A computational model of amoeboid cell migration

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We present a two-dimensional computational model of amoeboid cell migration characterised by cell shape changes due to the formation and extension of protrusions known as blebs. Using this model, we numerically study the deformation of the cell membrane during blebbing, as well as the effects of obstacles, such as protein fibres in the extracellular matrix, on the motion of the blebbing cell. The model is established in the framework of Stokes flow. Cell membrane deformation is coupled to membrane tension, membrane bending, membrane–cortex adhesion and cortical activities via the intracellular and extracellular fluid field described by the Stokes equation. By assuming that actin monomers move at constant speed towards the membrane and polymerise when they approach the membrane, our model shows that the cell movement in unconfined space can be sustained. We also study how a migrating cell interacts with obstacles hydrodynamically, allowing us to model cell migration in confined environments and to investigate the effects of confinement on the cell migration speed. Our model can be used to further study how tumour cells move through the extracellular matrix during cancer metastasis.

Keywords: cell migration; cellular blebs; amoeboid protrusions; Stokes flow; boundary integral method

1. Introduction

Cell migration plays an indispensable role in the development and maintenance of multicellular organisms. Many vital processes such as wound healing, germ cell migration during embryonic development and organogenesis require the precise movement of cells to specific locations. The failure of cells to migrate properly has serious consequences such as birth defects during development, ineffective wound repair or even the metastasis of tumours. As such, a deeper understanding of the underlying mechanism by which cells migrate may lead to the development of new therapeutic strategies for controlling invasive tumour cells.

In general, cells migrating in vitro in the extracellular matrix or in vivo on a substrate can be broadly classified into either the mesenchymal mode or the amoeboid mode, based on the morphology of the migrating cell. Figure 1 depicts cell morphologies of the two migration modes as the cell moves in the extracellular matrix. Mesenchymal cell migration is associated with sheet-like and spindleshaped membrane protrusions called lamellipodia and filopodia, respectively, that are supported by polymerising actin filaments. They are characterised by the existence of stress fibres, focal adhesions and strong cell–matrix or cell–substrate adhesion. While moving through the extracellular matrix, mesenchymal cells may remodel the extracellular matrix via pericellular proteolysis, in order to make space for the cell to go through the matrix. However, amoeboid cell migration is associated with rounded cell membrane protrusions called blebs that are powered by hydrodynamical cytoplasmic flow. These membrane protrusions are void of cytoskeletal elements such as stress fibres. Adhesion between an amoeboid cell and the extracellular matrix or the substrate is minimal. Instead of going through the matrix via pulling and pushing force generated through cell–matrix adhesion like a mesenchymal cell, an amoeboid cell anchors itself to the matrix via formation of blebs and squeezes through the matrix via contraction of cell body. Unlike mesenchymal cell migration, pericellular proteolysis is not observed in amoeboid migration (Friedl and Wolf 2003, 2010).

Amoeboid protrusions are regarded as an important mechanism of cell motility after blebs were observed in the migration of some tumour cells through three-dimensional matrices (Friedl and Wolf 2003; Sahai and Marshall 2003; Wolf et al. 2003), in the migration of zebrafish primordial germ cells during gastrulation (Blaser et al. 2006), in the migration of neutrophils in circulation, as well as during the chemotaxis of slime mold, *Dictyostelium discoideum* (Yoshida and Soldati 2006). In some cells, bleb protrusions can cooperate with mesenchymal protrusions to power cell motility (Yoshida and Soldati 2006; Diz-Munoz et al. 2010). Some cells can carry out mesenchymal–amoeboid transition to switch between the two protrusion modes depending on their environment (Friedl and Wolf 2003; Sahai and Marshall 2003; Wolf et al. 2003; Bergerta et al. 2012). However, in contrast to the numerous studies that had been carried out for mesenchymal migration, how bleb
protrusions contribute to amoeboid migration is still not well studied. There have been previous studies on modelling the formation and dynamics of cellular blebs (Young and Mitran 2010; Lim et al. 2012; Strychalski and Guy 2012), but the question of how bleb formation eventually leads to cell motility is still not fully addressed.

