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Traveling Wave Model to Interpret a Wound-Healing Cell Migration Assay for Human Peritoneal Mesothelial Cells

PHILIP K. MAINI, D.Phil.,[1] D.L. SEAN McELWAIN, Ph.D.,[2] and DAVID I. LEAVESLEY, Ph.D.[3]

ABSTRACT

The critical determinants of the speed of an invading cell front are not well known. We performed a "wound-healing" experiment that quantifies the migration of human peritoneal mesothelial cells over components of the extracellular matrix. Results were interpreted in terms of Fisher's equation, which includes terms for the modeling of random cell motility (diffusion) and proliferation. The model predicts that, after a short transient, the invading cell front will move as a traveling wave at constant speed. This is consistent with the experimental findings. Using the model, a relationship between the rate of cell proliferation and the diffusion coefficient was obtained. We used the model to deduce the cell diffusion coefficients under control conditions and in the presence of collagen IV and compared these with other published data. The model may be useful in analyzing the invasive capacity of cancer cells as well in predicting the efficacy of growth factors in tissue reconstruction, including the development of monolayer sheets of cells in skin engineering or the repair of injured corneas using grafts of cultured cells.

INTRODUCTION

Active cellular invasion is characteristic of both normal and pathological phenomena. In dermal wound healing, for example, fibroblasts migrate into the wound space in response to the presence of collagen and various growth factors while also producing both collagen and growth factors. When tumor cells invade they produce proteases, which degrade the extracellular matrix, and a similar process is seen in trophoblast implantation into the uterine wall. In wound-healing angiogenesis, capillary sprouts comprising endothelial cells emerge from the host vascular bed and invade the wound space. The intense worldwide interest in tissue engineering, where the proliferation and migration of cells is central to many of the novel tissue restoration techniques, drives the need for this basic research.

The wound-healing assay described below provides quantitative data on cell migration and this article provides a mathematical description of this invasion process in terms of Fisher's equation. This equation has terms that can be interpreted as random cell motion and cell proliferation, which is assumed to be of the logistic type. Under certain conditions, this equation exhibits traveling wave solutions that are waves of constant shape traveling at uniform speed.[1]

The experiments examined migration over different components of the extracellular matrix. As predicted from the model, the invading front appears to move at constant speed (different for different substrates) in all experiments. The model provides an expression for the wave speed in terms of the random cell motility coefficient and the rate of cell proliferation and, conversely, this expression can be used to determine a relationship

[1]Centre for Mathematical Biology, Mathematical Institute, University of Oxford, United Kingdom.
[2]School of Mathematical Sciences, Queensland University of Technology, Brisbane, Australia.
[3]School of Life Sciences, Queensland University of Technology, Brisbane, Australia.

between the random cell motility and the cell proliferation rate if the wave speed is known. A validated model such as this may prove useful in quantifying important aspects of cell migration.

As far as we are aware this is the first time that this well-known equation has been used to fit experimental data on wound healing, although Sherratt and Murray[2] examine a similar problem in experiments on epidermal wound healing involving a two-dimensional model and Sheardown and Cheng[3] have used a generalization of Fisher's equation in their model of corneal epithelial wound healing. More recently, Kobayashi *et al.*[4] have used a wound-healing assay to quantify the cell migration of rabbit anterior cruciate and medial collateral ligament cells. They use a diffusion equation, which is the same as Fisher's equation without cell proliferation, to determine the random motility coefficient. They find that they need to allow the random motility coefficient to change over time to obtain good fits to the experimental data.

This article is laid out as follows. In the next section, we describe the wound-healing assay and the third section outlines Fisher's equation. In the fourth section we discuss parameter estimation and experimental results and in the final section we compare the predicted values with those in the published literature and give suggestions for how this model might be used in further experiments.

