Dynamics of initial cell spreading:
a hydrodynamics-governed process

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Résumé

Nous comparons de façon systématique la phase initiale d’écoulement d’une cellule en suspension sur un substrat, sous l’effet de forces adhésives, avec des simulations numériques d’objets modèles. En effet, pour différents types cellulaires et modalités d’adhésion, l’aire étalée croît linéairement au cours du temps, puis plus lentement. De plus, nos expériences montrent que la transition entre ces régimes est déclenchée par l’évolution de la géométrie de la cellule et non par la valeur absolue de l’aire ou du temps écoulé. Nous montrons que cette dynamique est gouvernée par la dissipation dans le cortex d’actine de la cellule.

Abstract

The initial stages of spreading of a suspended cell onto a substrate under the effect of adhesion are systematically compared to the behaviour of model objects, simulated by finite elements. It has been reported that in different cell and adhesion types the spread area initially grows linearly as a function of time and then at a slower rate. In addition our experiments show that the transition between this power-law and the slower regime is triggered by geometry rather than absolute value of the area or elapsed time. We show that this dynamics is governed by dissipation in the actin cortex of the cell.

1 Introduction

Mechanical properties of live cells are now recognised as a crucial feature of their function. However, they are tremendously difficult to characterise, since cells are complex composite objects, undergoing perpetual reshaping and able to generate forces through the activity of molecular motors, and thus behave as complex systems whose highly nonlinear global properties are not easily understood from the properties of their components. Predictive modelling of cells is thus, so far, restricted to limiting cases in which only few of their mechanical features come into play.

Even in these cases, prediction is often impaired by the difficulty of measuring quantitative parameters (such as viscosity) in independent experiments, given the apparent variability of cell response to different mechanical probing. Predictive modelling thus often involves a large number of free parameters which undermine its definiteness. One possibility to escape this difficulty is to find situations in which experimental observations can reveal a robust, self-similar dynamical process. Indeed, self-similarity proceeds from a balance of leading-order processes (e.g., energy inputs or dissipation) which is unaffected by numerical values of parameters, and thus offers a discriminating test for predictive models.

Such a self-similar law has recently been evidenced by [1], who monitored the initial spreading rate of a cell driven by adhesion forces on a solid substrate. Cells suspended in a fluid assume a shape close to spherical. They can interact with a neighbouring solid substrate either by specific adhesion (due to binding receptors and ligands present on cell membrane and substrate) or nonspecific adhesion (electrostatic, van der Waals). In both cases, contact area is observed to grow approximately linearly for a few minutes for different cell types [e.g. 1, 2], then the growth usually assumes a slower rate, which can be fitted with a square root function of time. Some cells eventually stop spreading, others start active processes and migrate [3]. This last step is evidently out of the reach of a purely mechanical model of the cell, but on the other hand similarity of the earlier steps with dynamics of
spreading of a fluid drop is meaningful. The robustness of the behaviour observed with respect to cell type and adhesion type leaves room for purely mechanical interpretation of these observations. The objective of this paper is to investigate whether a simple mechanical model can reproduce the observed sequence of power-laws.

2 Experimental observations

Scale-free behaviours such as the one evidenced by [1] can originate from physical balances at any of the spatial scales present in the cell. In order to extract a relevant spatial scale out of the phenomenon, we focus on the transition between the two power-law regimes rather than on the power-law in itself. This transition defines a time \( t^* \) and contact area \( A^* \) from which the governing balance appears to change.

It is found in our experiments [4] that time \( t^* \) is highly variable from one cell to another, while \( A^* \) varies in proportion with each cell characteristic size \( A_0 \). Figure 1 summarises these findings. This validates the assumption that geometry triggers the transition, rather than elapsed time or absolute dimension of the contact patch. Thus it makes sense to apply the scaling theory, considering that phenomena are invariant when all spatial dimensions are normalized by the typical size of the suspended cell considered, \( A(t)/A_0 \). Further, since the characteristic spatial scales of molecular processes are the same in HeLa and T24 cells, we infer that these phenomena do not govern a cell-size dependent process, and thus that only phenomena occurring at the cell scale are relevant in this study.