Computational models have become increasingly important in unravelling the intricacies of cell migration due to the complex nature of the migration process. For example, a quantitative model that couples the stretch-sensitive proteins in the lamellipodia to the dynamics of the actin cytoskeleton has allowed for the prediction of the various phases of mesenchymal migration dynamics possible (Lai and Chiam 2011). A computational model to simulate a cell’s entry into a microchannel has also managed to reproduce observations in agreement with experiments (Leong et al. 2011). In addition, single-cell migration modelling including cell–matrix interactions with the individual matrix fibres has provided quantitative measures of the impact of extracellular matrix on cell migration (Schluter et al. 2012). Computational methodologies that employ the phase field method to model moving cell membranes under the effect of actin polymerisation, myosin contraction and cell–substrate adhesion have also allowed the study of mesenchymal cell motion and cell morphology (Shao et al. 2012).

In this article, we propose a computational model of bleb-associated cell motility. The model allows us to study bleb formation and dynamics and bleb-induced cell migration. In addition, we also use it to study the hydrodynamic interaction of the migrating cell with obstacles such as the protein fibres in the extracellular matrix or the confining walls of a microchannel. In Section 2, we describe the mathematical formulation of the model and the computational method used to solve the model, first for an unconfined cell free from any obstacles and then for a cell interacting hydrodynamically with obstacles. In Section 3, we present numerical results for bleb dynamics, bleb-induced cell migration and cell migration in confined microchannels. In Section 4, we discuss the implications of our findings and future applications of our computational model. Finally, we present our conclusions in Section 5.

2. Computational model and method

Bleb dynamics can be characterised into three stages: bleb nucleation, bleb growth and bleb retraction (Blaser et al. 2006; Charras 2008; Charras and Paluch 2008; Charras et al. 2008). Nucleation of a bleb may be caused by localised variations in the intracellular hydrostatic pressure that tear the cell membrane from the underlying actin cortex, by local weakening of cortex–membrane adhesion, or by local rupture of the actin cortex. Such localised effects can be caused by cortical instability or polarisation triggered by a variety of intracellular or extracellular cues (Paluch et al. 2006). Once a bleb is nucleated, excessive cytoplasmic pressure drives the detached cell membrane to expand and grow. Bleb growth is accommodated by further delamination of cell membrane from the cortex, flow of lipid into the bleb through the bleb neck and unwrinkling of excess folded membrane. Actin cortex that is not attached to cell membrane depolymerises during bleb growth and later polymerises beneath the bleb membrane when bleb growth slows down. Myosin-driven contraction of the reformed cortex retracts the bleb. At the same time,
2.1 Model for cells migrating in unconfined environment without obstacles

First, we will discuss the scenario of a blebbing cell that is free to migrate in an unconfined environment free of any obstacles. For simplicity, we will consider a cell suspended in an incompressible viscous fluid in an infinite two-dimensional domain. While our model can be extended to three dimensions, here we limit ourselves to the simplest geometry in two dimensions where we can still capture many of the qualitative trends. We restrict our model to the case where a bleb is nucleated due to local weakening of cortex–membrane adhesion and where bleb growth is supported only by further delamination of cell membrane from the actin cortex. In our model, a cell is represented by an elastic actin cortex with cortical tension $T_c$ and bending stiffness $B_c$ that is enclosed by an elastic cell membrane characterised by membrane tension $T_m$ and bending stiffness $B_m$. The cell membrane is uniformly adhered to the actin cortex, and the membrane–cortex adhesion is assumed to be Hookean. The spring constant of membrane–cortex adhesive springs is calculated from the adhesion energy $E_{ad}$ as $k_{ad} = \sqrt{2E_{ad}/\ell_c}$, where $\ell_c$ is the maximum length of membrane–cortex adhesive molecules, beyond which the membrane–cortex adhesion breaks down. The cytoplasm and extracellular fluid are assumed to be incompressible and to have the same viscosity $\mu$.

Due to the low Reynolds number of the cytoplasm, viscous forces dominate and inertia forces can be neglected. The cytoplasmic velocity field $\mathbf{u}$ and pressure field $p$ at any instant of time can therefore be described by the Stokes equation and the equation of continuity:

\begin{align}
\nabla p &= \mu \nabla^2 \mathbf{u} + \mathbf{f}, \\
\nabla \cdot \mathbf{u} &= 0
\end{align}

subjected to body force $\mathbf{f}$ that arises due to membrane bending $f_{bm}$, membrane tension $f_{m}$, and membrane–cortex adhesion $f_{ad}$. Calculation of these forces will be illustrated in detail in the later part of this section. The motion of the membrane at position $\mathbf{r}_m$ can be obtained from the no-slip boundary condition imposed on the Stokes equation, i.e.