EXPERIMENTAL METHODS

The experimental protocol is described in detail elsewhere[5]; only a brief outline of the relevant procedures is given here. Human peritoneal mesothelial cells (HPMCs) were harvested from discarded peritoneal dialysate and cultured. In the wound-healing assay, the HPMCs are grown as monolayers on collagen I until they reach confluence—typically 4 days. A 4-mm scrape wound was made in the confluent layer and the displaced cells were

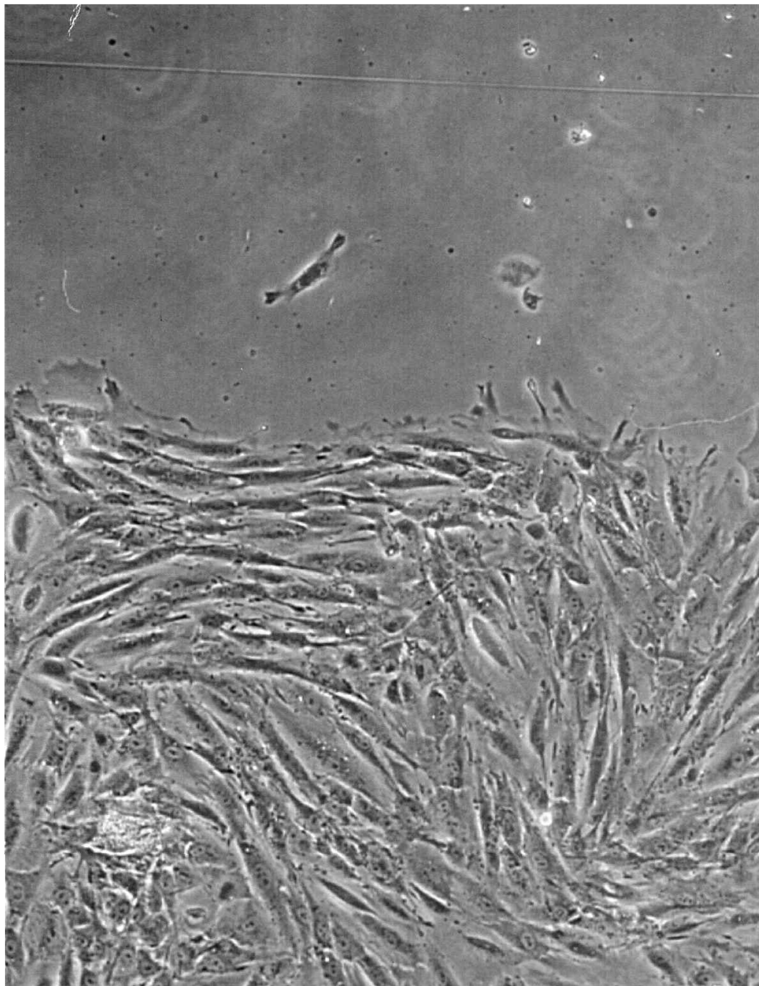

FIG. 1. Typical human peritoneal mesothelial cell front 10 h after wounding.

removed and the remaining cells were bathed in fresh culture medium. The position of the invading cell front was noted against a reference grid at 15 points (there were 5 points on each of 3 independent wounds). Fresh medium containing fetal calf serum was supplied each day. The wound-healing assay was carried out with HPMCs from a single patient, but with the cells migrating across different substrates: collagen I, collagen IV, laminin, fibronectin, vitronectin, and hyaluronic acid, all components of the extracellular matrix. For this particular patient's HPMCs, with which we have carried out this detailed analysis, collagen IV was the most effective at enhancing cell front progress and here we analyze results for the control and collagen IV. Figure 1 shows a typical situation after wounding (10 h). There is a sharp front of cells migrating into the scrape wound.

The results for all substrates are remarkably consistent, as shown in Fig. 2; after a short period of time, the cell fronts progress at what appears to be constant speed.

DESCRIPTION OF THE MODEL

The spatial spread of the invading cells is assumed to be a process in which individual cells undergo a dispersion process as well as proliferation. Fisher's equation captures these two features. It is of the form

The rate of change of cell number density at position x at time t	=	change in cell number density due to random diffusion processes	+	change in cell number density due to cell proliferation

or

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} + kc(c_0 - c) \qquad (1)$$

where $c(x,t)$ is cell density, D is the random diffusion (motility) coefficient, kc_0 is the intrinsic growth rate of the cells (the maximum rate at which the cells proliferate), and c_0 is the cell density at confluence. Fisher[6] was studying the propagation of an advantageous gene in a population when he proposed this equation, although it was Kolmogoroff *et al.*[7] who first examined the behavior of the solutions in detail. There is an extensive mathematical literature on Fisher's and related reaction-diffusion equations (see, e.g., Grindrod[8] and Murray[1]). Significant advances have been made concerning the ex-

