growth term. The discrete model arises naturally from the continuum model. Individual cells are simulated as continuous-time random walkers with nearest-neighbor transitions, together with a birth/death process. The migration and proliferation parameters are determined by analysing individual mice 3T3 fibroblast cell trajectories obtained during the development of a confluent cell monolayer and in a wound healing assay. The population-scale model successfully predicts the shape and speed of the traveling wave, while the discrete model is also successful in capturing the contact inhibition of migration effects.

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1. Introduction

Cell invasion is central to normal and pathological biological phenomena. Dermal wound healing, angiogenesis and tumor invasion are typical examples of cell invasion. Cell migration and proliferation are the two key cell functions responsible for cell invasion. Tissue engineering, whereby healthy tissue is cultured to repair damaged or missing tissue, depends on successful invasion of cells on a biodegradable scaffold.

The wound-healing cell migration assay provides a valuable in vitro tool for studying the combined processes of cell migration and proliferation and the role of cell interaction during these processes. The experimental procedure involves the incubation of cells to near confluence and the creation of an artificial wound empty of cells.

As cells invade the wound site, the cell population typically exhibits traveling wave-like behavior. This is true for a variety of cell types studied in vitro: examples include human peritoneal mesothelial cells (Maini et al., 2004a,b), endothelial cells (Takamizawa et al., 1997), airway epithelial cells (Savla et al., 2004; Zahm et al., 1997), fibroblasts (Boucher et al., 1998), and keratinocytes (Ura et al., 2004). Over time the cells fill the wound. Proliferation is density limited, as the cell population cannot grow without bound. The formation of a cell monolayer during the process of wound closure is typical of the cell types discussed in the literature.

Cells in a wound-healing scrape assay maintain close contact with each other and their motility has been observed to decrease with increasing local density by Zahm et al. (1997), Tremel (2006) and Tremel et al. (2006). This phenomenon is called contact inhibition of cell locomotion, first proposed by Abercrombie to describe contact between crawling cells. Cell collisions often cause cells to reorientate and move in a new direction (Abercrombie, 1980).

Several mathematical descriptions of the wound-healing assay are based on the Fisher equation. This equation describes the cell density, where the cell motility is prescribed by a diffusive flux, where the diffusivity is a constant.
(Maini et al., 2004a,b; Savla et al., 2004; Takamizawa et al., 1997), or the diffusivity is dependent on an external chemical factor (Dale et al., 1994). The Fisher equation has traveling wave solutions that move with a constant speed dependent on the diffusion constant, which is a measure of the cell’s random motility, and the cell proliferation rate, which is related to the cell doubling time. Maini et al. (2004a,b) experimentally measured the position of the wound wavefront with time and established that it moved at approximately a constant speed. They determined the wave speed for scrape wounds on different substrates. Analytical results from the Fisher equation then provided a simple relationship between the random motility and the doubling time of the cell. The details of the cell density behind the wavefront were not investigated. In contrast, Takamizawa et al. (1997) calculated numerical solutions to the Fisher equation with different values of the diffusivity and proliferation rate parameters and compared this to experimental data of cell density within the wound. A trial and error method was used to estimate the two parameters that gave the best fit to the data. Savla et al. (2004) slightly modified the Fisher equation to account for an initial time period when the cells spread out. For this work, the best fit parameter values between the numerical solutions and experimental data were obtained with a nonlinear least-squares algorithm. Dale et al. (1994) used a cell diffusivity that was a function of an external chemical factor and only compared the traveling wave speed with the observed speed in corneal epithelial wound healing.

These previous models do not include the observed effects of contact inhibition of migration in the wound-healing assay. With the availability of individual cell trajectory data, the contact inhibition effect can be quantitatively determined. As we demonstrate, this effect is in fact significant and should be included when modeling individual cell behavior. Increasingly contact inhibited cell motility is being modeled in other contexts, such as interacting cell populations (Painter and Sherratt, 2003; Sherratt, 2000), the migration of neurons (Cai et al., 2006) and tumor cells (Sherratt and Chaplain, 2001).

We present a multi-scale modeling approach to analyze the population-scale behavior and the individual cell-scale behavior in a wound-healing assay. We develop a general continuum model of invasion, which includes contact inhibition of migration. A discrete model, giving rise to individual-based simulations, is derived from the continuum model, using master equations similar to those of Anderson and Chaplain (1998), Othmer and Stevens (1997) and Painter and Sherratt (2003). The cell density profiles and the wound-healing speed are population-level behavior, which can be determined through continuum models. Individual cell behavior is reproduced with discrete simulations derived from the continuum models. Such simulations provide details of the significant differences in individual behavior in different regions of the invasion wave.

We apply these models to a real experimental system provided by Tremel (2006) and Tremel et al. (2006) using mice 3T3 fibroblast cells. Two experimental systems are investigated. The first is the development of a confluent cell monolayer and the second is the closure of a scrape wound in a wound-healing assay. Estimates of the model parameters are determined from time-lapse images of cell number distribution and individual cell trajectories. Model, simulation and experimental results are compared and are shown to match well. This approach is a significant extension to the previous work using the Fisher equation as a basis for modeling the wound-healing assay.

2. Theoretical models

2.1. Continuum cell population model

Continuum models of the wound-healing assay often use the Fisher equation. This model accounts for constant diffusive migration in one spatial dimension and proliferation to a carrying capacity, described by logistic growth. The Fisher equation exhibits traveling wave solutions which move with a constant speed. As discussed in the Introduction, previous studies (Maini et al., 2004a,b; Savla et al., 2004; Takamizawa et al., 1997) used the Fisher equation to match experimental observations of either the wave speed or the cell density distribution.

Here we generalize the Fisher equation to include the effects of cell density dependent diffusivity. The evolution of the cell density $\hat{u}(x,t)$, at position $x$ and time $t$, is modeled with a diffusion equation with logistic growth as

$$\frac{\partial \hat{u}}{\partial t} = D_0 \frac{\partial}{\partial x} \left( D(\hat{u}) \frac{\partial \hat{u}}{\partial x} \right) + x\hat{u} \left( 1 - \frac{\hat{u}}{\hat{u}_*} \right),$$

(1)

where $D_0$ is the diffusivity of an isolated cell, so that the (dimensionless) diffusivity function $D(\hat{u}/\hat{u}_*) \equiv 1$ as $\hat{u} \to 0$. The standard Fisher equation corresponds to constant diffusivity, where $D(\hat{u}/\hat{u}_*) \equiv 1$ for all values of $\hat{u}$. Logistic growth is a model of proliferation which includes crowding effects by reducing the growth rate as the density approaches the carrying capacity, or threshold value, $\hat{u}_*$. Here $x$ is the mitotic index, which corresponds to the proliferation rate of a cell in an uncrowded environment.