$$\mathbf{u} |_{\mathbf{r}_m} = \frac{d\mathbf{r}_m}{dt}.$$  

The actin cortex is assumed to be permeable and to interact with the cell membrane only via the membrane–cortex adhesion term $f_{ad}$. The motion of the actin cortex at position $\mathbf{r}$, is obtained by solving for the force balance on a cortical element, assuming a uniform cortical viscosity $\nu_c$,

$$f_{bc} + f_{lc} + f_{ad} + f_{com} = \nu_c \frac{dr_c}{dt},$$

where $f_{bc}$ and $f_{lc}$ are the cortical bending force and cortical tension force, respectively. In addition, a compression force, $f_{com}$, is exerted on each cortical spring in the tangential direction to model the cortical pre-stress. A schematic of the model and forces involved are shown in Figure 2(a), (b).

During bleb growth, the part of the actin cortex that was detached from the cell membrane was observed to depolymerise and to later polymerise beneath the bleb membrane (Charras et al. 2006; Charras 2008; Charras et al. 2008). Reformation and contraction of the new actin cortex leads the bleb to retract. In order to incorporate the depolymerisation and polymerisation of actin cortex during bleb retraction, we introduce the concept of diffusive cortical elements into the model. These diffusive cortical elements are the cortical elements that are detached from the cell membrane upon breakage of membrane–cortex adhesive springs. Instead of following Equation (4), we assume that these elements move towards the bleb membrane with a constant speed $v_c$ in the direction towards the corresponding membrane elements that were attached to the cortex. Physically, these diffusive elements represent the actin monomers that make up the reformed cortex underneath the bleb membrane while $v_c$ is the speed of the actin monomers moving into the bleb. Once the actin monomers are within a distance $D_{eq}$ from the cell membrane, membrane–cortex adhesion is reformed, and the newly formed actin cortex is integrated into the original cortex of the main cell body. Cortical tension will then drive the bleb to retract. Schematics of bleb formation, cortical reformation and bleb retraction for a cell that is confined in a microchannel are illustrated in Figure 2(c)–(f). In our model, the volume enclosed by the cell membrane is conserved due to incompressibility of the fluid, but the volume between the cortex and cell membrane need not be conserved as the cortex is permeable and can expand or contract.

2.2 Computational method

A cell of size $r_m$ initially takes a two-dimensional circular shape with its intracellular pressure higher than the surrounding environment. In order to make sure that the cell is in a stable state before any bleb formation event, the system is first allowed to equilibrate for a sufficient amount of time. After equilibration, bleb nucleation is initiated by instantaneous detachment of a small patch of membrane–cortex adhesion, under the assumption that a bleb nucleates due to local weakening of cortex–membrane adhesion. The loss of equilibrium in forces...
results in cytoplasmic flow towards the direction of the detached membrane. Bleb growth is supported by the bending and stretching of membrane and further detachment of membrane from the cortex when the total energy exceeds the adhesive energy $E_{ad}$.

The cell membrane is parameterised in the deformed configuration by $r_m(\zeta) = (x_m(\zeta), y_m(\zeta))^T$, with $\zeta = [0, L_m]$, where $L_m$ is the perimeter of the membrane. Similarly, the actin cortex is parameterised in the deformed configuration by $r_c(\zeta) = (x_c(\zeta), y_c(\zeta))^T$, with $\zeta = [0, L_c]$, where $L_c$ is the perimeter of the cortex. As mentioned earlier, the total body force $f(r)$ in Equation (1) consists of the forces from membrane bending $f_{bm}$, membrane tension $f_{tm}$ and membrane–cortex adhesion $f_{ad}$. This total body force is, therefore,

$$f(r) = \int_0^{L_m} f_m(r_m) \delta(r - r_m) d\zeta$$

$$= \int_0^{L_m} [f_{bm}(r_m) + f_{tm}(r_m) + f_{ad}(r_m)] \delta(r - r_m) d\zeta,$$

where $\delta(r)$ is the two-dimensional Dirac delta function which is 1 if $r = r_m$ and 0 otherwise. In terms of the
bending energy density \( E_b = B y^2 / 2 \) with curvature 
\( \gamma = (x^4 y^4 - y^4 x^4) / (x^2 + y^2)^{3/2} \), the stretching energy density 
\( E_t = T e^2 / 2 \) with strain \( e = \sqrt{x^2 + y^2} - 1 \), and the membrane–cortex spring constant \( k_{ad} \), the bending forces, tensile forces and adhesive forces in Equations (4) 
and (5) can be evaluated in a straightforward way:

\[
\begin{align*}
\mathbf{f}_j(r_j) &= - \left[ \left( \frac{\partial E_{b,j}}{\partial x_j} - \frac{\partial \phi_j}{\partial x_j}, \frac{\partial E_{b,j}}{\partial y_j} - \frac{\partial \phi_j}{\partial y_j} \right) - \left( \frac{\partial E_{b,j}}{\partial x_j} - \frac{\partial \phi_j}{\partial x_j}, \frac{\partial E_{b,j}}{\partial y_j} - \frac{\partial \phi_j}{\partial y_j} \right) \right], \\
\mathbf{f}_j(r_j) &= - \left[ \left( \frac{\partial E_{t,j}}{\partial x_j} - \frac{\partial \phi_j}{\partial x_j}, \frac{\partial E_{t,j}}{\partial y_j} - \frac{\partial \phi_j}{\partial y_j} \right) - \left( \frac{\partial E_{t,j}}{\partial x_j} - \frac{\partial \phi_j}{\partial x_j}, \frac{\partial E_{t,j}}{\partial y_j} - \frac{\partial \phi_j}{\partial y_j} \right) \right], \\
\mathbf{f}_{ad}(r_m) &= -k_{ad}(r_m - r_c), \\
\mathbf{f}_{ad}(r_c) &= -\mathbf{f}_{ad}(r_m).
\end{align*}
\]

In these equations, the index \( j \) represents either \( m \) for cell membrane or \( c \) for actin cortex. For numerical calculation, both the cell membrane and actin cortex are represented by \( N \) discrete points. The discretised membrane points and 
cortical points are denoted by \( r_m \) and \( r_c \), for \( i = 1, 2, \ldots, N \). Various forces involved are then calculated by 
treating the derivatives in Equations (6)–(9) by second-order centred finite differencing.

We solve Equations (1) and (2) with body force 
Equation (5) using the boundary integral method with 
regularised Stokeslets (Cortez 2001). In this method, the 
body force at each discretised point \( f(r_j) \) is assumed to 
spread over a small blob of radius \( \varepsilon \), i.e.

\[
f_{\text{reg}}(r) = f(r_i) \phi_{\varepsilon}(r - r_i).
\]

This is termed the regularised body force. The blob \( \phi_{\varepsilon}(r) \) is 
a radially symmetric smooth function that satisfies 
\( \int_{R^2} \phi_{\varepsilon}(r) \, dr = 1 \), where \( \varepsilon \) controls the spreading of the 
blob function. Regularised forces were introduced for the 
purpose of removing the singularity in the Stokeslets, 
which are solutions of the Stokes equation subjected to 
point forces. There are several choices of \( \phi_{\varepsilon}(r) \) in 
two-dimensional space and we choose the following (Tlupova 
and Cortez 2009):

\[
\phi_{\varepsilon}(r) = \frac{2 \varepsilon^4}{\pi (r^2 + \varepsilon^2)^3},
\]

where \( r = |r| \). With this choice of blob function, the 
solution of Equations (1) and (2) subjected to the body force 
Equation (5) is then given by (Tlupova and Cortez 
2009):

\[
p(r) = \frac{1}{2\pi} \sum_{j=1}^{N} \left[ \mathbf{f}_m \cdot (r - r_m) \right] \\
	imes \left[ \frac{|r - r_m|^2 + 2\varepsilon^2}{(|r - r_m|^2 + \varepsilon^2)^2} \right],
\]

\[
\mathbf{u}(r) = \frac{1}{4\pi \mu} \sum_{j=1}^{N} \left\{ \mathbf{f}_m \left[ \frac{1}{2} \ln(|r - r_m|^2 + \varepsilon^2) - \frac{\varepsilon^2}{|r - r_m|^2 + \varepsilon^2} \right] \\
+ \mathbf{f}_m \cdot (r - r_m)(r - r_m) \left[ \frac{1}{|r - r_m|^2 + \varepsilon^2} \right] \right\}.
\]

Having solved for the fluid velocity field \( \mathbf{u} \), the membrane 
marker positions \( r_m \) and cortical marker positions \( r_c \) 
can now be updated according to Equations (3) and (4) using 
the forward Euler method with a time step \( \delta t \).