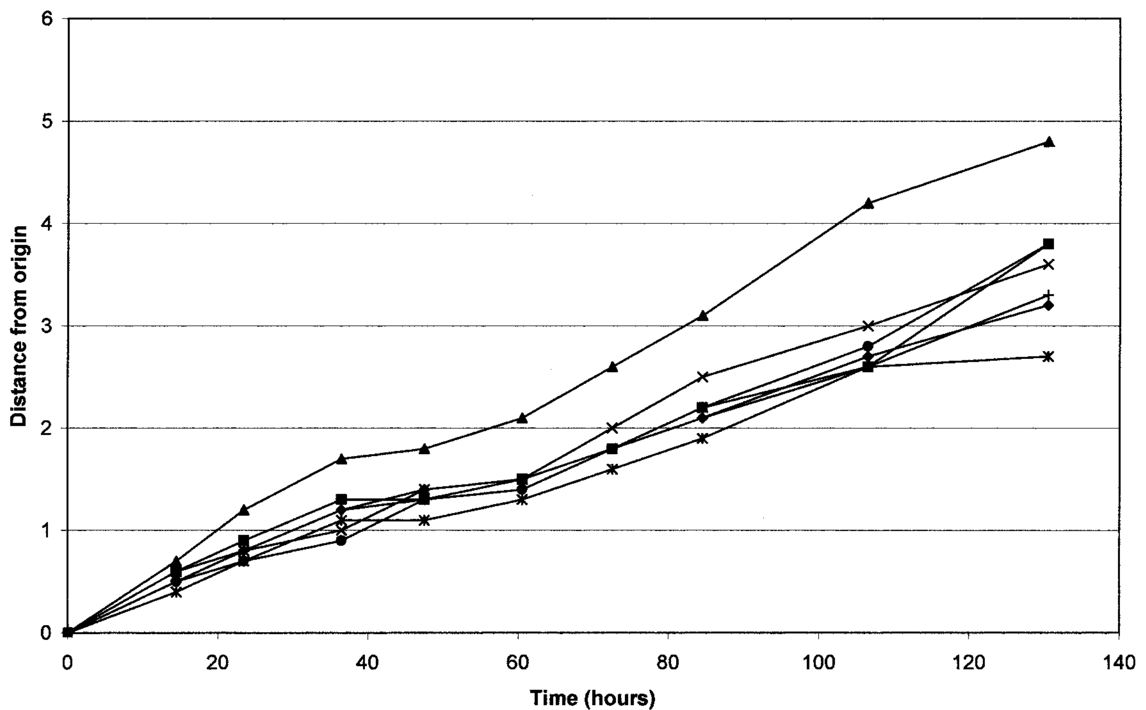

FIG. 2. Typical plots from experimental data for the position of the invading cell front (taking the origin as the position at 9.5 h and measuring time from that point) over different substrates. Distance units are 0.25 mm. Control (◆), collagen I (■), collagen IV (▲), laminin (×), fibronectin (*), vitronectin (●), hyaluronic acid (+).

istence and structure of solutions of such equations. Increasingly, they are also being used in models of a range of physical and biological phenomena including pattern formation in embryos, combustion waves in solids, and epizootic wave progression.

It is known that under certain conditions, Fisher's equation exhibits a wave of unchanging form (a traveling wave) at constant speed and that the speed of the wave is given by $2(c_0kD)^{1/2}$.[1] Figure 3 shows the numerical solution of Fisher's equation at equal times from an initial step function in which the cells are confluent for $x < 0$ and there are no cells for $x > 0$. The invading front takes some time to settle down to the traveling wave, which progresses at constant speed. The behavior of the solution is different from that of the pure diffusion case shown in Fig. 4 for the same diffusion coefficient and same initial state. The solution does not "fill in" because there is no reaction term and the solution does not exhibit wavelike solutions.

EXPERIMENTAL RESULTS AND MODEL PARAMETER ESTIMATION

Figure 5A and B shows the progression of the invading cell front as well as a regression line (least squares) passing through the origin.

