A computational model of cell migration coupling the growth of focal adhesions with oscillatory cell protrusions

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1. Introduction

Cell migration is a crucial process for many physiological and pathological events, such as embryogenesis and cancer metastasis. It is a highly integrated multistep mechanism comprising three well-defined steps, namely membrane protrusion, cell-substrate adhesion and finally contraction leading to cell translocation. Translocation comprises the forward displacement of the cell centre of mass and the detachment of the cell from the substrate. The molecular events orchestrating cell migration are still largely unknown. Nonetheless, some key regulatory molecules involved in each of these essential steps have now been identified (Giannone et al., 2007; Mylonas et al., 2006; Li et al., 2005; Firtel and Chung, 2000). Such knowledge has allowed for cell motility events to be both experimentally and mathematically reconstructed and further elucidated.

The process of formation of membrane protrusions has been the source of active debate since the eighties (see review by Mogilner, 2006). The solution/gelation cycle of the actin gel, mediated by calcium release, was first evoked to explain the protrusions (Oster, 1984). Subsequently, advances in cell imaging revealed that a high density of actin coincided spatio-temporally with the formation of membrane protrusions in migrating cells, indicating that actin polymerization is directly responsible for the protrusions formation (Borisy and Switkina, 2000; Carlier and Persoz, 2007; Mylonas et al., 2006; Li et al., 2005; Firtel and Chung, 2000). Such knowledge has allowed for cell motility events to be both experimentally and mathematically reconstructed and further elucidated.

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the highly spatio-temporally organized nature of the cell membrane protrusion dynamics was exhibited. Spatial organization was revealed by the observation that cells assume an isotropic shape. In addition, the measure of periodicities in the membrane oscillations confirmed the temporally organized nature of the spontaneous protrusion dynamics (Stéphanou et al., 2004). Membrane blebs forming at the tip of the protrusions suggest that the internal pressure, rather than a Brownian mechanism, may be responsible for initiating the protrusions (Charras et al., 2005; Abraham et al., 1999; Bereiter-Hahn and Luers, 1998). Under this mechanism, contractions generated by the actomyosin complex induce a cytoplasmic flux, which then pushes on the membrane and leads to protrusions at sites where the links between membrane and cytoskeleton are too weak to prevent membrane extension (Paluch et al., 2005, 2006). Simulations based on this hypothesis qualitatively reproduce the spatio-temporal membrane deformations observed in unstimulated keratocytes (Alt et al., 1995; Alt and Dembo, 1999) and fibroblasts (Stéphanou et al., 2004).

Actin-driven protrusion is the result of a cascade of molecular events initiated by the small-GTPases, in particular Rho-A, which are proteins involved in signal detection (Ridley, 2006; Hall, 2005; Marée et al., 2006). Such activation does not occur in unstimulated cells. Consequently it can be hypothesized that, even though both protrusion mechanisms dynamically coexist in a cell, the functional state of the cell dictates which one will predominate. For example, directional cell migration induced by either chemical (molecular gradients) or mechanical cues (alterations in substrate rigidity or topography) can be guided primarily by polymerization-driven protrusion. On the contrary, protrusions in an unstimulated cell can largely derive from passive hydrostatic pressure. This hypothesis, which aims to distinguish the unstimulated from the directionally polarized cell awaits further experimental and theoretical investigation.

In vivo cell migration, although physiologically imperative, is also energetically demanding and catastrophic if uncontrolled. For that, it is closely guided and fine tuned by both chemical and physical cues. Thus, little attention has been given to spontaneous or random cell migration. However, spontaneous cell migration is of major interest since it allows for isolation and study of cellular mechanisms, such as the coordination of actin turnover with the focal adhesion dynamics both spatially and temporally (Dunn and Zicha, 1995). In this paper, our approach is to develop a mathematical model where spontaneous membrane protrusions are induced by the hydrostatic pressure (Alt and Tranquillo, 1995). Cell membrane oscillations thus result from the interplay between this protrusive force and the counteracting stress from the actin filaments linked to the membrane. The actin fibre tension and actomyosin contractility depend on the local amount of actin, where actin turnover is regulated around a chemical equilibrium density. Various spontaneous pulsating cell behaviours can be generated depending on the choice of the cytomechanical parameters (cytoplasm viscosity, filaments and membrane elasticities, actomyosin contraction toxicity, etc.) (Stéphanou and Tracqui, 2002; Stéphanou et al., 2004).

The model is then further developed to take into account the formation of adhesions with the substrate. Once an adhesion is formed, the model aims to describe how it can mature into a more stable adhesion structure able to support cell traction thus allowing for cell translocation (Chen et al., 2003; Sastry and Burridge, 2000). With the present model, we aim to incorporate the key processes, rather than the detailed molecular events, of adhesion maturation during cell migration. Consequently, we will simply assume the existence of three successive types of adhesions (adhesion spot, A; focal complex, FX and focal adhesion, FA) (Kaverina et al., 2002; Zaidel-Bar et al., 2004), each being characterized by its lifetime and resistance to traction (Galbraith et al., 2002).

In the subsequent sections of this paper, we initially present the details of the computational model followed by the results, including experimental data presenting key features of fibroblast migration. These experimental data serve as the basis to scale our simulation results a posteriori. We then investigate the influence of two important parameters of the model on cell speed: (i) the adhesion strength, which is known to influence cell speed biphasically, thus serving as a test for the model and (ii) the adhesion proteins recycling time and adhesion lifetimes, which demonstrate how actin dynamics and adhesions dynamics are temporally interrelated.

2. Model formulation

As noted previously, cell migration is a multistep process, primarily comprising three distinct steps namely membrane protrusion, adhesion to the substratum, and translocation of the cell via application of traction forces by the contracting cytoskeleton. The cyclical repeat of these three steps allows the cell to migrate in its microenvironment. The model proposed herein comprehensively addresses each of these distinct processes. Since random migration is our main interest, we assume the substrate to be isotropic in all aspects (chemically, mechanically, etc.). Therefore, we do not describe its properties explicitly.

2.1. A model for spontaneous cell membrane protrusions

Many different hypotheses have been proposed to explain the spontaneous oscillatory movement of the cell membrane and the role of actin turnover (i.e. polymerization/denpolymerization cycles) in the recurring protrusion and retraction dynamic of the lamellipodium. Among the currently published models, we chose the model by Alt and Tranquillo (1995) as the basis. The main reason being that this model considers both the molecular events of actin turnover dynamics and the related mechanical properties of the cell membrane and cytoskeleton. This model has successfully described the appearance of rotating deformation waves around the cell body of keratocytes (Alt et al., 1995) and leukocytes (Alt, 1990), as well as more complex periodic pulsating patterns such as those observed in L929 fibroblasts (Stéphanou et al., 2004).