### 2.3 Model for cells interacting hydrodynamically with obstacles

We now consider the migration of a blebbing cell in the 
presence of fixed obstacles in the extracellular environment. 
Such obstacles may be the protein fibres of the 
extracellular matrix in the in vivo scenario or the walls of 
microchannels in in vitro experiments. We assume that the 
cell does not adhere to these obstacles. Therefore, the 
interaction between the cell and obstacles is purely 
hydrodynamical. The boundary integral method with 
regularised Stokeslets can be easily adapted to the case 
when fixed obstacles exist. This is done by imposing zero 
velocity boundary conditions on the obstacles and finding 
the forces exerted by the obstacles on the fluid (Cortez 
2001). Assuming that the obstacles are discretised by \( M \) 
points given at positions \( r_{\text{obs}} \), \( j = 1, 2, \ldots, M \), zero 
velocity boundary conditions require that

\[
0 = \mathbf{u}(r_{\text{obs}})
\]

\[
= \frac{1}{4\pi \mu} \sum_{j=1}^{N} \left\{ \mathbf{f}_m \left[ \frac{1}{2} \ln(|r_{\text{obs}} - r_m|^2 + \varepsilon^2) - \frac{\varepsilon^2}{|r_{\text{obs}} - r_m|^2 + \varepsilon^2} \right] \\
+ \mathbf{f}_m \cdot (r_{\text{obs}} - r_m)(r_{\text{obs}} - r_m) \left[ \frac{1}{|r_{\text{obs}} - r_{\text{obs}}|^2 + \varepsilon^2} \right] \right\}
\]

\[
+ \frac{1}{4\pi \mu} \sum_{j=1}^{M} \left\{ \mathbf{f}_{\text{obs}} \left[ \frac{1}{2} \ln(|r_{\text{obs}} - r_{\text{obs}}|^2 + \varepsilon^2) - \frac{\varepsilon^2}{|r_{\text{obs}} - r_{\text{obs}}|^2 + \varepsilon^2} \right] \\
+ \mathbf{f}_{\text{obs}} \cdot (r_{\text{obs}} - r_{\text{obs}})(r_{\text{obs}} - r_{\text{obs}}) \left[ \frac{1}{|r_{\text{obs}} - r_{\text{obs}}|^2 + \varepsilon^2} \right] \right\}.
\]
Equation (14) is a linear system of equations from which the unknown boundary forces \( f_{\text{obs}} \) can be solved. The solution of Equations (1) and (2) subjected to body force Equation (5) and boundary conditions is therefore given by

\[
p(r) = \frac{1}{2 \pi} \left\{ \sum_{i=1}^{N} [f_m \cdot (r - r_m)] \left[ \frac{1}{\epsilon^2} \frac{|r - r_m|^2 + 2 \epsilon^2}{(|r - r_m|^2 + \epsilon^2)^2} \right] \right\} + \sum_{i=1}^{M} [f_{\text{obs}} \cdot (r - r_{\text{obs}})] \left[ \frac{1}{\epsilon^2} \frac{|r - r_{\text{obs}}|^2 + 2 \epsilon^2}{(|r - r_{\text{obs}}|^2 + \epsilon^2)^2} \right],
\]

\( \text{(15)} \)

\[
u(r) = \frac{1}{4 \pi \mu} \left\{ \sum_{i=1}^{N} \left[ f_m \cdot \left( \frac{1}{2} \ln \left( \frac{|r - r_m|^2 + \epsilon^2}{|r - r_m|^2 + \epsilon^2} \right) - \frac{1}{|r - r_m|^2 + \epsilon^2} \right) \right] + \sum_{i=1}^{M} \left[ f_{\text{obs}} \cdot \left( \frac{1}{2} \ln \left( \frac{|r - r_{\text{obs}}|^2 + \epsilon^2}{|r - r_{\text{obs}}|^2 + \epsilon^2} \right) - \frac{1}{|r - r_{\text{obs}}|^2 + \epsilon^2} \right) \right] \right\}.
\]

\( \text{(16)} \)

Figure 3 summarises the algorithm of our model for computing bleb membrane dynamics and blebbing cell migration.

Figure 3. Flowchart of computational method to solve for bleb dynamics and cell migration.
migration in the presence of boundaries or obstacles. Tables 1 and 2 list the values of various physical and numerical parameters used in the simulations.