Transition occurs for \( A^*/A_0 \approx 0.8 \), which means that the contact area is smaller than the area of the equatorial section of the cell when it is spherical, figure 1(c). The approximative shape of the cell is thus the one of a truncated sphere, on which smaller-scale details such as microvilli and filopodia are superimposed. For a spread area \( A/A_0 = A^*/A_0 \approx 0.8 \), the deformation undergone by the cell is thus small (compared to a fully spread cell), and does not necessarily imply conformational changes in its cytoskeleton. This means that a purely mechanical process can still be the leading-order phenomenon setting the spreading rate even after transition.

3 Modelling and numerical technique

Modelling live cells is a formidable entreprise which is still far out of reach. However, in situations such as the initial spreading of cells, experimental results give some hope that only a few of the features of the living cell are setting the dynamics, which may allow for a simple model to explain them. Thus we proceed by introducing the
simplest possible model for this precise situation and gradually introducing features until a behaviour similar to the one observed in cells is obtained, and is robust to parameter variations.

Cells in suspension are very much spherical but they are strongly inhomogeneous in their composition. The central part (around 10% to 20% volume, or 50% radius) is occupied by a nucleus. Around the nucleus is the cytoplasm, a porous medium composed of polymerised, reticulated proteins (the cytoskeleton), membrane-bound compartments of sub-micrometric size (organelles) and a fluid (the cytosol). The mechanical properties of the cytoplasm are expected to arise from the fluid (incompressibility, viscosity) and the cytoskeleton (elasticity or viscoelasticity, active remodelling fuelled by chemical energy). Mechanical response is dominated by a viscous-type response at shear rates lower than 0.1 s$^{-1}$ [5] this can be understood because of the short lifetime of the reticulations and of the protein filaments themselves [order 1 s, see e.g. 6]. The cytoskeleton is far from having a homogeneous distribution in the cell. Microtubules are highly rigid filaments arranged in a star-like manner from the centre of the cell to its periphery. For geometric reasons, they are not expected to sustain much stress during initial spreading. Actin filaments are concentrated in a cortex in the periphery of the cell (thickness of order 1 µm, [7]). Thus one can expect a higher viscosity in this outer region. The cell is enclosed by a membrane, which is bound to the actin cortex. The membrane is a lipid bilayer which prevents large molecules to enter the cell and creates osmotic effects. It is fluid (in-plane shear viscosity is low) and (nearly) inextensible. This inextensibility constraint is modulated by the presence of membrane “reservoirs” (either ruffles or invaginations). From frustrated spreading experiments by [7], where spreading is limited because only a small circular patch is functionalized and creates osmotic effects. It is fluid (in-plane shear viscosity is low) and (nearly) inextensible. This inextensibility constraint is modulated by coalescence (and decoalescence) of small vesicles. This is an active biological process called exocytosis/endocytosis, happening on the time scale of 100 s [8]. We do not directly consider this in our model, however as long as one assumes that this process is not geometrically directed, its effects are similar to the presence of excess area.

Given the experimental observations, Stokes equations are expected to be a fair first-order approximation of the dissipative mechanisms in the dynamical process. Assuming that a lubrication layer thick of order 0.1 µm remains between cell and substrate, the shear rate is of order 1 s$^{-1}$ initially and decreases, and the Reynolds number is vanishingly small. In the cell, a typical distance is 1 µm (the thickness of the shell of actin around the cell), so the shear rate is one order of magnitude lower. This means also that viscoelastic properties of the proteins in the cell cytoplasm will be dominated by viscous response. Buoyancy effects are sufficient to make cells sediment, however they are too weak to deform cells and, when cells are close enough to the substrate, they become negligible with respect to the adhesion force which then provides the only first order driving force. This adhesion force, in our case, may result either from specific or nonspecific interactions, without modifying the scaling laws of spreading. Thus we choose the simplest model we can think of, that is, a van der Waals-type potential of magnitude $W$, as proposed by [9] for nonspecific adhesion:

$$W(z) = w \left( \left( \frac{d_0^2}{z^2} - 1 \right)^2 - 1 \right).$$

We take the equilibrium distance of the potential $d_0 = 0.05R_0$ in this model for simulations, the invariance of the spreading profile (up to a scaling factor in time) has been checked with $d_0 = 0.01R_0$ in some cases.