The model considers that cell protrusion dynamics depend on the biophysical properties (i.e. viscoelasticity and contractility) of the cortical network of actin and myosin filaments, which underlies the cell membrane and surrounds the cell body. Moreover this highly dynamic network is able to: (i) disassemble at locations where it becomes condensed and (ii) reassemble where it is weaker (i.e. in newly formed cell protrusions, such as lamellipodia). Thus, cell protrusions form as a response initially to a mechanical non-equilibrium state involving peripheral forces on the cell membrane and then the actin turnover induced by intracellular spatial variation. The model thus addresses cortical actin dynamics, which are mainly responsible for cell shape reorganization, by focusing on the ability of actin to:

- polymerize into F-actin and depolymerize into G-actin,
- interact with myosin to generate the contractile activity in the cell,
- move through the cytoplasm via convection.

The local amount of F-actin also determines the intensity of the resistive stress applied on the membrane as the result of crosslinks between the actin cytoskeleton and the membrane proteins.
This resistive stress together with the stress induced by the cortex–membrane curvature balance the intra-cellular hydrostatic pressure. This pressure acts as the “driving force” for membrane protrusion in the model. This pressure is reinforced by active polymerization of actin filaments, when adhesions have formed on the expanding membrane, thus clustering actin polymerization to form stress fibres.

2.1.1. Model equations

The model considers the two-dimensional annular domain bounded by the cell body and the cortex–membrane complex at the lamellar tip. Specifically, the cell body is represented with a fixed circular shape to withstand the tension/pressure in the annular cytoplasm ring, whereas the cortex–membrane complex at the lamellar tip defines a free radially moving boundary. We denote by $L(\theta, t)$ the width of the annular domain along any radial direction located by the angle $\theta$ ($0 \leq \theta \leq 2\pi$) (Fig. 1).

For simplicity, we transform the two-dimensional problem into a one-dimensional version. We assume, as in Alt and Tranquillo (1995), that the F-actin density as well as its convective tangential velocity, are constant in the radial direction. The second assumption implies that the cortical network can slide with respect to the membrane, which is partly justified considering that membrane proteins involved in the network–membrane connections are mobile within the membrane. Thus the three variables considered in the spontaneous cell oscillations model are:

1. the F-actin concentration in the cortex $a(t, t)$,
2. the F-actin tangential velocity $v(t, t)$,
3. the width of the cell cortex annulus $L(\theta, t)$, given by the membrane position as measured from the surface of the cell body.

The spatio-temporal evolution of these three variables is given in the following set of partial differential equations (PDEs) (full details are given in Stéphanou et al., 2004). The first equation represents the conservation of F-actin in the cortex. The rate of actin turnover (i.e. polymerization/depolymerization), characterized by $\eta$, depends on the local value of F-actin concentration relative to the stationary concentration at the chemical equilibrium $a_0$. The equation is thus

$$\frac{\partial a}{\partial t} = \beta a - \eta L(a - a_0)$$

(1)

The second equation describes the balance of forces applied at the lamellar tip on the cortex–membrane complex in the radial direction. The model takes into account:

- the viscous friction due to the sliding movement of the cortex–membrane over the substratum, linearly depending upon the actin density and modulated by the coefficient $\phi_1$,
- the intra-cellular hydrostatic pressure $\beta_1$,
- the resistive elastic stress due to the cortical network–membrane attachment which intensity linearly depends upon the actin density, with $\gamma_1$ the elasticity coefficient for the actin network,
- a curvature-dependent stress due to the surface tension of the cortex–membrane complex characterized by the coefficient $\gamma_1$.

The equation is given by

$$\phi_1 \frac{\partial L}{\partial \theta} = \beta_1 - \gamma_1 L a + \frac{\partial}{\partial \theta} \left( \gamma_1 \frac{\partial L}{\partial \theta} \right)$$

(2)

Finally, the third equation concerns the balance of forces applied on the cortical network in the tangential direction. It includes:

- the frictional force on the network moving within the cytosol, with magnitude controlled by a drag coefficient $\phi_0$ (related to the attachment of the network with the membrane and other types of filaments),
- a viscous stress with viscosity coefficient for the cytoplasm $\mu_0$,
- the membrane curvature-induced stress with coefficient $\tau_0$.

This equation is given by

$$\phi_0 \frac{\partial v}{\partial \theta} = \frac{\partial}{\partial \theta} \left( \mu_0 \frac{\partial v}{\partial \theta} + \sigma_0 (a) - \frac{\partial}{\partial \theta} \left( \tau_0 \frac{\partial L}{\partial \theta} \right) \right)$$

(3)

The contractile activity of the actomyosin network is modelled by the non-linear function $\sigma_0(a)$ (Fig. 2). Two mechanical states

![Fig. 1. Schematic representation of the cell in the theoretical model. Two zones are considered: (i) the cell body, which includes the nucleus surrounded by a dense network of filaments confined by a circular area with radius $R_0$ and (ii) the cell cortex where remodelling of the actin cytoskeleton mainly occurs and corresponds to the area bounded at one side by the outer boundary of the cell body and at the other side by the cell membrane. The width of the cortex in any angular direction $\theta$ is given by $L(\theta)$.](image1)

![Fig. 2. Profile of the actomyosin contractility as a function of the actin density $\sigma_0(a)$ (red curve). The contractility first increases with the actin density until it reaches a saturation density ($\sigma_{sat} = 4$). Above this point, contractility decreases due to a compaction effect of the network of filaments. Active pressure on the cell membrane due to the combination of fluid hydrostatic pressure $\beta$ and actin polymerization-induced pressure $\beta(a)$ (blue curve).](image2)
have to be distinguished according to the value of the F-actin concentration \( a(y, t) \) with respect to a saturation concentration \( a_{sat} \).

Specifically, if \( a(y, t) \) is lower than \( a_{sat} \), the contractile stress increases according to a parabolic law. However, above the saturation threshold \( a_{sat} \), the stress decreases exponentially as a consequence of the network compaction. The non-linear function proposed by Alt and Tranquillo (1995) is the following:

\[
\sigma_y(a) = \psi_0 a^2 e^{-2b/a_{sat}}
\]  

(4)

where the coefficient \( \psi_0 \) controls the magnitude of the contractile stress.