3. Results

3.1 Blebbing releases intracellular pressure

We first consider a cell migrating freely in an environment free of obstacles. The cell membrane and actin cortex are each discretised by 200 membrane and cortical markers. Bleb nucleation is initiated by ablating 15 out of the 200 membrane–cortex adhesion points contiguously at one end of the cell. The system is then allowed to run for 2.5 min in real time to model bleb growth and bleb retraction. It has been shown experimentally (Maugis et al. 2010) that, upon formation of a bleb, the intracellular pressure drops and as the bleb stabilises through actin polymerisation and contraction, the pressure rises again until the next bleb forms. A similar pressure profile is also observed in our model, thus validating its correctness. In Figure 4(a), we show that indeed the maximum intracellular pressure drops sharply after bleb nucleation, which implies that blebbing releases intracellular pressure. Eventually, the drop in pressure stabilises as the intracellular pressure is balanced by the increase in membrane tension and membrane bending. Upon reformation of an actin cortex underneath the bleb and bleb retraction, the pressure increases and eventually reaches the initial intracellular pressure.

3.2 Blebbing results in net displacement of the cell body

In our model, the nucleation of a bleb and retraction thereafter result in a net displacement of the cell in the direction of the bleb. At first sight, this was not expected as the cell is immersed in a low Reynolds number fluid, and the extending protrusion would push the cell body forwards while subsequent retraction would do the opposite leading to zero net translation. However, in our model, net movement can occur because the shape change of the cell during bleb formation is different from that during bleb retraction. Thus, the cell can achieve propulsion even in a low Reynolds number environment. Figure 4(b) shows the displacement of the centre of mass of a cell during the course of one blebbing event. Most of the displacement is observed to be achieved during bleb growth.

3.3 Cells in confined environments can form blebs spontaneously

We now consider a cell that is confined between two parallel obstacles as a model for a cell that is migrating through the protein fibres in the extracellular matrix or is being aspirated through a microchannel. The cell membrane and cell cortex are assumed to be cylindrical in shape at the beginning of the simulation and they are each discretised by 200 membrane and cortical markers. The system is allowed to equilibrate for at least 2 min in real time, after which a bleb nucleation event is initiated by ablating 15 out of the 200 membrane–cortex adhesion points contiguously at one end of the cell. The system is then allowed to run for 2.5 min in real time.

A few trends can be observed. First, the cell is only stable when the gap size is larger than a certain size, termed the threshold confinement. Below the threshold confinement, the cell becomes unstable and blebbing

Table 1. Physical parameters and their values used in computational model and simulations.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{m0}$</td>
<td>Rounded cell diameter</td>
<td>25 μm</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Membrane tension</td>
<td>$2 \times 10^{-6} \text{N m}^{-1}$</td>
</tr>
<tr>
<td>$B_m$</td>
<td>Membrane bending rigidity</td>
<td>$2 \times 10^{-19} \text{J}$</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Cortical tension</td>
<td>$4 \times 10^{-5} \text{N m}^{-1}$</td>
</tr>
<tr>
<td>$B_c$</td>
<td>Cortical bending rigidity</td>
<td>$1 \times 10^{-18} \text{J}$</td>
</tr>
<tr>
<td>$E_{ad}$</td>
<td>Membrane–cortex adhesion energy</td>
<td>$0.5 \times 10^{-6} - 8 \times 10^{-9} \text{N m}^{-1}$</td>
</tr>
<tr>
<td>$k_{ad}$</td>
<td>Membrane–cortex adhesion constant</td>
<td>$1 \times 10^{4} - 16 \times 10^{5} \text{N m}^{-1}$</td>
</tr>
<tr>
<td>$l_c$</td>
<td>Maximum length of membrane–cortex adhesive molecule</td>
<td>1 μm</td>
</tr>
<tr>
<td>$f_{com}$</td>
<td>Pre-stress of the actin cortex</td>
<td>10 – 50 Nm$^{-2}$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Cytoplasmic and extracellular fluid viscosity</td>
<td>1.2 Pa s</td>
</tr>
<tr>
<td>$v_c$</td>
<td>Cortical viscosity</td>
<td>12 kg m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$v_e$</td>
<td>Actin monomer diffusive speed</td>
<td>10 μm min$^{-1}$</td>
</tr>
<tr>
<td>$D_{equil}$</td>
<td>Critical distance of actin cortex reformation</td>
<td>0.01 μm</td>
</tr>
</tbody>
</table>