We consider a cylindrical flow domain of symmetry axis $(O, e_z)$, and denoted $\Omega \times [0, 2\pi]$ (using cylindrical coordinates, figure 2). This domain contains an incompressible Newtonian fluid of homogeneous viscosity $\eta = \eta_k$ except in the drop or vesicle, which occupies a subdomain $\Omega_d(t) \times [0, 2\pi]$ which is advected by the fluid velocity $u$, and where viscosity $\eta$ is equal to some function $\eta_d(x, t)$, also advected by the fluid velocity. This writes as:

$$\Omega_d(t) = \{X(x_0, t), x_0 \in \Omega_d(t_0)\} \quad \eta_d(X(x_0, t), t) = \eta_d(x_0, t_0)$$

with $t \mapsto X(x_0, t)$ the trajectory of a material point occupying $x_0$ at time $t_0$, defined by the Cauchy problem:

$$\begin{cases}
\frac{\partial X(x_0, t)}{\partial t} = u \circ X(x_0, t), & t \in [0, T], \\
X(x_0, t_0) = x_0,
\end{cases}$$

where $u \circ X(x_0, t)$ is understood as $u(X(x_0, t), t)$.

The structure of the problem should reflect our objective of describing a balance between adhesion force and viscous friction and be scale-invariant to a change in numerical value of any of the parameters. This is made clear by
Figure 2: (a) Computational domain and example of part of a finite element mesh used in numerical simulations. In the case of the viscous drop model, viscosity in $\Omega_d$ is equal to $\eta_d$. (b) Shape of the domain $\Omega_d(t_1)$ for the case of the composite drop and of the vesicle with membrane wrinkles. The grayed area has viscosity $\eta_d$, the white areas have viscosity $\eta_s \ll \eta_d$. 

non-dimensionalising the problem by length $R_0$ (initial radius of the drop or vesicle), viscosity $\eta_0$ (largest viscosity in the problem), time $T = R_0 \eta_0 / w$ and pressure $P = w / R_0$, which does not introduce any nondimensional group in the governing equations:

$$-\text{div} \left( 2\eta D(u) \right) + \nabla p = 0$$

$$\text{div} u = 0$$

with boundary conditions (see figure 2):

$$u = 0$$

$$u_r = 0, \quad \frac{\partial u_z}{\partial r} = 0$$

$$-p n_{\text{out}} + 2\eta D(u) n_{\text{out}} = 0$$

$$\left[ -p I + 2\eta D(u) \right]_{\text{int}} n_{\text{int}} = -\nabla W + f_{\text{int}}$$

where $[\phi]_{\text{int}}$ denotes the jump of $\phi$ across the interface $\Gamma_{\text{int}}(t)$, and $f_{\text{int}}$ are interfacial forces other than the adhesion force $\nabla W$.

For fluid drops suspended in a fluid in which they are immiscible, this force is equal to the capillary force. Since the dynamic balance we are looking for is between adhesion and viscous friction, we consider the limit of vanishing capillary tension (infinite capillary number), thus suppressing any competition between driving forces. This limit is relevant only in the first stages of drop spreading, which is the domain in which analogies between drop and cell spreading are investigated. Viscosity differs between the drop inside and medium, and is either supposed uniform within the cell or space dependent. Such models are called composite or compound drop models [10].

In the case of vesicles, the interfacial forces correspond to the forces exerted by the membrane on the fluids. They originate from the bending rigidity, shear viscosity and inextensibility of the membrane. For the same reason as for drops, we consider the limit of zero bending rigidity. Lipid bilayers have a low in-plane shear viscosity and, even for spectrin-lined membranes, has a negligible contribution to dynamics compared to bulk-viscosity in cells. (From the value of in-plane shear viscosity found by [11] for red blood cells, we expect dissipation in the eukaryotic cell membrane to be about 3 orders of magnitude smaller than in the bulk.) Inextensibility, however, is a constraint imposed to membrane flow and thus to fluid flow in its vicinity. It can be expressed in terms of the surface divergence of the (tangential) velocity along the membrane:

$$\text{div}_s u = 0$$

A detailed study of this type of modelling of the interactions of a viscous fluid and a membrane is given in [12].