The dimensionless system of equations is given by

\[
\frac{\partial}{\partial t} \left( \frac{a L}{\epsilon^2} \right) = -\frac{\partial}{\partial y} \left( \frac{a L}{\epsilon^2} \right) + \gamma L + \frac{\partial}{\partial y} \left( \frac{a L}{\epsilon^2} \right)
\]  

(5)

and

\[
\frac{\partial}{\partial t} \left( \frac{a v}{\epsilon^2} \right) = \beta + \gamma L a + \delta \frac{\partial}{\partial y} \left( \frac{a L}{\epsilon^2} \right)
\]  

(6)

These friction and pressure effects are modelled by two additional terms in the equation describing the cortex–membrane movements. The modified equation is given by

\[
\left( a + \delta a_{adh} \right) \frac{\partial}{\partial t} \left( \frac{a L}{\epsilon^2} \right) = \beta + \beta(a) \delta_{FA} - \gamma L a + \delta \frac{\partial}{\partial y} \left( \frac{a L}{\epsilon^2} \right)
\]  

(10)

where \( a \) is a friction coefficient and \( \beta(a) \) is modelled by the function

\[
\beta(a) = \frac{2\beta}{\pi} \text{arctan}(a)
\]  

(11)

This pressure increases with the density of actin and rapidly reaches a plateau corresponding to the maximum of the actin polymerization rate (Fig. 2). The Dirac functions are used to materialize the adhesion sites and are given by

\[
\delta_{adh}(\theta, t) = \delta_{X}(\theta, t) + \delta_{FA}(\theta, t)
\]  

(12)

with

\[
\delta_{X}(\theta, t) = \begin{cases} 1 & \text{if } \theta \text{ corresponds to an adhesion site of type } X \\ 0 & \text{elsewhere} \end{cases}
\]  

(13)

Note that in this model formulation, the adhesions affect only the membrane movements (Eq. (6)) and do not directly affect the actin dynamics (Eqs. (5) and (7)).

### 2.2. Formation and maturation of the adhesions

Three different types of cell adhesions are considered in the model, the adhesion point (A), the focal complex (FX) and the focal adhesion (FA). Each adhesion type corresponds to a particular level of maturation characterized by its lifetime and resistance to traction (Fig. 3). A is thermodynamically unstable and assumed to have a short lifetime and a weak resistance to traction (Nicolas et al., 2004). Nonetheless, it can mature into a moderate traction force, since too strong a traction breaks it.

**Fig. 3.** Diagram showing the adhesion strength (blue curve) and lifetime (red spots) for each adhesion type as used in the coming simulations. Each type of adhesion needs to reach a threshold force to mature to the next level (a positive force for \( A \rightarrow FX \), \( R_{FA} \) for \( FX \rightarrow FA \). \( T_{adh} \) represents the threshold for cell translocation, and \( T_r \), the adhesion protein recycling time.

**Table 1** Dimensionless parameters which define the cell mechanical and chemical properties.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protrusive hydrostatic pressure</td>
<td>( \beta )</td>
<td>0.5</td>
</tr>
<tr>
<td>Actin network elasticity</td>
<td>( \gamma )</td>
<td>0.5</td>
</tr>
<tr>
<td>Cytoplasm viscosity</td>
<td>( \mu )</td>
<td>2.0</td>
</tr>
<tr>
<td>Membrane elasticity</td>
<td>( \epsilon )</td>
<td>1.5</td>
</tr>
<tr>
<td>Curvature-related coefficient</td>
<td>( \delta )</td>
<td>0.001</td>
</tr>
<tr>
<td>Contractile coefficient</td>
<td>( \psi )</td>
<td>5.0</td>
</tr>
<tr>
<td>Saturation density of actin</td>
<td>( a_{sat} )</td>
<td>4.0</td>
</tr>
</tbody>
</table>

2.2.1. Adhesion spot formation

Since migration is unstimulated, we assume that small and weak adhesion spots \( A \) can form spontaneously with
a minimum of conditions, due to energy fluctuations induced by the chemical reactions within the cell (Nicolas et al., 2004). We thus simply assume that the probability to form an adhesion is higher: (i) where the membrane extension is maximum, that is where the cell offers a greater surface of contact with the substrate and (ii) where the amount of actin is sufficient since the density of integrins, which are the main protein constituents of cell to substrate attachment, depends on it.

In the model, $A$ occurs spontaneously at the extremity of the protrusive membrane, where the two aforementioned conditions are fulfilled. That is, where the membrane extension is maximum ($L_{\text{max}}$) with this maximum above a given threshold ($L_{\text{max}} > L_{\text{thr}}$) and associated with a sufficient local amount of actin ($a > a_{\text{thr}}$). The flowchart in Fig. 4 summarizes the different steps of the maturation process.

$A$ is characterized by a short lifetime and weak adhesion strength. It is therefore too weak to support traction forces leading...
to cell translocation. It needs to mature first into a more stable structure (Kaverina et al., 2002).

2.2.2. Maturation process

A matures into FX and then into FA through the incorporation of additional molecular components in a hierarchical way (Zaidel-Bar et al., 2004). The first molecules involved are \( \alpha_5\beta_1 \)-integrin and phosphotyrosine, closely followed by talin and paxillin. As the FX keeps growing, other proteins, such as \( \beta\)-actin, FAK, VASP, Arp2/3, and vinculin are further recruited. This leads to a close connection with the matrix, where the distance between the membrane and the substrate is significantly reduced as observed with interference reflection microscopy (Izzard, 1988). Whereas FX maturation occurs during cell protrusion, the transformation of FX into FA is mechanically induced by an increase in tension from actin filaments (Galbraith et al., 2002) concomitant with the recruitment of zyxin and tensin.

Back in the model, we assume that maturation of A into FX simply occurs if the adhesion is mechanically solicited through tension forces exerted by the actin filaments of the cell cytoskeleton. In order to decide whether an adhesion should grow or not, we define a force-related criterion, by calculating the resulting force \( R_F(\theta_j, t) \) existing at time \( t \) for each adhesion \( j \). This resulting force corresponds to the sum of the contributions of all the individual forces \( F(\theta_j, t) \) balancing the movements of the cortex–membrane complex for each directions \( \theta_j \). These forces are then projected on the \( \theta_j \)-direction supporting the adhesion \( i \) (\( \theta_i = \frac{2\pi i}{m} \) with \( m \) the number of points defining the membrane boundary), namely

\[
R_F(\theta_j, t) = \sum_{i=0}^{m} F(\theta_j, t) \cos(\theta_j - \theta_i) \tag{14}
\]

with

\[
F(\theta_j, t) \text{ such that } \frac{\partial x}{\partial t} + F(\theta_j, t) = 0 \tag{15}
\]

\( F(\theta_j, t) \) is derived from Eq. (10), neglecting the curvature-related term since its contribution is small. It thus comprises: (i) the adhesion-related term responsible for an increased tension between the cell body and the adhesion site, if an adhesion has formed, (ii) the passive tension from the actin filaments existing everywhere in the cell and modulated by the local membrane extension and local density of actin, and (iii) the pressure term, which tends to repel the cell body from the adhesion site. \( F(\theta_j, t) \) for site \( j \) at time \( t \) is given by

\[
F(\theta_j, t) = \delta_{\text{adhesion}}(\theta_j, t) \gamma_2 [L(\theta_j, t + \Delta t) - L(\theta_j, t)] + \gamma_2 L(\theta_j, t) \text{ passive tension pressure force} \tag{16}
\]

with \( \gamma_2 = \alpha / \Delta t \), \( \Delta t \) being the time step of the numerical scheme. Note that \( F(\theta_j, t) \) strongly depends on the nature of the site \( j \). For example, if \( j \) is not an adhesion site then only the passive contributions remain, i.e. \( F(\theta_j, t) = \gamma L(\theta_j, t) a(\theta_j, t) - \beta \).