Note that, in the usual way, we have simplified the two-dimensional spatial geometry of a cell monolayer to only one spatial dimension, by effectively averaging the cell density (cell number per unit area) over the second dimension to give us the variable $\hat{u}$ (cell number per unit length).

For convenience we introduce dimensionless variables for cell density and the space and time variables as

$$u = \frac{\hat{u}}{\hat{u}_*}, \quad \tau = x t, \quad \xi = x \sqrt{\frac{x}{D_0}}.$$

(2)
Then the dimensionless generalization of the Fisher equation (1) becomes

$$\frac{\partial u}{\partial \tau} = \frac{\partial}{\partial \xi} \left( D(u) \frac{\partial u}{\partial \xi} \right) + u(1 - u).$$

(3)

We now discuss the dimensionless diffusivity function. For contact inhibition of locomotion, increasing cell density restricts the movement of the cells and, therefore decreases the diffusivity. Thus, we require a functional form which satisfies $D(0) = 1$ and $D(u) < 0$. A function that fits these requirements is

$$D(u) = \frac{A}{A + u^2},$$

(4)

where $A$ is a measure of contact inhibition. This function was used by Cai et al. (2006) to model the contact inhibition of neurons migrating from a dense cluster. Of course, other monotonically decreasing functional forms could be used to model contact inhibition. For the purpose of this paper we will consider Eq. (4); it is smooth and it introduces only one fitting parameter.

### 2.1.1. Minimum wave speed of traveling wave solutions

The constant diffusivity of Fisher equation supports traveling wave solutions with a minimum wave speed. To determine the minimum wave speed of traveling wave solutions to the contact inhibition model, we follow the standard phase plane analysis used for the Fisher equation (Murray, 2002). Let $u(z, \tau) = u(z)$, where $z$ is the traveling wave coordinate $z = \xi - \bar{c} \tau$ and $\bar{c}$ is the scaled wave speed. The nonlinear diffusion model has two critical points, $u = 0$ and $u = 1$. By restricting the eigenvalues of $u = 0$ critical point to be strictly real, and since $D(0) = 1$, we obtain the same expression for the minimum wave speed as obtained for the Fisher equation, namely

$$\bar{c} \geq \bar{c}_{\text{min}} = 2.$$

(5)

We note that imaginary eigenvalues give oscillations about $u = 0$, hence giving negative densities that are not physical. The eigenvalues of $u = 0$ are negative and so $u = 0$ is a stable node. Stability analysis about $u = 1$ shows it is a saddle point.

It is well known that the wave speed of numerically generated traveling waves to the Fisher equation depends on the spatial rate of decay of the initial condition. In wound-healing assay experiments, a scrape across the cells produces a sharp-fronted wound. Therefore, the appropriate initial condition has compact support. Such an initial condition evolves to a traveling wave with the minimum wave speed (Murray, 2002).

### 2.1.2. Asymptotic solutions

Following the approach of Canosa (1973), approximate solutions to the shape of traveling wave solutions can be determined (Simpson et al., 2006) as

$$u(z) = u_0(z) + \frac{1}{\bar{c}^2} u_1(z) + O\left( \frac{1}{\bar{c}^3} \right).$$

(6)

where

$$u_0(z) = \frac{1}{1 + e^{z/\bar{c}}},$$

(7)

and $u_1(z)$ is given in the Appendix. Since the function $u_0(z)$ is independent of $A$, the first terms of the asymptotic solution of both the modified Fisher equation and the Fisher equation are the same. The function $u_0(z)$ is a useful approximation to the traveling wave solutions of the contact inhibition model, as demonstrated by the excellence of the fit in Fig. 1.

To summarize, the Fisher equation and the modified Fisher equation that accounts for contact inhibition are similar at the continuum level. They both exhibit traveling wave solutions that have the same minimum wave speed and the same dominant term in their asymptotic solutions. Hence, both models are equally suitable to model the population-level behavior of the cells. However, during the period of time before the cell population has established the traveling wave, the transient behavior of the cell population is dependent on contact inhibition effects. The contact inhibition model takes longer to establish a traveling wave compared to the Fisher equation with the same initial condition, due to the reduced diffusivity. We have compared the length of time for the two models to establish a traveling wave. This establishment time increases as the contact inhibition parameter $A$ decreases (recalling that $A \to \infty$ gives the Fisher equation). However, discerning such differences in the population-level behavior during an experiment will be difficult.

An alternative more revealing method to examine the differences between the two models is considered next.

### 2.2. Discrete individual cell model

We describe a general treatment of reaction diffusion continuum equations leading to continuous-time discrete-space random walk expressions that can be used to simulate individual cells. This treatment provides a direct correspondence between the continuum and discrete models. An alternative approach is to write down a discrete model for individual cell motion at the outset. Zygourakis and coworkers (Cheng et al., 2006; Lee et al., 1995) have developed discrete models based on cellular automata, where various rules of cell locomotion and division are proposed. However, when cell interaction rules are non-trivial, the extraction of population-level continuum equations is difficult (Liggett, 1985; Turner et al., 2005).

The decomposition of general diffusion equations to continuous-time master equations is well studied (Othmer and Stevens, 1997; Painter and Sherratt, 2003). The transition probabilities of individuals between lattice sites
gives the interaction between neighboring individuals and the interaction between individuals and any chemical signals present in their environment. A similar procedure, although in discrete time, has been used to model and simulate the response of neurons to a signaling molecule (Cai et al., 2006) and the growth of blood vessels (Anderson and Chaplain, 1998).

Simulations of nonlinear diffusion reaction equations are generally discrete in space and time. For example, a modified Fisher equation has been simulated with the diffusion term modeling the random modeling walk behavior of each individual, the convection term modeling the bias of the random walk, and the logistic growth term modeling the birth and death processes (Scho¨nborn et al., 1994). The individual cells make nearest-neighbor transitions only. The Fisher equation has also been simulated using a particle method, where the particles are not restricted to make nearest-neighbor transitions and each particle has a mass associated with it (Tompson and Dougherty, 1992). The logistic term was integrated to find the cell density at the next time step; the change in net mass was implemented by adjusting the particle number or the particle masses. A limitation of discrete time simulation is the use of a fixed order of operations at each time step, for example, convection then diffusion followed by reaction (Scho¨nborn et al., 1994). This is equivalent to the operator split method used to solve partial differential equations (Simpson and Landman, 2006; Tompson and Dougherty, 1992).

To avoid this complication, we consider events to take place in continuous time and follow previous approaches (Othmer and Stevens, 1997; Painter and Sherratt, 2003). Individual cells are treated as continuous-time nearest-neighbor random walkers. Cell mortality is controlled by a birth and death process, which is derived from the logistic growth term.