Table 2. Numerical parameters and their values used in computational model and simulations.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>Number of discretised membrane and cortical elements</td>
<td>200</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Blob function width of regularised force</td>
<td>2 μm</td>
</tr>
<tr>
<td>$\delta t$</td>
<td>Time step</td>
<td>$5 \times 10^{-3}$ min</td>
</tr>
<tr>
<td>$T_1$</td>
<td>Relaxation time</td>
<td>$\geq 2.0$ min</td>
</tr>
<tr>
<td>$T_2$</td>
<td>Simulation time</td>
<td>2.5 min</td>
</tr>
</tbody>
</table>
occurs spontaneously at both ends of the cell. This occurs because the cell becomes too squeezed, resulting in a high intracellular pressure. Membrane–cortex springs that hold the cell membrane to the cell cortex are stretched beyond their threshold lengths, resulting in their breakages and spontaneous formation of blebs. Such profuse blebbing behaviour can be overcome by increasing the adhesion strength of the membrane–cortex springs or by decreasing the strength of cortex compression otherwise known as the cortical pre-stress. The phase diagrams in Figure 5 depict the regions whereby the cell is stable for different degrees of confinement at various membrane–cortex adhesion strengths (Figure 5(a)) and cortical pre-stresses (Figure 5(b)).

Figure 4. Intracellular pressure and displacement of a cell during bleb formation and retraction. (a) Cell shape and intracellular pressure profile. The graph (top row) plots the maximum intracellular pressure and is observed to decrease at the onset of bleb nucleation and to gradually increase to its initial value during bleb retraction. The labels (A)–(F) denote time points where the cell shape and intracellular pressure profile are plotted in the middle and bottom rows, respectively. For the cell shapes (middle row), red denotes the cortex, green the reforming cortex and blue the membrane. (b) Displacement of the cell’s centre of mass. The labels (A)–(F) correspond to the times indicated in panel (a).
When the membrane–cortex adhesion energy is high, membrane–cortex springs are able to resist the intracellular pressure. As a consequence, the membrane–cortex springs will be stretched to a lesser extent and thus less likely to tear and form a bleb. Such spontaneous blebbing behaviour is also observed in unheated Escherichia coli cells that are squeezed between a microscope slide and cover slip (Scheie and Ehrenspeck 1973), in which mechanically increased internal pressure results in blebbing in E. coli cells. Spontaneous blebbing is also affected by the extent of cortical pre-stress. When the strength of cortex compression decreases, it can be observed in Figure 5(b) that spontaneous blebbing occurs less readily. Reducing cortex compression reduces intracellular fluid pressure and thus reduces the tendency of spontaneous blebbing.

3.4 Cell migrating speed depends on extent of cell confinement

For cells to make use of blebbing to affect net migration, they need to create blebs preferentially at the leading edge. Hence, we study the migration of confined cells due to the nucleation of a single bleb. The results, as shown in Figure 6(a), can be classified into two regimes according to the average cell migration speed and the ratio of the confinement gap size $B$ to the cell diameter $D$. The average cell migration speed is obtained by taking the average of the cell migration speed at each time step. The cell migration speed at each time step is the difference of the cell’s centre of mass between adjacent time steps divided by the value of the time step. The two regimes are denoted as ‘Regime 1’ where the confinement gap size is large and ‘Regime 2’ where the confinement gap size is small. In Regime 1, characterised by $B/D > 0.6$, the average cell migration speed is observed to increase as the cell becomes more confined (decreasing $B/D$). This increase in cell speed can be attributed to the rapid increase in intracellular pressure caused by the increasingly narrow confinement (Figure 6(b)). The rapid increase in intracellular pressure leads to formation of a larger bleb and thus an increase in cell speed. Two examples are shown in Figure 6(c), (d). For gap sizes $0.6 \leq B/D \leq 0.68$, the intracellular pressure is sufficiently high such that the blebs are unable to retract completely and another bleb starts forming at the site of nucleation soon after retraction. However, in Regime 2 where the cell is being very confined, $B/D < 0.6$, cell migration speed is observed to decrease as the cell becomes more confined (decreasing $B/D$). Thus, the decrease in cell speed is because, in this regime, the intracellular pressure is so high (Figure 6(b)) that nucleation of a bleb at the front of the cell is insufficient to release the intracellular pressure and a bleb forms spontaneously at the rear of the cell. This prevents directed motion as blebs are formed at both ends of the cell. An example is shown is Figure 6(e). Taken together, the existence of both regimes suggests that an optimum confinement gap size exists in order for a cell to translate blebbing into motility. This optimum confinement exists due to the inability of the membrane–cortex adhesive springs to withstand excessive pressure. Extreme confinement results in a high intracellular pressure such that blebs spontaneously form at both ends of the cell leading to less directed motion and hence slower motility.