If the resulting tension force \( R_F(\theta_j, t) \) exerted between the adhesion site and the cell body is positive (i.e. if the force is a traction force) then \( A \) matures into FX. If this condition is not fulfilled during the lifetime \( t_A \) of \( A \), then the adhesion breaks (Fig. 4). Similarly, the maturation of FX into FA occurs if \( R_F(\theta_j, t) \) applied on the adhesion reaches a threshold tension \( R_{thr} \) during the lifetime \( t_{FX} \) of FX. Once FA is formed, recruitment of actin occurs at the adhesion site to promote the formation of stress fibres. Fig. 5 shows an epifluorescent photomicrograph of an adherent cell (B.2), which exhibits the FA and actin cytoskeleton locations and interconnection in the cell. FA are mainly located at the cell periphery and connect the stress fibres, formed by bundles of actin filaments. The fibres contract and contribute in pulling the cell body forward.

2.3. Cell translocation

The model assumes that stress fibres connect the cell body to FA, hence only this adhesion type is considered able to transmit and sustain adequate traction force for effective cell translocation. In order to determine the direction \( \theta_{thr} \) for cell translocation (Fig. 6), the resulting traction force \( T_F \) existing in each stress fibre \( i \) is calculated as follows:

\[
T_F(\theta_j, t) = \sum_{j=0}^{m} \left\{ \gamma_2 [L(\theta_j, t + \Delta t) - L(\theta_j, t)] \right\} + \gamma_2 L(\theta_j, t) a(\theta_j, t) \cos(\theta_j - \theta_i) \tag{17}
\]

The cell translocation then corresponds to the displacement of the cell centroid (i.e. centre of the nucleus) in the \( \theta_{thr} \)-direction for which the resulting traction force \( T_F \) is the greatest. We note that in the translocation process, only the cell centroid is moving while the points forming the membrane do not.

In order to evaluate the amplitude of the displacement of the cell centroid, we simply assume that it is proportional to the resulting calculated force. Such proportionality corresponds to an “elastic release” whereby the stress fibre suddenly breaks once a threshold force \( T_{thr} \) is reached. The displacement \( r \) of the cell centroid is thus given by

\[
T_F(\theta_{thr}, t) = k r \text{ therefore } r = \frac{T_F(\theta_{thr}, t)}{k} \tag{18}
\]

where \( k \) is a coefficient characterizing the elasticity of the medium (stress fibre immersed in the cytosol) (parameters \( T_{thr} \) and \( k \) are given in Table 2).

Fig. 5. Epifluorescent photomicrograph of FA and cytoskeleton of an adherent MCF7 cell. FA are revealed by the staining of paxillin, which is one of the early proteins of FA. Phalloidin stains actin cytoskeleton, whereas DAPI [blue] is used as a DNA marker to reveal the nucleus. Magnification bar corresponds to 20 \( \mu \)m.
cell displacement (left diagram). The cell centroid is pulled in the direction corresponding to the greatest traction force from the filaments anchored to the related focal adhesion. Vector $r$ represents the cell centroid displacement from its initial position (dark grey) to its final position (light grey).

Fig. 6. Snapshot of a simulated cell showing the various adhesion types (A, FX and FA) that have formed at the cell periphery. Only FA contributes in the calculation of the cell displacement (left diagram). The cell centroid is pulled in the direction corresponding to the greatest traction force from the filaments anchored to the related focal adhesion. Vector $r$ represents the cell centroid displacement from its initial position (dark grey) to its final position (light grey).

Table 2
Simulation parameters which define the conditions for the formation and maturation of the cell adhesions and the cell translocation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane extension threshold</td>
<td>$L_{th}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Actin density threshold</td>
<td>$a_{th}$</td>
<td>1.1</td>
</tr>
<tr>
<td>Tension threshold</td>
<td>$R_{th}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Friction coefficient for adhesion</td>
<td>$g$</td>
<td>5.0</td>
</tr>
<tr>
<td>Translocation threshold</td>
<td>$T_{th}$</td>
<td>2.3</td>
</tr>
<tr>
<td>Stress fibre elasticity</td>
<td>$k$</td>
<td>14</td>
</tr>
</tbody>
</table>

3. Results

3.1. Experimental background

Fibroblast cells were chosen as an experimental model, since we have previously studied and characterized the non-migrating pulsating behaviour of this cell type. In addition, these cells are easily isolated and studied on a single-cell basis. The main focus of this paper is the spontaneous migratory behaviour of fibroblasts. Unlike directional migration, spontaneous migration is neither elicited nor guided by a gradient of chemicals or other factors. In our experimental set-up, spontaneous fibroblast migration was elicited nor guided by a gradient of chemicals or other factors. In this paper is the spontaneous migratory behaviour of fibroblasts.

3.2. Numerical simulations

In the simulation of cell migration we take as initial condition a small random perturbation $\varepsilon$ ($|\varepsilon| \leq 0.05$) of the homogeneous steady state $(L, a, v)$, with

\[ L(0, 0) = 1, \quad a(0, 0) = 1 \pm \varepsilon, \quad v(0, 0) = 0 \]  \hspace{1cm} (19)

This corresponds to the state of a cell just after mitosis, where its shape is circular and actin density relatively homogeneous. The model parameters are chosen so as to allow selection of an oscillating state according to the stability analysis of the PDEs system presented in Appendix A.2. This choice of parameters thus corresponds to a Hopf bifurcation point. The parameter $\psi$ representing the contractile activity of the actin network, which is directly deduced from $\mu$, is taken as the bifurcation parameter. In order to destabilize the bifurcation state, the value of $\psi$ is increased, corresponding to an intensification of the actomyosin complex contractility. The parameters $\beta$ and $\gamma$ which represent the protrusive hydrostatic pressure and the elastic coefficient of the actin network respectively, are not involved with the mode selection and are chosen independently.