When written in terms of a scaled cell density \[ u(x, t) = \frac{\hat{u}}{u^*} , \] Eq. (1) is a reaction diffusion equation of the general form

\[
\frac{\partial u}{\partial t} = D_0 \frac{\partial}{\partial x} \left( D(u) \frac{\partial u}{\partial x} \right) + F(u)u .
\] (8)

In our application, \( F(u) = x(1 - u) \), but in outlining the discrete modeling approach, we allow \( F(u) \) to be general.

We simulate the evolution of a population of individuals in continuous time based on the governing mechanisms, diffusion and reaction, by letting the individuals have internal clocks signaling when a particular event is to occur. The main events are transitions between sites, birth and death. We consider only nearest-neighbor transitions on a discrete lattice, \( x = i\Delta x \). Transitions are governed by the diffusivity \( D_0D(u) \), and the birth/death process is governed by \( F(u) \).
Let \( n_i(t) \) be the number of individuals at lattice site \( i \) at time \( t \) and let \( n^* \) be the maximum number of individuals at each lattice site (corresponding to the carrying capacity density \( u^* \)). Then we define a scaled number of individuals as

\[
u_i(t) = \frac{n_i(t)}{n^*}
\]

and relate this to the scaled cell density \( u \) and threshold density \( u^* \) by

\[
u_i(t) = \Delta x \, u(i\Delta x, t), \quad n^* = \Delta x \, u^*.
\]

First consider the motion of cells. We apply a centered differencing scheme to Eq. (8) to obtain a master equation of a continuous-time nearest-neighbor random walk process:

\[rac{d\nu_i}{dt} = T(i|i+1)\nu_{i+1} + T(i|i-1)\nu_{i-1} - (T(i+1|i) + T(i-1|i))\nu_i,
\]

where \( T(i|i) \) represents the transition rate of individuals from site \( j \) to \( i \). Here the nearest-neighbor transition rates are given by

\[T(i \pm 1|i) = D_0 \frac{D_{i+1/2}}{(\Delta x)^2} = T(i|i \pm 1),\]

where

\[D_{i+1/2} \approx \frac{D(u_{i+1}) + D(u_i)}{2}.
\]

To simulate individuals obeying this random walk process, let \( \lambda_\ell \) be the mean waiting time of a transition event (which includes remaining at the current site). At each transition event, let the individual have a probability \( p \) of making a transition to the left, probability \( q \) of making transition to the right, and probability \( r = 1 - p - q \) of remaining at the current site. The master equation becomes

\[rac{d\nu_i}{dt} = \frac{1}{\lambda_\ell}[pu_{i+1} + qu_{i-1} - (p + q)\nu_i].
\]

We scale the waiting time and the transition probabilities with a factor of two to ensure that \( p \) and \( q \) can be interpreted as probabilities and satisfy \( p + q \leq 1 \) (this is due to \( \max D(u) = 1 \)). Thus

\[
\frac{1}{\lambda_i} = 2 \frac{D_0}{(\Delta x)^2}, \quad p = \frac{D_{i+1/2}}{2}, \quad q = \frac{D_{i-1/2}}{2}, \quad r = 1 - p - q.
\]

Note that with this choice, as \( u \to 0 \), we obtain the usual definitions for an unbiased random walk that \( p = q = \frac{1}{2} \).

For birth and death events, we consider the reaction term in Eq. (8), as

\[
\frac{d\nu_i}{dt} = F(u_i)\nu_i,
\]

which is a rate equation governing the individuals at lattice site \( i \). The proliferation rate \( F(u) \) is related to the mean waiting time for a birth or death event \( \lambda_p \) by

\[
|F(u)| = \frac{1}{\lambda_p},
\]

There is a birth when \( F(u_i) > 0 \) and a new cell is placed at the same site as the original cell. If \( F(u_i) < 0 \), the event is treated as cell death or removal, and the cell is removed from the simulation.

### 2.2.1. Simulation process

The main data structures include: (i) an array representing the one-dimensional lattice with cells represented by nodes of doubly linked lists, (ii) a binary search tree with nodes referencing the cells on the lattice (to sort the order in which events occur), and (iii) a list used to gather the histories of the cells. Such data structures were chosen due to the highly dynamic nature of the simulations, where numerous insertions and deletions occur. Each cell is given a waiting time for its next event. From the two mean waiting times, \( \lambda_\ell \) for transition and \( \lambda_p \) for birth/death, we calculate the waiting time for each of these events. The actual waiting time is exponentially distributed (Hughes, 1995). The exponential distribution is a memoryless distribution. This means the time one waits for the next event to occur is independent of the time one has already waited. To maintain the one-to-one correspondence between the solution of the master equation and the solution of a continuous-time random walk process, the time between successive moves must be independent, identically distributed, exponential variables (Hughes, 1995). To obtain exponentially distributed random numbers \( f(X) \), we use

\[
f(X) = -\lambda \ln(1 - X),
\]

where \( X \) is a uniformly distributed random number between 0 and 1 and \( \lambda \) is either \( \lambda_\ell \) or \( \lambda_p \). This generates the time the cell must wait for the corresponding event. The event with the shorter waiting time is assigned to the cell as its next event. The cell is then inserted in the order of waiting times into the events search tree. The top cell of the search tree is deleted and the event for that cell is carried out. After the event, the cell is assigned a new waiting time for the next event and it is reinserted into the events search tree. After each event, the local density will change, except in cases where the cell is to remain at the same site. Consequently, all the cells that are affected by the event will have their waiting times reset.

### 3. Results and discussion

We apply the continuum and discrete models to an experimental system of Tremel (2006) and Tremel et al. (2006), using mice NIH 3T3 fibroblast cells. The first experiment investigates the development of a confluent cell monolayer. From estimates of the cell diffusivity at low and high density, an estimate of the strength of the contact inhibition effect is made. The resulting
functional form of the diffusivity is used in our model of the wound-healing assay, which is the second experimental system.

The cells were grown in tissue culture polystyrene (TCP) flasks and examined with an inverted microscope attached with a digital camera. Population density profiles and individual cell trajectories were obtained using the imaging analysis software ImagePro Plus, together with manual counting and tracking of cells.

3.1. Uniform density experiment

This experiment involves uniformly seeding the substrate with a small number of cells and allowing the cells to proliferate over time. The cell density remains reasonably uniform throughout the experiment, as shown in Fig. 2. A confluent cell monolayer evolves over time.