For example, in Fig. 5A, the speed, as determined from the least-squares fit, is 0.0254 units h^{-1}, where the distance units are 0.25 mm. That is, the speed is 0.00635 mm h^{-1}. Now, given that the Fisher equation predicts a wave speed of $2(c_0kD)^{1/2}$, we have $D = v^2/4c_0k$ mm^2 h^{-1}, where c_0k is in hours and v, the speed, is in mm h^{-1}. Noting that $c_0k = \ln 2/t_d$, where t_d is the cell doubling time (hours),

$$D = \frac{v^2 t_d}{4 \ln 2} \ \text{mm}^2\ \text{h}^{-1}$$

or

$$D = \frac{v^2 t_d}{4 \ln 2 \times 60 \times 60} \ \text{mm}^2\ \text{s}^{-1} \qquad (2)$$

For example, when the doubling time, t_d, is 24 h, and the speed, v, is 6.35×10^{-3} mm h^{-1}, then we predict that the random motility (diffusion) coefficient, D, is 9.70×10^{-8} mm^2 s^{-1}. Table 1 gives an estimation of the diffusion coefficient for a range of healing speeds and cell cycle times using Eq. (2).

Table 2[9,10] and Table 3[3,4,11,12] give experimentally determined estimates of the cell doubling time and the diffusion coefficient for various cell lines. If we use a value of 4.3 days for the cell cycle time based on the Neidbala *et al.*[10] data for HPMCs on a plastic substrate, the estimated diffusion coefficient under control conditions is $D = 4.17 \times 10^{-7}$ mm^2 s^{-1}. For migration over collagen

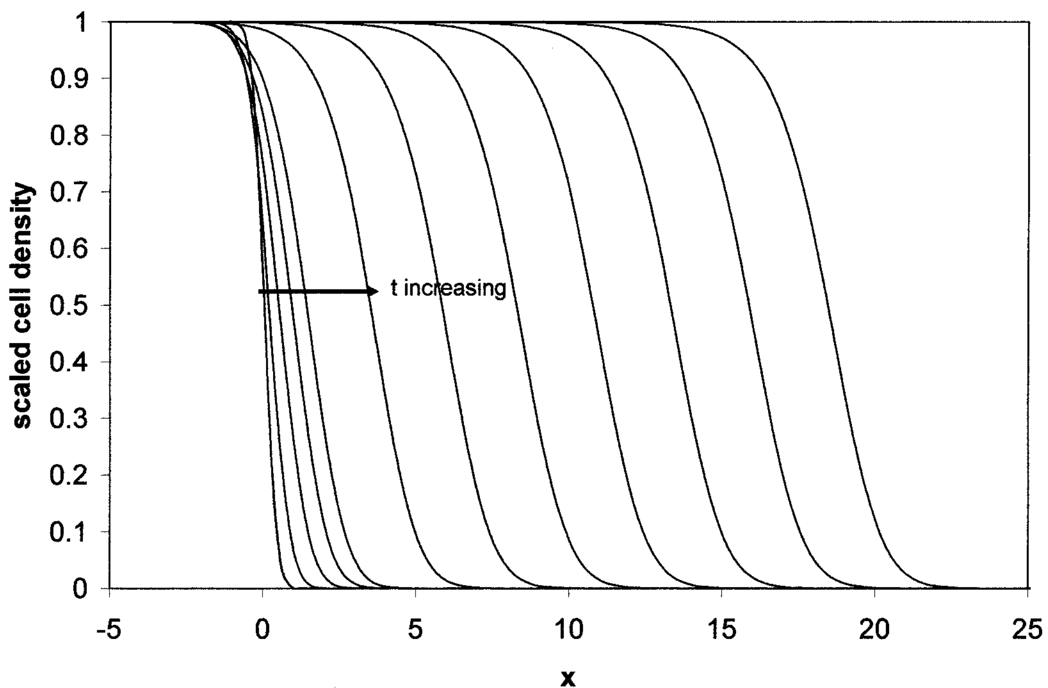

FIG. 3. Numerical solution of Fisher's equation, showing the developing wave front. The distributions are at equal time steps of 9.5 h. Here the doubling time, t_d, is 24 h and the diffusion coefficient, D, is 9.709×10^{-8} mm^2 s^{-1}. The cell density is scaled by the confluent cell density and the distance is in grid units.