The model parameters used to simulate spontaneous cell oscillations, select a pulsating state with mode $4$. This state, which involves a membrane pulsation between two perpendicular protrusive directions, corresponds to the spontaneous pulsating state observed and characterized on isolated fibroblast cells (Stéphanou and Tracqui, 2002). These parameters are:

\[ \mu = 2, \quad \varepsilon = 1.5, \quad \delta = 0.001, \quad \beta = \gamma = 0.5, \quad a_{act} = 4, \quad \psi = 5.6 \]  \hspace{1cm} (20)
Cell membrane oscillations and the related actin fluctuations in the cortex can lead locally to the formation of an adhesion spot, if the membrane extension and actin density both reach given thresholds (Table 2). These thresholds reflect the fact that the highest probability to form an adhesion occurs if the membrane offers a greater contact area to the substrate, with a sufficient amount of actin to connect/nucleate the integrins. Then the maturation of the adhesions, according to the rules described in the flowchart (Fig. 4), ultimately leads to cell translocation, that is effective cell migration. All the parameters related to the formation and maturation of the adhesions are given in Tables 2 and 3.

Fig. 10 presents snapshots of migration of the virtual cell. Each snapshot shows: (i) the cell shape, (ii) the cell migratory path (trajectory) and (iii) cell adhesion spots with their corresponding level of maturation (A, F, X or A). The snapshots are not evenly distributed in time, as the aim is rather to show some representative examples of the distribution of the different types of adhesions along the cell membrane during migration.

Three different phases in the migration process can be identified in the simulation (Fig. 10), which correspond to the three different migrating behaviours experimentally observed. First a bi-directional migrating phase (palindromic migration) from snapshot A–C then a slow migrating phase from D to E, where the cell centroid randomly moves with a very small amplitude around an “equilibrium” spot, and finally a fast migrating phase from F to H characterized by a persistent direction of migration.

Fig. 7. Time-lapse microscopy of NIH 3T3 fibroblasts. Cells were seeded on an uncoated polystyrene cell culture dish and their spontaneous migratory tracks were captured every 10 min for 4 h. The phenotypic alterations of a slow (Cell 1), a fast (Cell 2) and a palindromic (Cell 3) migrating fibroblast are depicted in 40-min intervals. Magnification bar represents 20 μm.
Concerning the adhesions, the simulation shows that all the different types often co-exist (Fig. 10D–F). Moreover, several adhesions appear simultaneously since the conditions for adhesion concern an area of the cell membrane more than a single spot (Fig. 10C–F).

In each case, the formation of focal adhesions indicates the direction of the next step of the cell trajectory.

3.2.1. **A posteriori scaling of the model with experimental data**

Spatial and temporal scaling of the model are based on the measurements previously performed on the 3T3 fibroblasts. Specifically, spatial scaling is realized from measurements of the radius of the cell nucleus ($R_0 = 7\, \mu m$). Temporal scaling is performed by fitting the time it takes for the fast migrating cell to move along a given distance. Measurements from the experimental data give a distance of about $140\, \mu m$ covered by the cell in 4 h. During its fast moving phase the virtual cell (Fig. 10E–H) is covering a distance of about $70\, \mu m$ in 50,000 iterations, that we assimilate to 2 h.

With this calibration, the evolutions of the simulated cell speed and the associated cell area (Fig. 11) can be plotted and compared with the experimental measurements. As the model calibration was performed from the fast migrating phase, the
Concerning the mean cell area, the value measured for the simulated cell is 730 \mu m^2. As observed experimentally, the minimum cell speed decreases drastically from 24% to 15% for \( g_{\text{min}} = 31 \mu m/min \). The mean cell speeds measured for the palindromic and slow phases of the simulated cells are 0.60 \pm 0.14 \mu m/min. The mean cell speeds measured for the short recycling time, 24% of the cell adhesions just broke, therefore the conditions for rapid formation and growth of new adhesions are optimal. On the contrary, when the recycling time \( T_r = 115 \text{s} \) lasts longer, the cell is given more time to fully restabilize its shape. This leads to a much slower cell speed of 0.35 \mu m/min. Interestingly, the decrease in the cell speed is not linear to the increased recycling time but rather occurs stepwise.

To better understand how the recycling time of adhesion proteins influences cell speed through the formation and maturation of adhesions, the distribution of the different types of adhesions \((A, FX, and FA)\) developed by the cells is presented in Fig. 14 for three different recycling times, namely short \((T_r = 14 \text{s})\), long \((T_r = 115 \text{s})\) and intermediate \((T_r = 72 \text{s})\). Each case corresponds to a migration time of 6h. The simulation results show that for the short recycling time, 24% of the cell adhesions matured to FA, 30% of the adhesions remain at stage A and 45% do not maturate beyond the FX stage. Increasing the recycling time first leads to a marked decrease in the amount of cell adhesions with the substrate. Specifically, 1.5 and 1.8 times less adhesions are formed during intermediate and long recycling times respectively. The amount of adhesions reaching the FA stage also decreases drastically from 24% to 15% for \( T_r = 72 \text{s} \) and only 9% for \( T_r = 115 \text{s} \). For the intermediate recycling time, the same amount of adhesions remains at stage A (30%) but less adhesions maturate to stage FA (15%), whereas for the longer recycling time the maturation becomes even more difficult at an earlier stage as more adhesions remain at stage A (40%).

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Iterations</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion proteins recycling time</td>
<td>( T_r )</td>
<td>400</td>
<td>58</td>
</tr>
<tr>
<td>Adhesion point lifetime</td>
<td>( \tau_A )</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>Focal complex lifetime</td>
<td>( \tau_{FX} )</td>
<td>500</td>
<td>72</td>
</tr>
<tr>
<td>Focal adhesion lifetime</td>
<td>( \tau_{FA} )</td>
<td>500</td>
<td>72</td>
</tr>
</tbody>
</table>

The parameter \( \gamma_2 \) appears as a friction coefficient in the model equations. Consequently, it can be interpreted as a representation of the strength of the adhesion of the cell to its substrate. The influence of this parameter on the cell speed is evaluated through simulations. Fig. 12 shows that the increase in the adhesion strength from \( \gamma_2 = 5 \) to 30, first leads to an increase in the cell speed from 0.51 up to 0.64 \mu m/min. However, further increase in \( \gamma_2 \) decreases the cell speed to 0.54 \mu m/min. This biphasic dependency of the cell speed on the adhesion strength is in complete agreement with well-established experimental results (Palecek et al., 1997). Palecek and colleagues demonstrated that cell speed increased to fibronectin concentration. However, when fibronectin concentration became too high, the attachment of the cell to the substrate was so great that migration was impeded.