3.1.1. Estimation of model parameters from experimental data

The number of cells is counted at various times, giving density versus time data shown in Fig. 3. The carrying capacity is estimated from our experiments to be approximately \( \frac{1 \times 10^3}{\text{cells/}\mu\text{m}^2} \) and this value is used in all our models and simulations. By assuming uniform cell density, the spatial dependence in Eq. (3) can be ignored. The change in mean scaled cell density \( \langle u \rangle \) is then given by logistic growth as

\[
\frac{d \langle u \rangle}{dt} = \alpha \langle u \rangle (1 - \langle u \rangle) \tag{19}
\]

The solution is given by

\[
\langle u(t) \rangle = \frac{u_0}{e^{-\alpha t} + u_0(1 - e^{-\alpha t})} \tag{20}
\]

where the initial scaled density is \( u_0 = \langle u(0) \rangle \). Almost no cell division was observed for the first two days; hence the solution (20) is fitted to the data from the start of day three. The time-scale has been adjusted accordingly.

The best fit function is obtained with a mitotic index \( \alpha = 1.8 \times 10^{-3} \text{/min} \). Boundary effects can be ignored as the size of the cell flask is much greater than the camera field, which was far from the cell flask’s physical boundaries.

Several cells were tracked during the first 48 h when individual cells are predominantly isolated from each other and cell density is low. Another group of cells was tracked for the last 10 h when there is essentially a monolayer of confluent cells and the cell density is high. These cell trajectories are shown in Fig. 4. We tracked cells that did not undergo division during the tracking period. Cells undergoing division tend to decrease motility, break

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Fig. 2. Images of the uniform density experiment. All cell images presented in this paper were obtained using the Olympus IX-70 inverted microscope with a 4× objective lens. Images courtesy of A. Tremel, unpublished.

Fig. 3. The uniform density experiment. The scaled cell density data (points) fitted with the logistic solution (20) (solid line). Time \( t = 0 \) corresponds to the beginning of day three. Here \( u^* = 1 \times 10^3 \text{cells/}\mu\text{m}^2 \) and initial scaled density \( u_0 = 0.06 \), giving \( \alpha = 1.8 \times 10^{-3} \text{/min} \) for best fit.
adhesive contacts from the substrate and neighboring cells. The influence of the time delay caused by mitosis on the healing wave speed is investigated by Landman et al. (2006).

We introduce the appropriate statistics for analysing our trajectory data. We want to test whether we can distinguish any anisotropy or bias in the cell motion. For this analysis, the position of a given cell at time \( t \), relative to its position at time 0, is taken to be a random two-dimensional vector \( \mathbf{R}_t = (X_t, Y_t) \), where \( X_t \) and \( Y_t \) are Cartesian coordinates associated with the probability density function \( p(x, y, t) \), so that for any plane region \( \Omega \) the probability that the cell is found there at time \( t \) is

\[
\Pr((X_t, Y_t) \in \Omega) = \int_{\Omega} p(x, y, t) \, dx \, dy.
\]  

(21)

In choosing to model the system at this level, we have lost the ability to treat the swarm of motile cells as interacting stochastic motions. Instead we consider one tagged cell moving in a prescribed environment. We have already seen how we may infer rules to describe the motion of an individual cell given its environment (especially the abundance of cells at neighboring positions). In the uniform density experiment, the environment has only short-time-scale fluctuations in cell density, so the motion of a tagged cell is naturally modeled as a motion in a uniform environment, inviting the use of simple models based on the diffusion equation.

If the cells are undergoing homogeneous isotropic diffusion, then

\[
\frac{\partial p}{\partial t} = D_0 \nabla^2 p,
\]

(22)

and so with the appropriate initial condition \( p(x, y, 0) = \delta(x)\delta(y) \), we have

\[
p(x, y, t) = \frac{1}{4\pi D_0 t} \exp \left[ -\frac{(x^2 + y^2)}{4D_0 t} \right].
\]

(23)

The marginal probability density functions for Cartesian coordinates are

\[
p_1(x, t) = \int_{-\infty}^{\infty} p(x, y, t) \, dy, \quad p_2(y, t) = \int_{-\infty}^{\infty} p(x, y, t) \, dx.
\]

(24)

As the system is isotropic, it is natural to consider the probability density function for the radial displacement \( |\mathbf{R}_t| \). We denote this probability density function by \( \phi(r, t) \). From standard change of variables considerations we have

\[
\frac{\phi(r, t)}{2\pi r} = p(x, y, t),
\]

(25)

and from Eq. (23) we deduce the Rayleigh distribution

\[
\phi(r, t) = \frac{r}{2D_0 t} \exp \left[ -\frac{r^2}{4D_0 t} \right].
\]

(26)

We compute the mean values and other positive integer moments of the coordinates \( X_t, Y_t \) and \( |\mathbf{R}_t| \) in the standard way as

\[
\langle X_t^k \rangle = \int_{-\infty}^{\infty} x^k p_1(x, t) \, dx, \quad \langle Y_t^k \rangle = \int_{-\infty}^{\infty} y^k p_2(y, t) \, dy,
\]

(27)

\[
\langle |\mathbf{R}_t|^k \rangle = \int_0^\infty r^k \phi(r, t) \, dr.
\]

(28)

The following relationships are obtained:

\[
\langle X_t \rangle = 0, \quad \langle Y_t \rangle = 0, \quad \langle |\mathbf{R}_t|^2 \rangle = \pi D_0 t,
\]

(29)

\[
\langle X_t^2 \rangle = 2D_0 t, \quad \langle Y_t^2 \rangle = 2D_0 t, \quad \langle |\mathbf{R}_t|^4 \rangle = 4D_0 t.
\]

(30)

This gives us four ways to estimate the diffusivity \( D_0 \) from experimental observation of the trajectories of individual cells. The trajectory of one cell corresponds to one experimental realization of the diffusion process. For a given time interval \( 0 \leq t \leq T \) we may take a number of
experimental realizations and use these to compute estimates of \( \langle X_t^2 \rangle \), \( \langle Y_t^2 \rangle \), \( \langle |R_t|^2 \rangle \) and \( \langle |R_t|^2 \rangle \) for \( 0 \leq t \leq T \).