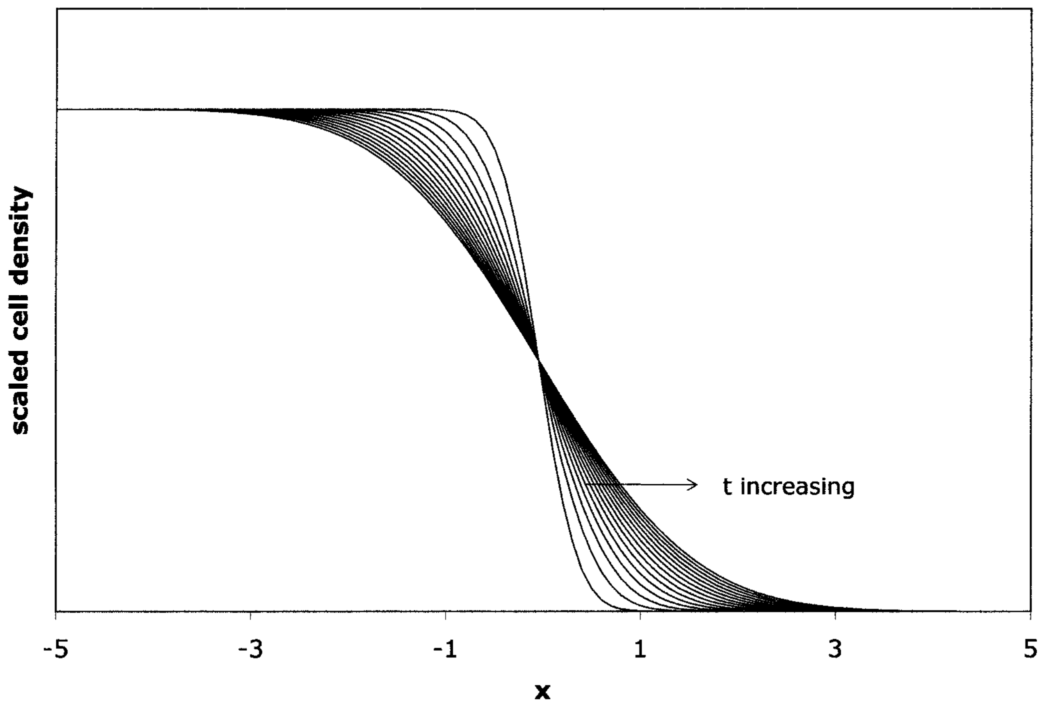

FIG. 4. Solution of the pure diffusion equation (Fisher's equation without the cell proliferation term) for the same times as in Fig. 3, using the same diffusion coefficient. The distributions are at equal time steps of 9.5 h. The cell density is scaled by the confluent cell density and the distance is in grid units.

IV, the front speed, v, as determined from the least-squares fit (see Fig. 5B), is 9.43×10^{-3} mm h^{-1}, so that the estimated diffusion coefficient is $D = 9.18 \times 10^{-7}$ mm^2 s^{-1}.

DISCUSSION

In this article we have used Fisher's equation to describe the movement of the cell front. As mentioned above, in a similar wound-healing assay for anterior cruciate (ACL) and medial collateral ligament (MCL) cells, Kobayashi *et al.*[4] use a description based on the diffusion equation (which is Fisher's equation without the proliferation term). The duration of the experiments outlined in Kobayashi *et al.* is much shorter, 6 and 12 h, than those considered in this article and, as the authors point out, proliferation may not be important. However, it is interesting to note that, using the diffusion equation model, the random motility coefficient for both cell types appears to be greater at 12 h than at 6 h, although the difference is not statistically significant.

In the Fisher equation, the proliferation term enables the density c to exhibit constant profile, constant speed traveling waves (compare Fig. 3 and Fig. 4). It turns out that such waves exist for a wide range of initial conditions (see, e.g., Murray[1]). Note that, as a result, the propagation speed (which in Fisher is proportional to $D^{1/2}$, whereas in the pure diffusion case it is proportional to D) can be greatly increased. As seen in Fig. 1, the cell front is quite sharp and an extension of this model would be to adopt a generalization of Fisher's equation, which has sharp front solutions, such as

$$\frac{\partial c}{\partial t} = D\frac{\partial}{\partial x}\left(c\frac{\partial c}{\partial x}\right) + kc(c_0 - c) \qquad (3)$$

although the good fit obtained with the standard Fisher equation would not appear to justify this.