#### 3.2.2. Influence of the adhesion strength on cell speed

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#### 3.2.3. Influence of the recycling time of adhesion proteins on cell speed

The recycling time of adhesion proteins \((\text{e.g. integrins}) \ (T_r)\) corresponds to a refractory period during which the cell remains unable to form new adhesions with the substrate. This event occurs immediately after cell translocation when all adhesions are released. Fig. 13 shows that the cell speed decreases with increased recycling time. For a short recycling time \( T_r = 14 \text{s} \), the cell is moving much faster with a speed reaching 0.91 \mu m/min. This is due to the fact that the cell remains partially polarized with higher actin density at the leading edge, where the focal adhesions just broke, therefore the conditions for rapid formation and growth of new adhesions are optimal. On the contrary, when the recycling time \( T_r = 115 \text{s} \) lasts longer, the cell is given more time to fully restabilize its shape. This leads to a much slower cell speed of 0.35 \mu m/min. Interestingly, the decrease in the cell speed is not linear to the increased recycling time but rather occurs stepwise.

To better understand how the recycling time of adhesion proteins influences cell speed through the formation and maturation of adhesions, the distribution of the different types of adhesions \((A, FX, and FA)\) developed by the cells is presented in Fig. 14 for three different recycling times, namely short \((T_r = 14 \text{s})\), long \((T_r = 115 \text{s})\) and intermediate \((T_r = 72 \text{s})\). Each case corresponds to a migration time of 6h. The simulation results show that for the short recycling time, 24% of the cell adhesions matured to FA, 30% of the adhesions remain at stage A and 45% do not maturate beyond the FX stage. Increasing the recycling time first leads to a marked decrease in the amount of cell adhesions with the substrate. Specifically, 1.5 and 1.8 times less adhesions are formed during intermediate and long recycling times respectively. The amount of adhesions reaching the FA stage also decreases drastically from 24% to 15% for \( T_r = 72 \text{s} \) and only 9% for \( T_r = 115 \text{s} \). For the intermediate recycling time, the same amount of adhesions remains at stage A (30%) but less adhesions maturate to stage FA (15%), whereas for the longer recycling time the maturation becomes even more difficult at an earlier stage as more adhesions remain at stage A (40%).
In all three cases, the fraction of adhesions that effectively lead to cell translocation represents only 1% of the total amount of cell-to-substrate adhesions. However, small, this fraction remains coherent with observations on unstimulated fibroblasts, which mainly exhibit membrane fluctuations rather than effective migration.

3.2.4. Influence of the adhesions lifetimes on the cell speed

Each adhesion type is characterized by a limited lifetime. Adhesion lifetime increases with the adhesion maturation level, as adhesions become progressively more stable. This means that $\tau_A < \tau_{FX} \leq \tau_{FA}$. To evaluate the influence of adhesion lifetime on cell speed, simulations were run for a range of values for $\tau_A$ with increasing magnitude $m$ for $\tau_{FX} = m\tau_A$ relative to $\tau_A$. In other words, $\tau_{FX} = \tau_{FA} = m\tau_A$, with $m = 2, 3$ and 5 successively.

We note that in our simulations the same lifetime value is consistently taken for $\tau_{FX}$ and $\tau_{FA}$. This stems from the conditions we defined for cell translocation, where a threshold tension needs to be reached. This threshold has to be reached within a few iterations after the formation of a focal adhesion at a site of optimal conditions. Otherwise the threshold is never reached and further maintaining the focal adhesion causes numerical instabilities.

Fig. 15 presents curves obtained for three different magnitude $m$. It appears that changes between the different magnitudes of...
Focal adhesion lifetimes for the three curves in Fig. 15 for lifetimes has a homogenizing effect (mental data). An autocorrelation analysis (Dunn and Zicha, 1995) trajectories considered are small (23 points only for the experimental data). The cells sense these physical and chemical cues in their microenvironment, process the information and respond appropriately by changing their morphology and/or motility pattern. Numerous proteins are actively produced and recruited to generate the cell’s response. In other words, the “cell protrusive machinery” is switched on. Although most migratory phenomena are induced and directionally guided in vivo, random migration in...
vitro is of great interest to study the cell state, without external stimulation, when the cell is considered unactivated and its protrusive machinery switched off.

Additionally, directional cell movement is orchestrated by different effector molecules than those involved in random migration, thus different mechanisms are activated. For instance, pressure-driven membrane protrusion through cytoplasmic flux (Charras et al., 2005; Abraham et al., 1999; Bereiter-Hahn and Luers, 1998) was for long opposed to actin polymerization-driven protrusion (Borisy and Switkina, 2000; Carlier and Pantaloni, 1997; Theriot and Mitchison, 1991), when in fact these two processes co-exist. Specifically, pressure-driven protrusion is predominant in resting cells (i.e. non-migrating cells), where spontaneous actomyosin contractility remains the main cell activity. In contrast, actin polymerization-driven protrusion is predominant in activated cells, where a number of actin regulation proteins are recruited to produce efficient actin network turnover (with induced branching, treadmilling, etc.) (Carlier and Pantaloni, 2007; Borisy and Switkina, 2000). It is thus important to identify the cell dynamic state. Hence, investigation of the unactivated (unstimulated) state of a cell is fundamental if one wants to properly describe the cell reaction to an external signal.

Unstimulated cells have been shown to exhibit spontaneous membrane protrusions, oscillating and spatio-temporally organized, with the appearance of specific cell shapes, and periodically repeating patterns (Germain et al., 1999; Stéphanou et al., 2004; Giannone et al., 2004). A simple mathematical model based on actin turnover and contractility, where pressure-driven protrusion competes with actin filaments retraction on the membrane, has allowed us to reproduce typical self-organized features of fibroblasts’ spontaneous movements (Stéphanou et al., 2004). In this paper, we proposed to extend this model by coupling the spontaneous membrane deformations with the formation of adhesions, essential for cell migration.

Energy fluctuations can account for the formation of simple adhesion spots (Nicolas et al., 2004). However, to generate the force required for cell translocation, i.e. to pull the cell forward, actin recruitment at the cell’s leading edge is necessary to increase the traction force from the filaments and overcome the adhesion force. Thus our model proposes a “protrusive switch”, whereby passive hydrostatic pressure on the membrane is reinforced by an active actin polymerization-induced pressure. This process occurs at the adhesion site, once the adhesion matures to a focal adhesion. This phenomenon is considered autocatalytic, since cell contractility is responsible for the maturation of the adhesion, which progressively leads to the protrusive switch.