We use all four ways to estimate the diffusivity and compare the estimates. A large difference between the diffusivities obtained from \( \langle X_t^2 \rangle \) and \( \langle Y_t^2 \rangle \) will indicate the existence of some anisotropy in the cell trajectories. The standard model of homogeneous anisotropic diffusion (with the coordinates aligned with the anisotropy) has the probability density function \( p_A(x,y,t) \) governed by

\[
\frac{\partial p_A}{\partial t} = D_x \frac{\partial^2 p_A}{\partial x^2} + D_y \frac{\partial^2 p_A}{\partial y^2},
\]

where the unequal constants \( D_x \) and \( D_y \) represent the diffusivities in the two orthogonal coordinate directions. The corresponding probability density function initialized with \( p_A(x,y,0) = \delta(x)\delta(y) \) is

\[
p_A(x,y,t) = \frac{1}{4\pi t D_x D_y} \exp \left[ -\left( \frac{x^2}{4D_xt} + \frac{y^2}{4D_y t} \right) \right],
\]

with

\[
\langle X_t^2 \rangle = 2D_xt, \quad \langle Y_t^2 \rangle = 2D_y t.
\]

We also need to consider the possibility of a systematic drift in some direction and develop a test statistic to reveal its presence. The standard model for homogeneous isotropic diffusion with bias is provided by

\[
\frac{\partial p_B}{\partial t} = D_0 \nabla^2 p_B - \mathbf{v} \cdot \nabla p_B.
\]

Here \( \mathbf{v} = (v_x, v_y) \) is a constant drift velocity or bias. The fundamental solution of this equation can be expressed in terms of the fundamental solution (23) for unbiased isotropic diffusion via

\[
p_B(x,y,t) = p(x - v_xt, y - v_y t, t),
\]

and we have means and variances given by

\[
\langle X_t \rangle = v_xt, \quad \langle Y_t \rangle = v_y t, \quad \sigma_x^2 = \langle (X_t - \langle X_t \rangle)^2 \rangle = 2D_0 t, \quad \sigma_y^2 = \langle (Y_t - \langle Y_t \rangle)^2 \rangle = 2D_0 t.
\]

Each of the statistics used to infer a drift velocity or bias \( \mathbf{v} \), a diffusivity \( D_0 \) or anisotropic diffusivities \( D_x, D_y \) has predicted linear variation with time over the time interval \( 0 \leq t \leq T \) during which observations are recorded. The data is therefore analysed by constrained linear regression (with the regression line forced to pass through the origin). Ideally we want a large number of realizations \( N \) and a long time interval \( 0 \leq t \leq T \). However, we have only a small number of realizations of cell trajectories (5 at low cell density and 15 at high cell density). We increase the number of realizations by dividing the observed trajectories into smaller segments. Consequently, the number of paths increases, but the number of time points over a trajectory path decreases. The validity of this technique, which relies on independence between successive steps, is assessed below.

Estimates of the diffusivities and the biases of the trajectories obtained at early times at low cell density and at later times at high cell density are summarized in Tables 1 and 2 respectively. The tabulated values displayed under \( \langle X_t \rangle \) and \( \langle Y_t \rangle \) are the biases \( v_x \) and \( v_y \) determined from the gradient of a constrained linear regression through the origin. The values displayed under \( \langle X_t^2 \rangle \),

<table>
<thead>
<tr>
<th>Paths</th>
<th>Time points</th>
<th>Bias estimates (μm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \langle X_t \rangle )</td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>-0.127 0.96</td>
</tr>
<tr>
<td>29</td>
<td>20</td>
<td>-0.086 0.89</td>
</tr>
<tr>
<td>39</td>
<td>15</td>
<td>-0.084 0.96</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>-0.085 0.95</td>
</tr>
<tr>
<td>125</td>
<td>5</td>
<td>-0.080 0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paths</th>
<th>Time points</th>
<th>Diffusivity estimates (μm²/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \langle X_t^2 \rangle ) ( R_{adj}^2 )</td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>8.7 0.84 22 0.76 3.2 0.86 27 0.75 15 0.78 15 0.79</td>
</tr>
<tr>
<td>29</td>
<td>20</td>
<td>9.3 0.92 10 0.92 9.0 0.93 11 0.92 8.6 0.92 9.8 0.93</td>
</tr>
<tr>
<td>39</td>
<td>15</td>
<td>6.8 0.91 8.0 0.91 6.6 0.92 8.2 0.91 6.5 0.92 7.4 0.91</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>4.9 0.91 6.3 0.91 4.7 0.92 6.4 0.91 5.0 0.91 5.6 0.91</td>
</tr>
<tr>
<td>125</td>
<td>5</td>
<td>3.3 0.95 4.2 0.91 3.2 0.96 4.2 0.93 3.2 0.93 3.7 0.94</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>2.4 0.96 4.7 0.86 2.3 0.97 4.8 0.86 3.1 0.91 3.6 0.90</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>4.1 0.97 4.4 0.92 3.7 0.97 4.3 0.91 3.3 0.95 4.3 0.95</td>
</tr>
</tbody>
</table>
Table 2
Bias and diffusivity estimates from the uniform density experiment obtained from cell trajectories during the last 10 h of the experiment, when cell density is high. The time interval between time points is 10 min.

<table>
<thead>
<tr>
<th>Paths</th>
<th>Time points</th>
<th>Bias estimates (µm/min)</th>
<th>Diffusivity estimates (µm²/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(X̂ₙ)</td>
<td>R²ₐₐj</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>-0.021</td>
<td>0.87</td>
</tr>
<tr>
<td>45</td>
<td>20</td>
<td>-0.023</td>
<td>0.91</td>
</tr>
<tr>
<td>60</td>
<td>15</td>
<td>-0.016</td>
<td>-0.31</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>-0.017</td>
<td>0.77</td>
</tr>
<tr>
<td>180</td>
<td>5</td>
<td>-0.011</td>
<td>0.20</td>
</tr>
</tbody>
</table>

(Ŷₙ), σ̂₂ₙ, σ̂ᵢ, ⟨|Rᵢ|⟩, and ⟨⟨|Rᵢ|⟩⟩ are the estimated diffusivities obtained by the same method.

A measure of the goodness-of-fit is needed. Our constrained fitting is a linear regression without an intercept; consequently, there is one degree of freedom less than there is for a standard linear regression. The adjusted coefficient of determination, R²ₐₐj, is used to account for the constraint imposed on the linear regression (Devore and Peck, 2001). The adjusted coefficient of determination is defined in the following way. For a set of n data points (xᵢ, yᵢ), i = 1, 2, ..., n, the total sums of squares around the mean, Sₜ, and sums of squares of the residuals around the regression line, Sᵣ, are computed. The coefficient of determination R²ₐₐj is the normalized difference given by

\[ R²ₐₐj = 1 - \frac{Sᵣ}{Sₜ/n}, \]  

where

\[ Sᵣ = \sumᵢ (yᵢ, measured - yᵢ, fit)², \]

\[ Sₜ = \sumᵢ (yᵢ, measured)². \]  

Values of R²ₐₐj close to unity correspond to a good fit. Although R²ₐₐj is usually positive, the definition does allow R²ₐₐj to become negative when the fit is poor for small sample sizes (as in the top row of Table 1) and when the fit is poorer than the average value.