The numerical solution of Fisher's equation is shown in Fig. 3 and the "position" of the front is shown in Fig. 5A and B together with the regression line and the experimental data. The curves for $u = 0.01$, $u = 0.1$, and $u = 0.5$ (▲, ■, and ◆, respectively, in Fig. 5) were obtained by finding where on the evolving front the scaled cell density took on these values. As can be seen, eventually the slopes of these curves (the speed) are the same, indicating that the wave has settled down to a traveling wave (uniform shape and speed). The speed, as measured by examining the movement of such a point on the developing wave, can initially be negative if the point is taken near the top of the wave (near $x = 0$, the initial response is for the "cells" to diffuse into the empty space for $x > 0$ as can be seen in Fig. 3 and the point at which

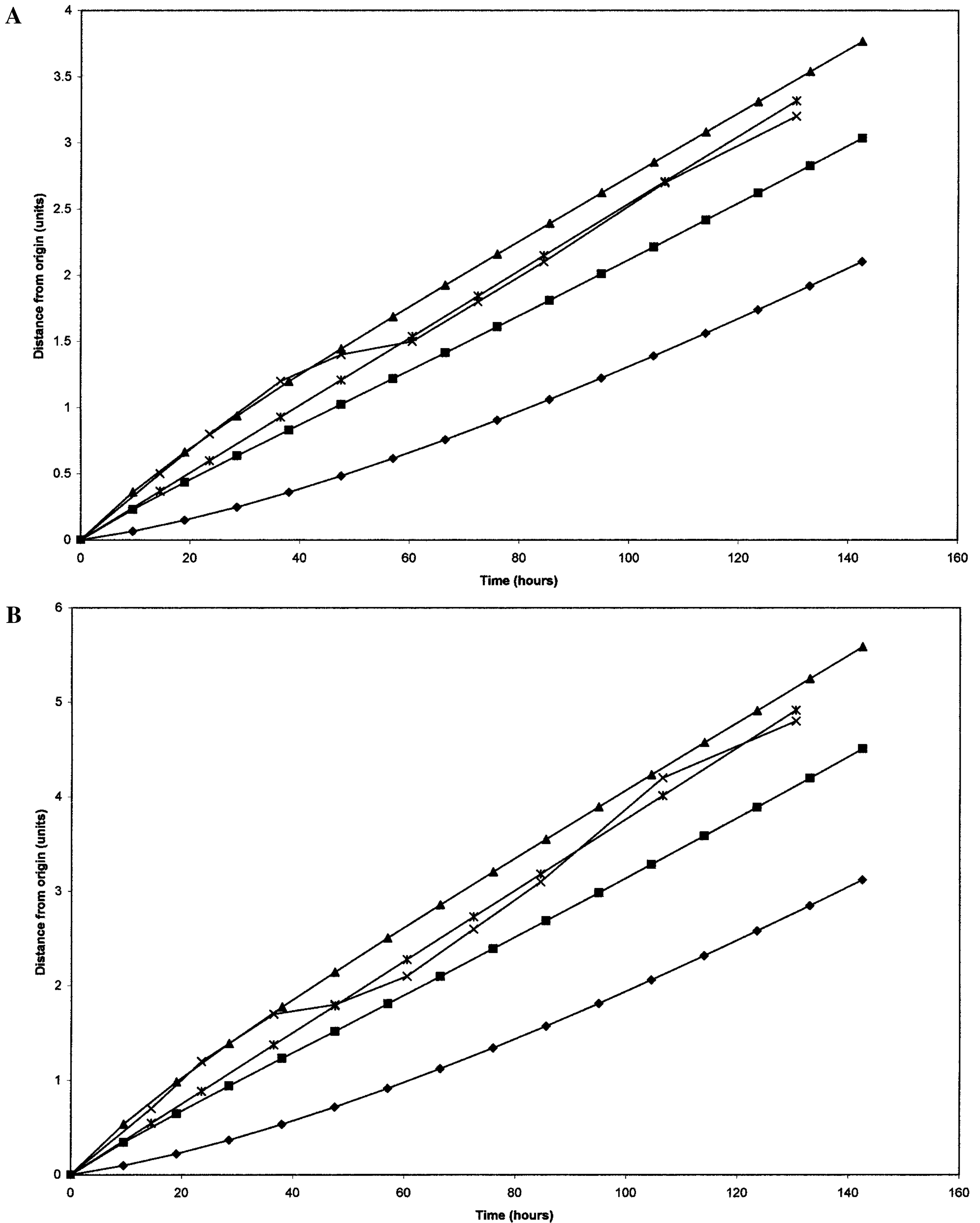

FIG. 5. (**A** and **B**) Plots of the experimental data for the position of the invading cell front (taking the origin as the position at 9.5 h and measuring time from that point) together with a least-squares straight-line fit of the data passing through the origin and the "position" of the front based on Fisher's equation. The model front position depends on the criterion used to estimate the front position (see text for explanation). Distance units are 0.25 mm. (**A**) Control; (**B**) collagen IV; experimental data (×), least-squares fit (*), $u = 0.5$ (◆), $u = 0.1$ (■), $u = 0.01$ (▲).

TABLE 1. PREDICTED CELL DIFFUSION COEFFICIENTS FROM THE FISHER MODEL FOR A TYPICAL RANGE OF WOUND-HEALING SPEEDS AND CELL CYCLE TIMES[a]

Speed ($mm\ h^{-1}$)	*Cell cycle time (days)*	*Estimated diffusion coefficient ($mm^2\ s^{-1}$)*
0.006	0.5	4.3×10^{-8}
	1.0	8.6×10^{-8}
	2.0	1.7×10^{-7}
	4.0	3.4×10^{-7}
	8.0	6.9×10^{-7}
0.008	0.5	7.7×10^{-8}
	1.0	1.5×10^{-7}
	2.0	3.1×10^{-7}
	4.0	6.1×10^{-7}
	8.0	1.2×10^{-6}
0.010	0.5	1.2×10^{-7}
	1.0	2.4×10^{-7}
	2.0	4.8×10^{-7}
	4.0	9.6×10^{-7}
	8.0	1.9×10^{-6}

[a]Using Eq. (2).

$u = 0.95$ on the curve, e.g., moves to the left before moving to the right).