Numerical simulations performed show that the model is able to reveal the main features of fibroblast cell migration. That includes alternating phases where the cell can either explore a very short perimeter (slow migration), move bi-directionally (palindromic migration) or transiently assume a persistent directional migration (fast migration). In agreement with our experimental data, simulations also show that the cell speed is correlated with the maximum cell area, as a consequence of the protrusive switch phase.

To further validate the model, we verified the influence of adhesion strength upon the cell speed. It is well known that cell speed and adhesion strength have a biphasic relationship. Specifically, cell speed increases with the adhesion strength until further increase in cell adhesion strength hinder migratory speed (Palecek et al., 1997). The model managed to reveal this relationship within the authorized numerical limits.

The influence of the temporal parameters of the model, more specifically the adhesion proteins recycling time and the adhesion lifetimes were also investigated. In the model, we made the simplifying assumption that all adhesions are released while the cell translocates (Kirfel et al., 2004). The adhesion proteins recycling time induces a refractory period immediately after translocation. This corresponds to the time during which the virtual cell is not allowed to make new adhesion with the substrate. A longer recycling time means that the cell is given more time to recover its spontaneous pulsating state. Once this state is reached it becomes more difficult to destabilize the cell, that is to recreate the conditions for migration. This implies that...
the cell is potentially more reactive, with a quicker migration speed, when in a perturbed state rather than in equilibrium. This happens because actin distribution is more heterogeneous, which favours the formation of focal adhesions, thus promoting migration.

Interestingly, increasing the adhesion lifetime has the opposite effects on migration speed. Specifically, increasing the adhesion lifetime increases the probability that an adhesion will reach the next level of maturation. Then, tension forces have more time to develop and produce efficient cell translocation, resulting in higher migration speeds. Simulations also suggest that the persistence in the direction of migration can be affected by the choice of the relative adhesion lifetimes.

Actin and adhesion dynamics are interrelated (Gupton and Waterman-Storer, 2006), with complex relationships between intracellular events (actin turnover, maturation of the adhesions, recycling of the proteins, etc.). The model demonstrates that alterations of the temporal parameters can modify the spontaneous cell migration behaviour, thus providing with insight on how the cell would potentially react to an external stimulation. A future step in this direction is to investigate the explicit coupling between the cytoskeleton elements with the extracellular matrix through its mechanical and topographical properties. Such a model is essential for disciplines such as tissue engineering and the developing nano-patterning technologies, since it will provide the conceptual framework required to interpret the growing body of experimental observations of the individual and collective cell behaviours on micro- and nano-patterned substrates.

Acknowledgements

We would like to thank Dr. G. Vassilopoulos (IIBEA, Athens, Greece) for kindly providing the NIH 3T3 and Dr. O. Collin for providing the fibroblast cell image used in Fig. 1. This work was partially supported by MIRG-CT-2006-044992 European Commission FP6 Marie Curie Actions to EM and MRTN-CT-2004-503661 M3CS-TU TH European Commission FP6 Marie Curie Actions.

Appendix A. Non-dimensionalization and linear stability analysis

A.1. Non-dimensionalization

The system of PDEs Eqs. (1)–(3) is non-dimensionalized by setting the following dimensionless variables:

\[ \tilde{t} = \frac{t}{\Lambda}, \quad \tilde{a} = \frac{a}{a_0}, \quad \tilde{L} = \frac{L}{a_0} \]

The normalized parameters are then

\[ \tilde{\beta} = \frac{\beta}{\gamma_1 \Phi_1 \Phi_0 \Phi_0}, \quad \tilde{\gamma} = \frac{\gamma_1}{\Phi_1 \Phi_0}, \quad \tilde{\rho} = \frac{\rho}{\Phi_0 \Phi_0}, \quad \tilde{\psi} = \frac{\psi_0 \Phi_0}{\Phi_0 \Phi_0} \]

\[ \tilde{a}_{at} = \frac{a_{at}}{a_0}, \quad \tilde{\tau} = \frac{a}{\Phi_0 \Phi_0}, \quad \tilde{\delta} = \frac{\gamma_1 \Phi_0}{\Phi_0 \Phi_0} \]

Dropping the tildes for notational simplicity, we obtain the following system of dimensionless PDEs:

\[ \frac{\partial (La)}{\partial t} = \frac{\partial (Lav)}{\partial \tilde{t}} + L(1 - a) \] (21)

\[ \frac{\partial L}{\partial \tilde{t}} = \tilde{\beta} - \gamma L a \tilde{a} + \left( \alpha \phi \tilde{a} + \phi \sigma(a) \right) \] (22)

\[ \frac{\partial a}{\partial \tilde{t}} = \frac{\partial a}{\partial \tilde{a}} \sigma(a) - \frac{\partial a}{\partial \tilde{a}} \left( \alpha \phi \tilde{a} + \phi \sigma(a) \right) - \frac{\partial \tilde{a}}{\partial \tilde{a}} \left( \alpha \phi \tilde{a} + \phi \sigma(a) \right) \] (23)

\[ \sigma(a) = \psi \phi a e^{-\phi a} \] (24)

A.2. Linear stability analysis

The linear stability analysis of the dimensionless system (Eqs. (21)–(23) for periodic boundary conditions) considers the behaviour of small perturbations around the homogeneous steady state, which is given by

\[ L_1 = \frac{\beta}{\gamma}, \quad a_1 = 1, \quad v_1 = 0 \] (25)

We then look for each variable \( L, a, \) and \( v, \) a solution with the general form:

\[ w - w_1 = e^{\xi t + i \omega t} \] (26)

where \( m \) represents the spatial mode of deformation. By substitution in the linearized system of equations one obtains

\[ \begin{pmatrix} \lambda + \gamma + m^2 \beta t \\ \beta \gamma (1 + \lambda) \\ im \beta t \\ a - a_1 \end{pmatrix} = 0 \] (27)

where \( \rho = \frac{a}{a_0} \). If \( M \) denotes the matrix above, the dispersion relation between \( \xi \) and \( m \) which has to be satisfied for the system to have a non-trivial solution is given by

\[ \det(M) = (\lambda + \gamma + m^2 \beta t)(1 + \lambda)(1 + \mu m^2) - m^2 \rho \frac{\beta}{\gamma} = 0 \]

\[ \frac{\partial}{\partial \tilde{a}} \lambda^2 + \left( m^2 \beta t + 1 - \frac{m^2 \rho}{1 + \mu m^2} \right) + \frac{m^4 \beta t}{1 + \mu m^2} \]

\[ + \left( \gamma + m^2 \beta t \right) \left( 1 - \frac{m^2 \rho}{1 + \mu m^2} \right) = 0 \]