An alternative widely accepted model of cell motion is the persistent random walk. The diffusivity is estimated by the following relationship:

\[ ⟨⟨|Rᵢ|⟩⟩ = 4D₀(t - P[1 - e^{-t/P}]), \]

where P is the persistence time of the cells (Dunn, 1983; Gail and Boone, 1970; Othmer et al., 1988; Stokes et al., 1991). Using our data of cells at low density, we found that the nonlinear fit for the two parameters P and D₀ was sensitive to the sample size. Random samples of 50 paths from 125 paths with 5 time points produced estimates of diffusivity ranging from 5 to 8 µm²/min and persistence time from 7 to 17 min. Our estimation of diffusivity typically ranged from 3 to 5 µm²/min, as seen in Table 1. Stokes et al. (1991) analysed 10 and 8 microvessel endothelial cell trajectories per sample and found the presence of an atypical cell (one with speeds 2 standard deviations from the mean) greatly affected the value of the estimated diffusivity. In Stokes et al. (1991) and other studies by Dunn (1983) using eight chick fibroblasts and Gail and Boone (1970) using 20 mouse fibroblasts, it seems that the sample sizes are too small to obtain a reliable estimate for the persistence time. The discarding of observations that differ from the typical case according to some subjective criterion is undesirable from a statistical point of view. In our methodology we choose to increase the sample size by decomposing trajectories into smaller segments, rather than discounting cell trajectories. The estimation of diffusivity has stabilized at 5 time points, as shown by the final three rows of Table 1. The variation in the estimates between 125 paths and 50 paths is small. The 50 paths are randomly chosen from the 125 paths. We have tested for consistency with other randomly chosen sets.

At high densities, the cells are almost in constant contact with neighboring cells, hence a measure of cell persistence time is irrelevant.

To test for bias, we compare the slopes of the constrained regression lines for σ²₂ₙ, σ²ᵢ, ⟨X̂ₙ⟩, and ⟨Ŷₙ⟩. For a sufficiently large number of realizations of an unbiased isotropic diffusion process these slopes should be...
identical. For trajectories obtained during the first 48 h (Table 1), the bias effects are small, with little difference between the four diffusivity estimates. Possible anisotropic effects are observed if there is a significant difference between $X_i^2$ and $Y_i^2$. For this data, the differences between these values decreases as the number of paths increases, so the differences could be due to insufficient data rather than an actual anisotropic effect. For trajectories obtained during the last 10 h (Table 2), the bias effects are small, and the measured diffusivities from all four estimation methods correspond well. Consequently, the trajectories are considered unbiased and isotropic.

Overall, the diffusivity of the cells at low density is greater than the diffusivity at high density. This is consistent with our assumptions regarding modeling contact inhibition, including a diffusivity function that decreases with density.

Taking into consideration the compromise between the number of paths and the number of time points, the accuracy of the linear fits, and the errors from manually tracking cell trajectories, we estimate approximate values of the diffusivity to be $D_0 = 3 \mu m^2/min$ at low density and $D_0D(1) = 0.3 \mu m^2/min$ at high density. Substituting these values into Eq. (4) gives an estimate of the dimensionless contact inhibition parameter $A \approx 0.1$. In summary, the parameter values estimated from the uniform density experiments of Tremel (2006) and Tremel et al. (2006) are $D_0 = 3 \mu m^2/min$, $\alpha = 1.8 \times 10^{-3}/min$, $A = 0.1$. (41)

### 3.1.2. Simulations

All the main parameter values have now been estimated (Eq. (41)) for the uniform density experiment, allowing us to simulate the experiment. All simulations in this paper are carried out in one space dimension and in scaled coordinates $\xi$ and $\tau$ as given in Eq. (2).

First we simulate the uniform density experiment at early times when the cells are at low density and assume no contact inhibition and negligible proliferation. The variances of the displacements $\sigma^2$ of four simulations as a function of dimensionless time are presented in Fig. 5. The total time of each simulation corresponds to 24 h. The expected trend $\sigma^2 = 2\tau$ is plotted together with the simulation data. We observe that the fit to the expected linear trend improves as the number of cell trajectories increases. The estimated diffusivity converges to unity as expected (and the corresponding $R^2$ value also converges to unity) as the number of cells used in the simulation increases.

![Fig. 5](image-url)

**Fig. 5.** The variances of cell trajectories as a function of time obtained from simulating the uniform density experiment at early times. All variables are dimensionless. The cells are assumed isolated and non-proliferating. The expected variances of the cell trajectories, $\sigma^2 = 2\tau$ (dashed line), and the lines of best fit given by a constrained linear regression through the origin (solid line) are shown. The diffusivities are estimated by halving the slope of the line of best fit (solid line), giving $D(u)$ values of $1.9$ ($R_{adj}^2 = 0.94$), $0.89$ ($R_{adj}^2 = 0.89$), $0.94$ ($R_{adj}^2 = 0.99$) and $0.97$ ($R_{adj}^2 = 1.0$), when using 10, 50, 100, 200 cells in the simulations.
Next we simulate the cell population for 72 h with proliferation, using the estimated diffusivities appropriate for both the Fisher equation and the contact inhibition model. A lattice with 100 sites, with no flux boundary conditions, is used. The size of the lattice is chosen so that the effects of the boundary conditions are small. Fig. 6 shows the mean cell density over time compared to the logistic growth solution from three simulations with different site carrying capacities for both of the models. For both models, large site carrying capacity achieves a better fit of the mean values to the expected values given by the logistic solution (20), and the deviations about the mean also decrease with the increasing size of the population. The deviations about the mean are in general greater for the Fisher equation than in the simulations of the contact inhibition model due to the higher diffusivity of the cells. Fig. 7 illustrates the cell density fluctuations across the lattice site at different times in a simulation, using a carrying capacity of 100 cells per site. Again, the fluctuations are greater in the Fisher model.

Since the experiments demonstrated that the cells at low densities (early times) have a much larger diffusivity than cells at high densities (later times), providing evidence of contact inhibition, we must compare the diffusivities of the simulated cells at early time $0 \leq \tau \leq 1$ and at later time $6.8 \leq \tau \leq 7.8$. Once again, the diffusivity is estimated from the gradient of the variances $\sigma^2$ over time as $D = \sigma^2/2t$. The $\sigma^2$ values of the simulated cells, along with the constrained lines of best fit, are displayed in Fig. 8 for both the Fisher equation and the contact inhibition model.