This article has established a relationship between the proliferation rate and the diffusion (random motility) coefficient provided the wave speed is known and the front moves as a traveling wave solution of Fisher's equation. To validate the model further, both proliferation data and cell front movement need to be obtained for the same cell line. If no actual cell proliferation data are available, then the following could be used. If the proliferation rate is increased by a fraction r, for example, by the addition of a growth factor, and the random motility is unchanged then, if the model is valid, the speed of the front will increase by $r^{1/2}$.

The estimates of the random motility coefficient derived for HPMCs are smaller than those published in the literature (see Table 2). However, the cells used in this study were derived from a patient undergoing continuous ambulatory peritoneal dialysis and it is possible that this patient's HPMCs were migration impaired.

TABLE 2. ESTIMATES OF DOUBLING TIMES FOR VARIOUS PERITONEAL MESOTHELIAL CELL LINES

			Reference	
Cell type	*Doubling time*	*Conditions*	*Authors*	*Ref. no.*
Rat peritoneal mesothelial cells	18 h	Suspended in growth medium	Hjelle *et al.*	9
Human peritoneal mesothelial cells	4.3 days	Plastic	Niedbala *et al.*	10
	2.4 days	Bovine corneal endothelial cell ECM		

TABLE 3. EXPERIMENTALLY DETERMINED ESTIMATES OF THE DIFFUSION COEFFICIENT FOR VARIOUS CELL LINES

			Reference	
Cell type	*Doubling time*	*Estimated diffusion coefficient ($mm^2\ s^{-2}$)*	*Authors*	*Ref. no.*
Rabbit corneal epithelial cells	25 h	$(1.61 \pm 0.43) \times 10^{-6}$	Sheardown and Cheng	3
Not specified	Not specified	1.2×10^{-5}	Tracqui	11
Neutrophil leukocytes	Not specified	3×10^{-6}	Maheshwari and Lauffenburger	12
ACL cells	Not specified		Kobayashi *et al.*	4
At 6 h		$(2.51 \pm 0.31) \times 10^{-6}$		
At 12 h		$(4.39 \pm 0.63) \times 10^{-6}$		
MCL cells				
At 6 h		$(4.63 \pm 0.65) \times 10^{-6}$		
At 12 h		$(6.59 \pm 1.47) \times 10^{-6}$		

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Address reprint requests to:
Sean McElwain, Ph.D.
School of Mathematical Sciences
Queensland University of Technology
G.P.O. Box 2434
Brisbane QLD 4001, Australia

E-mail: s.mcelwain@qut.edu.au