The roots of the dispersion relation above, which give the temporal evolution of perturbations with spatial mode \( m \), are given by the following pair of eigenvalues of the matrix \( M \):

\[ \lambda(m) = \frac{1}{2} \left[ -1 + \frac{m^2 \rho}{1 + \mu m^2} - m^2 \beta t \right] \]

\[ \pm \frac{1}{2} \sqrt{\left( 2\gamma - 1 - \frac{m^2 \rho}{1 + \mu m^2} + m^2 \beta t \right)^2 - 4 \gamma \left( \gamma + m^2 \beta t \right) - 4 \frac{m^4 \beta t}{1 + \mu m^2} } \] (28)

A.2.1. Conditions for the existence of an oscillating state

A simple way to look for self-sustained oscillations of the plasma membrane is to find model parameters which correspond to a destabilization of the uniform steady state of the variable \( L \). If this destabilization occurs through a Hopf bifurcation, then standard theory predicts the existence of limit cycle type solutions. Here, we are interested in spatially non-uniform solutions corresponding to the appearance of extending/retracting protrusions along different axes. These axes will be determined by the existence of unstable spatial modes for the cytomechanical model solutions. A Hopf bifurcation then occurs for complex eigenvalues, i.e. when \( \text{Re} \lambda(m) = 0 \). The value of \( m \) which maximizes the function \( \text{Re} \lambda(m) \) is given by

\[ m_0 = \sqrt{\frac{1}{\rho} \left( \sqrt{\frac{\rho}{\gamma} - 1} \right)} \] (29)
medium evaporation. The plate was then placed on a heated stage plate tip and its inverted lid with silicon grease to prevent HEPES to completely fill the plate. A seal was created between the (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, were fixed with 4% paraformaldehyde at room temperature for low serum medium (DMEM + GM). Following 2 h to allow for cell adhesion, GM was replaced by a experimental day, cells were trypsinized and re-plated on uncoated polystyrene P35 plates at a density of 1 x 10^5 cells/P35 in GM. Following 2 h to allow for cell adhesion, GM was replaced by a low serum medium (DMEM + 0.5% FBS) buffered with 25 mM HEPES to completely fill the plate. A seal was created between the plate tip and its inverted lid with silicon grease to prevent medium evaporation. The plate was then placed on a heated stage electronically maintained at 37°C (eba 100 mc-L, Lastungselektronik JENA GmbH, Germany). Digital images of migrating cells were acquired every 10 min for a total of 4 h using a Leica DM IL microscope equipped with a DFC 480 DCC digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Photoexposure of the cells between recording intervals was minimized to avoid phototoxicity.

We note that the coefficients β and γ, which represent the protrusive hydrostatic pressure and the elasticity coefficient of the network controlling the retraction of the lamellipod, respectively, have no influence on the modes selection. The key parameters are the cytoplasm viscosity μ and the actin–membrane complex elasticity c controlling the surface tension. As intuitively expected, large values of c and μ, which both tend to increase the stiffness of the system correspond to small value of m0, i.e. to a small number of potential membrane protrusions. Conversely, large values of the parameter ρ (i.e. ψ), which monitors the contractility of the F-actin network, favour the destabilization of a larger number of spatial modes (Fig. 17).

Appendix B. Cell culture, immunostaining, and image analysis

B.1. Cell culture

NIH 3T3 fibroblasts were maintained in DMEM + 10% FBS + P/S (GM) at 37°C and 5% CO2 in a humidified chamber. On the experimental day, cells were trypsinized and re-plated on uncoated polystyrene P35 plates at a density of 1 x 10^5 cells/P35 in GM. Following 2 h to allow for cell adhesion, GM was replaced by a low serum medium (DMEM + 0.5% FBS) buffered with 25 mM HEPES to completely fill the plate. A seal was created between the plate tip and its inverted lid with silicon grease to prevent medium evaporation. The plate was then placed on a heated stage electronically maintained at 37°C (eba 100 mc-L, Lastungselektronik JENA GmbH, Germany). Digital images of migrating cells were acquired every 10 min for a total of 4 h using a Leica DM IL microscope equipped with a DFC 480 DCC digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Photoexposure of the cells between recording intervals was minimized to avoid phototoxicity.

B.2. Immunocytochemistry and staining

For immunocytochemistry and staining, cells from a human mammary adenocarcinoma cell line (MCF7) were used. Cells were maintained as described above and seeded (1 x 10^4 cells/cm²) on glass coverslips coated with 0.15 mg/ml Collagen I. After overnight incubation in a humidified environment at 37°C and 5% CO2, cells were fixed with 4% paraformaldehyde at room temperature for 15 min, followed by a 2 min incubation in permeabilization buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 mM NaCl, 3 mM MgCl₂, 0.5% Triton X-100, and 300 mM sucrose in PBS). Non-specific staining was blocked with 0.1% Triton X-100 and 10% goat serum for 1 h at room temperature. Following incubation with a mouse monoclonal anti-paxillin antibody (1:750 in 0.04% Triton X-100 and 1% bovine serum albumin) at 4°C overnight, cells were incubated with Alexa Fluor 594 goat anti-mouse secondary antibody (1:200) and Alexa Fluor 488 phalloidin (1:200) for 1 h in the dark to reveal focal adhesions and actin cytoskeleton, respectively. Cell nuclei were visualized by the addition of 1 μg/ml 4’,6-diamidino-2-phenylindole (DAPI) in the final wash. Coverslips with cells were mounted with Pro-Long Gold antifade reagent and sealed. Focal adhesions, actin cytoskeleton, and cell nuclei were observed under a Leica DML microscope equipped with epifluorescence and images acquired with a Leica DFC 480 DCC digital camera and Leica imaging software (LAS: Leica Microsystems). The secondary antibody, phalloidin and Pro-Long antifade reagent were purchased from Molecular Probes (Carlsbad, CA, USA), whereas all other reagents and chemicals were purchased from SIGMA (St. Louis, MO, USA).

B.3. Analyses

Migration analyses were performed on cells that remained in the field of view for the entire recording period. Cells actively proliferating or contacting other cells during imaging were excluded from the analyses. The outline, centroid (i.e. centre of nucleus), and migratory track were manually drawn for each cell for the entire recording period using Adobe Photoshop version 7.0 (Adobe Systems Incorporated, San Jose, CA, USA). Adjustments of image brightness and contrast were performed equally throughout an entire image. Migration distance and cell area were measured using ImageJ software with calibrated settings (NIH, Bethesda, MD, USA). Cell speed (μm/min) was accordingly calculated. Graphs were generated using GraphPad Prism version 4.0.