As expected, the Fisher model simulations gives a constant estimate of the diffusivity (close to unity), for both early and later times, which is inconsistent with the experimental observations. In contrast, the results of the contact inhibition model are quite different and predict the experimental observations. For the early time data, the estimated diffusivity is approximately 0.5. With contact inhibition parameter estimate $A = 0.1$ from Eq. (41), this corresponds to a cell density values $u/\bar{C}^2 \approx 0.1$. As we are making the assumption that the density remains reasonably
uniform throughout this time period, we identify $u$ with $\langle u \rangle$. We calculated a mean value of $\langle u(t_1) \rangle \approx 0.1$ from the simulation, where $t_1 = 0, 0.05, \ldots, 1$. Hence, the estimates from the simulation are consistent. Similarly, for the later time data, the estimated diffusivity is approximately 0.1. This corresponds to $u \approx 0.9$–1.0. This estimate matches with the mean value of $\langle u(t_1) \rangle = 0.98$ from the simulation, where $t_1 = 6.80, 6.85, \ldots, 7.80$.

In summary, the simulation results with the contact inhibition model are consistent and are able to reproduce the experimental observations. The Fisher equation is unable to reproduce the experimental observations. Therefore, the essential ingredients of the uniform density experiments are captured with the contact inhibition model, using our estimates of the density dependent diffusivity and proliferation rate.

3.2. The wound-healing assay experiment

In the wound-healing assay experiments of Tremel (2006) and Tremel et al. (2006), a 3T3 fibroblast cell population was incubated for up to seven days, forming a confluent cell monolayer. A section of monolayer was then scraped away producing a wound. The resulting migration of cells into the empty site was examined. The cells were observed for two days from the time of scraping. Images of the cell population at 2 time points are shown in Fig. 9.
To obtain data on the cell density as a function of the horizontal coordinate $x$, we divide the images into vertical strips of width 50 $\mu$m and count the number of cells in each strip. Cell number data are gathered from images taken over 24–48 h after wound formation. We use the cell density data to estimate the diffusivity $D_0$ and mitotic index $\alpha$. A measure of the contact inhibition cannot be obtained directly from this experiment, due to the narrowness of the wavefront and the movement of the wavefront, which results in difficulties in obtaining cell trajectories in regions of low uniform density. The uniform density experiments were carried out for the express purpose of estimating the contact inhibition parameter $A$.

3.2.1. Estimation of model parameters from experimental data

The counting technique provided values of cell density as a function of distance $x$ at equal time intervals between 24 and 48 h after the formation of the wound. By fitting this data to the asymptotic form of the traveling wave solution, we will obtain estimates of $D_0$ and $\alpha$. The traveling wave solutions to both the Fisher equation and the contact inhibition model proposed here have the same dominant asymptotic term given by Eq. (7). It is appropriate to use the minimum wave speed, since a compact support initial condition imitates a scrape wound. The uniform density experiments were carried out for the express purpose of estimating the contact inhibition parameter $A$.

\[ u(x, t) = \frac{1}{1 + \tilde{C} \exp[(4x - 2\tilde{B}t)/2]} \quad (42) \]

Here $\tilde{A}$, $\tilde{B}$, and $\tilde{C}$ are the fitting parameters. From Eq. (7) these parameters must be identified with $\tilde{A} = \sqrt{\alpha/D_0}$ and $\tilde{B} = \alpha$. We must include a translation factor $\tilde{C}$, since previously without any loss of generality we assigned $u(2\sqrt{\alpha D_0} t, t) = 1/2$. The previous estimated values of $D_0$ and $\alpha$ from the uniform density experiments provided the initial inputs for $\tilde{A}$ and $\tilde{B}$ to the fitting function.

Using the NonlinearFit function in Mathematica, the values of

\[ D_0 = 5.5 \, \mu m^2 / \text{min}, \quad \alpha = 2.7 \times 10^{-3} / \text{min} \quad (43) \]

were obtained as the best fit values. This corresponds to a minimum wave speed of $2\sqrt{\alpha D_0} = 0.24 \, \mu m / \text{min} = 351 \, \mu m / \text{day}$. These values are greater than those obtained from the uniform density experiment (Eq. (41)) but are of the same order of magnitude.

The fitted function (42) with the corresponding best fit parameter values are shown with the experimental data in Fig. 10(a). At early times the fit is not very good since the cell population must evolve from a sharp scrape edge to a traveling wave, which takes a period of time. However, the fit improves at later times when the traveling wave has almost established. Although data was gathered some time after scraping, it is apparent that the cell population has yet to fully settle into a traveling wave.

We compare the fitted data to the full numerical solutions of the Fisher equation and the contact inhibition model (3). These partial differential equations are solved using the NAG Fortran Library D03PCF routine. The parameter values used are those in Eq. (43) together with $A = 0.1$, the value estimated from the uniform density data.
experiment. The profile fitted to the first data set at $t = 24$ h used as an initial condition. No flux boundary conditions are applied and a large domain (dimensionless width 65) is used to avoid any boundary effects. The numerical solutions of both models match reasonably well with the experimental data, as shown in Fig. 10(b). Alternatively, the parameter values could be estimated by optimizing the fits of the numerical solution to the experimental data as carried out by Savla et al. (2004).

We tracked 10 cells for 12 h, as shown in Fig. 11. The black points indicate the initial positions of the cells. Initially, five of these cells were behind the wavefront and the other five cells were at the leading edge. All the trajectories are biased in the direction of travel of the wave, the positive $x$ direction. We analyse the trajectories using the same techniques as used in the uniform density experiment. Due to the biases that exist in these trajectories, we will ignore $\langle X_i^2 \rangle$ and $\langle Y_i^2 \rangle$ and present only $\langle X_i \rangle$.
Statistics gathered from cell trajectories at the wavefront are presented in Table 3, while those from cell trajectories behind the wavefront are presented in Table 4. These show a bias in the \(x\) direction with \(\langle X_i \rangle > \langle Y_i \rangle\).

Using \(\langle X_i \rangle = v_x t\), the bias in the \(x\) direction corresponds to \(v_x = 0.3 \mu\text{m/} \text{min}\) for the tracked cells at the front of the wave. For cells behind the front, the bias is estimated as \(v_x = 0.1 \mu\text{m/} \text{min}\). Hence, the bias is more significant in cells at the wavefront than those behind the wavefront. Consequently, the cells at the front tend to be more persistent in moving into the wound site. The variances of both populations show the cells at the front have greater motility than those behind the front. These findings are consistent with the effects of contact inhibition. Cells behind the front are in regions of high density and will experience greater contact inhibition than the cells at the wavefront, hence the motility is reduced. Cells at the wavefront are biased to migrate into the wound site as these cells encounter less contact inhibition in that direction. Differences between the position variances \(\sigma_x^2\) and \(\sigma_y^2\) indicate possible anisotropy effects in the cell movement. However, these differences decrease as the number of paths increases.

### 3.2.2. Simulations

The shape and wave speed of the traveling wave solutions to both the Fisher equation and the contact inhibition model are almost indistinguishable at the population level, as discussed previously. At the individual level, the differences between the models will become apparent through simulations presented here.