A finite element technique is employed to calculate an approximate solution for a sequence of times $t_n$, and $\Omega_d(t_{n+1})$ is obtained by advecting $\Omega_d(t_n)$ with the approximation of velocity $u(t_n)$. This Lagrangian tracking of the interface is such that meshes of both the bulk and the interface coincide. Compared to methods where the bulk is meshed independently of the interface (such as level-set, phase-field and immersed boundary methods), the advantage is a greater accuracy due to the possibility of localising interfacial forces on the interface. Compared to
boundary integral methods, the advantage is versatility with respect to bulk-flow governing equations and geometry. See [12] for details.

Lagrange multipliers and the velocity are calculated using a saddle-point approach [13, 14] with Uzawa algorithm. The residual was $10^{-12}$. The method is implemented in the C++ open-source, free software rheolef [15]. The numerical method has been tested and validated with $O(h^{1.5})$ convergence against the analytical solution for drop oscillation modes of [16] (for the case of drops or composite drops) and the Stokes solution of the free fall of a spherical vesicle [14].

4 Numerical results

Numerical simulations of three simple models are performed: the viscous drop (in the limit of zero surface tension), the composite drop (i.e., a thin spherical shell of highly viscous fluid surrounding a low-viscosity interior) and the vesicle with membrane wrinkles, see figure 2(b). In all models, the dynamics are set by a balance between the adhesion energy gain when spreading on the substrate and dissipation due to deformation. The difference between models lays in the geometric pattern of dissipation.

4.1 Initial spreading

The corresponding spreading time profiles are obtained figure 3. High-viscosity drops are attracted to the substrate without exhibiting deformation, while the film between the drop and substrate is drained due to adhesion forces. The drop then starts to spread. However, for a viscosity contrast $\eta_d/\eta_s > 10^3$, the rate of spreading is found to be like $Ct^{0.5}$, with $C$ some constant depending on $T$ only. This is not similar to cell spreading, which is in $Ct$ initially. Thus this model cannot account for cell spreading dynamics.

Composite drops with a viscosity ratio up to $10^6$, which corresponds to the estimates for live cells, happen to spread at a linear rate, similar to cells. This model is thus retained.

Vesicles with excess membrane, again, spread like $Ct^{0.5}$ at the relevant viscosity contrast, and this model is also discarded.

4.2 Transition to a slower regime

It was found in experiments on live cells that the linear spreading regime ends when spread area $A$ is approximately $0.8A_0$. A validation for the retained model of composite drops is thus to verify that it exhibits the same transition. This is actually what is observed in figure 3: for $A \gtrsim 0.75A_0$, the spreading rate decreases. This check is significant because we have verified numerically that this transition, like the linearity of the spreading rate, is not influenced by the parameters of the model (i.e. $T$ and geometrical parameters). Thus the composite drop model robustly reproduces the transition between the linear and slower regimes.
5 Discussion

Based on our experimental and additional numerical evidence in [4], we show that the spreading dynamics of cells initially suspended in a fluid can be described using a simple model where viscous dissipation balances adhesion force.

This does not amount to claiming that cells are viscous objects, and that molecular friction governs their behaviour. Indeed, what we model as viscous dissipation can correspond to any dissipation proportional to an amount of local deformation. Thus the correspondence between the behaviour of the composite drop model and the cells means that the retarding force that balances adhesion is a dissipation in the cell cortex.

Biophysical knowledge of the cell cortex can then lead us to understand the nature of this dissipation: indeed, aside of lower order molecular friction, rearranging the cell cortex in order to spread implies to break and re-form a large number of crosslinking bonds between large actin filaments. This process has an energetic cost, which must be met by the adhesion energy that the cell gains in spreading.

Thus, our simple mechanical model allows to measure the unit cost of such a rearrangement process: it is an effective viscosity of this living material we are gaining access to.

References