We next consider blebbing in an equally spaced confined channel but with different membrane–cortex adhesion strengths to probe the dependence of cell migration speed with adhesion energy $E_{ad}$. The cell speeds under various confinement gap sizes and membrane–cortex adhesion energies are plotted in Figure 7. As can be seen, the range of gap sizes for which the migration speed is optimal becomes smaller as the adhesion strength increases, because the ability of the cell to withstand pressure increases with increasing adhesion strength.
4. Discussion

Observations from our numerical results suggest some experiments that could be carried out to validate our computational model. In particular, the biphasic behaviour of cell migration speed for different confinement gap sizes (Figure 6) can be readily tested against the results from experiments where blebbing of a confined cell can be initiated by local perfusion of ezrin, radixin and moesin protein inhibitors that locally weaken the membrane–cortex adhesion. The occurrence of spontaneous blebbing in extreme confinement (Figure 5(a)) as well as the variation of the biphasic cell speed behaviour at different membrane–cortex adhesion energies $E_{ad}$ (Figure 7) can be used to determine $E_{ad}$. Furthermore, the spontaneous blebbing phase diagram of Figure 5(b) can be applied to probe the intracellular pressure of a cell under confinement since the pre-stress of the actin cortex directly affects the intracellular pressure.

The non-zero cell speed in microchannel shows that a cell can migrate through a confined environment via blebbing without establishing any cell–substrate adhesion. Furthermore, before spontaneous blebbing takes place, a blebbing cell migrates faster as the confinement gets narrower.

Finally, even though our numerical results are only obtained through a cell confined in an equally spaced channel, we do not expect the migration behaviour to

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Effects of confinement gap size on cell speed and blebbing behaviour. (a) Cell migration speeds at various confinement gap sizes. Schematics of cell shape and blebs formed corresponding to point marked by (C)–(E) are shown in panels (c)–(e) below. Regime 1: cell forms a bleb at cell front. As confinement gap size decreases, intracellular pressure increases and a bigger bleb is formed, leading to higher cell migration speed. Regime 2: intracellular pressure within the cell is too high such that blebs are spontaneously formed at both cell front and rear. This results in smaller net movement of the cell and slower cell speed. (b) Maximum intracellular pressure for various confinement gap sizes. The labels (C)–(E) correspond to panels (c)–(e). (c) Bleb formation in Regime 1. In panels (c)–(e), red denotes the cortex, green the reforming cortex and blue the membrane. (d) Bleb formation at the boundary of Regime 1 and Regime 2. (e) Spontaneous bleb formation in Regime 2.

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Cell migration speed for various confinement gap sizes and membrane–cortex adhesion strengths. Range of confinement that results in maximum cell speed gets narrower as adhesion strength gets stronger.
differ much when the cell is put in an environment with more complex geometries. However, in order to realistically model a migrating cell in extracellular matrix, it would be necessary to further develop our current model in three-dimensional space.

5. Conclusion
Using a two-dimensional model of a cell comprising elastic membrane connected to a permeable elastic actin cortex via membrane–cortex adhesion, coupled with the assumption that depolymerised actin monomers move at constant speed towards a detached cell membrane, we have shown that amoeboid cell migration can be achieved via the formation and retraction of a cellular bleb, in both unconfined and confined environments in the absence of cell–substrate adhesion. This is in contrast to mesenchymal migration, in which cell movement is attained through forces generated from pushing on or pulling of the substrate via integrant adhesion. We have also shown that, when a blebbing cell is confined between two parallel obstacles such as a microchannel, cell migration speed is affected by the confinement gap size. Cell speed is observed to increase with decreasing gap size, until the microchannel becomes so narrow that blebs are formed spontaneously even without ablation of membrane–cortex adhesion. In such narrow confinement, amoeboid migration becomes less effective due to simultaneous bleb formation at all sides of the cell. Thus, there is an optimal confinement size for which migration speed is maximum. These results obtained from our computational model can be readily validated in experiments that we have also discussed.

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References