A lattice with 100 sites, with no flux boundary conditions, is again used. The simulation domain is \(0 \leq x \leq 200\). The carrying capacity chosen is 600 cells per site. The first data set at \(t = 24\) h are used as an initial condition.

The simulation results are compared to the previously generated numerical solutions to Eq. (3). The results for the Fisher equation are shown in Fig. 12(a) and those for the contact inhibition model are shown in Fig. 12(b). The simulated points are close to the numerical profiles for all times for both cases. Therefore, at a population level the results are again similar.

We now look for evidence of contact inhibition across the wavefront. To do so, we select several groups of cells...
with fixed horizontal position at 24 h after the scrape (corresponds to $\tau = 4$). We ask the following questions. Where do such groups of cells move over a 24 h period? Are there differences in the mean displacement as a function of time between the groups? For ease of visualization, each selected cell group is allocated a different color. We now describe this procedure.

We consider the initial cell density at $\tau = 4$ and divide the region at the wavefront into six regions, as illustrated in Fig. 13(a). We consider the cells allocated to each color-coded region and track their movement in time. We compare the resulting cell trajectories from each colored region. A day later, corresponding to $\tau = 8$, the simulated cell density (for both models) as a function of position is shown relative to the fixed colored regions in Fig. 13(b) and (c). We observe that the wavefront at this time has nearly overtaken all six regions. The mean position of the cells from the different colored regions is determined as a function of time, as shown in Fig. 13(d) and (e). The color of the plot corresponds to the color of the region where the cell group originated.

Cells in the Fisher equation model are expected to be unbiased and perform independent random walks. From the simulation data, cells from different regions are biased in different directions, showing no consistent bias to either direction (Fig. 13(d)). The corresponding variances are all similar (Fig. 13(f)), hence each group has a common motility.

The results for the contact inhibition model contrast these starkly! Cells in the contact inhibition model are persistently biased to the right, with a consistent increase in bias for regions with lowest cell density, closest to the wavefront (Fig. 13(e)). Similarly, the variances are now spread out (Fig. 13(g)), showing that cells in regions of low density are more motile compared to the cells in regions of high density.

The contact inhibition model is able to distinguish the motility ability of cells from different regions of the invasion wave, whereas the Fisher model is unable to do this. The cell behavior produced by simulations derived from the contact inhibition model is consistent with that observed in the experiment.

4. Conclusions

Diffusion and proliferation have long been considered the dominant biological mechanisms of cell migration in wound healing. This is evident in the prevalent use of the Fisher equation as a wound-healing cell migration model. However, this model excludes contact inhibition of migration.

Using a systematic approach (based on random walks and statistical techniques) to estimate the cell diffusivity at low and high density from a uniform density experiment, we show that the contact inhibition effect is significant; indeed, the cell diffusivity decreases by a factor of 10 from isolated cells to cells in a confluent monolayer. From this a contact inhibition effect parameter is deduced and used to build a nonlinear cell diffusivity. This functional form for the diffusivity is used in our model of the wound-healing assay.

For the mouse 3T3 cells, the uniform density experiment yielded parameter values $D_0 = 3 \text{mm}^2/\text{min}$, $\alpha = 1.8 \times 10^{-3}/\text{min}$, and contact inhibition parameter $A = 0.1 \text{cells/mm}^2$ (where $D(u) = A/(A + u)$), whereas the
Fig. 13. A comparison of cell behaviors across the wavefront for the Fisher equation and the contact inhibition model. (a) The initial cell density for the simulations is divided into six color-coded regions at time $\tau = 4$. (b) and (c) The simulated cell density as a function of position relative to the fixed colored regions at time $\tau = 8$. (d) and (e) The mean cell position versus time. (f) and (g) The variance versus time.
wound-healing assay experiment provided estimates as $D_0 = 5.5 \mu m^2/min$ and $\alpha = 2.7 \times 10^{-3}$/min. Recall that $D_0$ is the diffusivity at low cell density. The increase in $D_0$ may indicate that other mechanisms are at play in the wound-healing assay experiment. For example, if the confluent monolayer of fibroblasts builds a collagen substrate (Lanas et al., 1998), then some collagen substrate may remain after the creation of the scrape wound. This remaining substrate will be different from the collagen-free substrate in the uniform low density experiment. The collagen may enhance the cell migration and cause an increase in the wound-healing assay experiment. For example, migration and cause an increase in diffusivity, that is, the contact inhibition effect. There is no contact inhibition model. Of course, in vivo wound healing, such as dermal wound healing, there are strong growth factors which drive fibroblast migration, which gives rise to a chemotactically driven model (Haugh, 2006).

This multi-scale approach is a significant extension to previous modeling of the wound-healing assay. This approach can be generalized to other reaction diffusion equations. In particular, the development of simulation techniques for tissue engineering applications will be important for determining cell seeding strategies and scaffold surface modifications, where cell proliferation and migration are vital to the successful recruitment of cells into biodegradable scaffolds. Furthermore, recent advances in time-lapse imaging provide observations of individual cell trajectories which will lead to more physically and biologically accurate models.

Appendix

The order $c(1/\bar{c}^2)$ term in the expansion (6) is

$$u_1(z) = \frac{e^{\bar{c}^2/\bar{c}}}{(1 + e^{\bar{c}^2/\bar{c}})} \left[ \frac{A}{1 + A + Ae^{\bar{c}^2/\bar{c}}} + \frac{\kappa}{1 + A + Ae^{\bar{c}^2/\bar{c}}} \right]$$

$$+ \frac{e^{\bar{c}^2/\bar{c}}}{(1 + e^{\bar{c}^2/\bar{c}})^2 (1 + A)} \times \left[ \frac{A^2}{\bar{c}^2} - (1 + 2A) \ln(1 + A + Ae^{\bar{c}^2/\bar{c}}) \right], \quad (44)$$

and the constant $\kappa$ is a function of the contact inhibition parameter $A$, given by

$$\kappa = \frac{2A}{1 + 2A} + \frac{(1 + 2A) \ln(1 + 2A)}{1 + A}. \quad (45)$$

We can interpret the Fisher equation as the $A \to \infty$ case of the contact inhibition model. Then, as expected, the $u_1(z)$ term with $A \to \infty$ is consistent with the $c(1/\bar{c}^2)$ term obtained for the Fisher equation (Murray, 2002), namely

$$\lim_{A \to \infty} u_1(z) = \frac{e^{\bar{c}^2/\bar{c}}}{(1 + e^{\bar{c}^2/\bar{c}})^2} \ln \left[ \frac{4e^{\bar{c}^2/\bar{c}}}{(1 + e^{\bar{c}^2/\bar{c}})^2} \right]. \quad (46)$$

Acknowledgments

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