Viscous-Fingering-Like Instability of Cell Fragments

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We present a novel flow instability that can arise in thin films of cytoskeletal fluids if the friction with the substrate on which the film lies is sufficiently strong. We consider a two-dimensional, membrane-bound fragment containing actin filaments that polymerize at the edge and depolymerize in the fragment. Performing a linear stability analysis of the initial state due to perturbations of the fragment boundary, we find, in the limit of large friction, that the perturbed actin velocity and pressure fields obey the same laws governing the viscous fingering instability of an interface between immiscible fluids in a Hele-Shaw cell. A remarkable feature of this instability is that it is independent of the strength of the interaction between actin filaments and myosin motors.

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Shape change and motility allow cells to respond to their environment and play central roles in many biological processes such as embryonic development and wound healing. The crawling of cells, such as keratocytes or fibroblasts, involves the protrusion of a thin leading edge, the lamellipodium, driven by the polymerization of the actin cytoskeleton, the adhesion to the substrate via specific proteins, and molecular motor-enabled contraction of the cytoskeleton to translocate the trailing cell body [1]. Cells lying on extracellular matrix, *in vivo*, or on a substrate, *in vitro*, can be stationary or motile. Recently, cells *in vitro* have been observed to spontaneously switch from the stationary state in which their shape is roughly circularly symmetric, with the nucleus at the center surrounded by the lamellipodium, to a motile and anisotropic, usually crescent-shaped state [2]. A crucial aspect of the motility transition is the shape polarization of the cell occurring prior to motility, in which a preferred direction is chosen and which determines the direction of motion [2]. It is not known yet to what degree the process of shape polarization is biochemical or mechanical in nature.

Remarkably, physical units far simpler than eukaryotic cells can undergo shape polarization and the transition to self-sustained motion: the work of Ref. [3] showed that flat cell fragments, containing only actin cytoskeleton and myosin II motors enclosed by a plasma membrane, undergo transitions between circularly symmetric, nonmotile and crescent-shaped, motile states. That cell fragments show similar shape transitions and motile behavior as full cells has led us to study theoretically cell shape dynamics in these simpler systems with fewer structural elements and fewer measurable parameters.

The actin cytoskeleton is a highly complex medium: it is polar as actin polymerizes at its ''plus'' end, facing the membrane abutting the lamellipodium; it is viscoelastic; and it is active and driven out of equilibrium by ATP hydrolysis, needed for continuous polymerization (treadmilling) and to generate myosin motor-induced stresses. The interaction of the cell with its environment is also very important; for example, cells crawling on a heterogeneous substrate tend to migrate to regions of greater substrate adhesion [4]. Cytoskeletal actin in a cell or cell fragment is able to transmit forces to its substrate through transmembrane proteins [5–7], namely, integrins, which bind reversibly to the substrate. If the actin velocity relative to the substrate is small compared to a/τ_b, where a is a molecular size and τ_b is the average time during which an integrin remains bound, then the force exerted by the moving filaments on the substrate can be expressed as a friction force, proportional to the actin velocity [8–11].

In this Letter, we demonstrate that polymerization and large friction forces are sufficient to destabilize the shape of an initially circular cell fragment. We start by considering a very simplified model of actin cytoskeletal flow in a cell fragment, as shown schematically in Fig. 1. The fragment is very thin and there is no flow or any spatial dependence in the thickness direction; also we consider

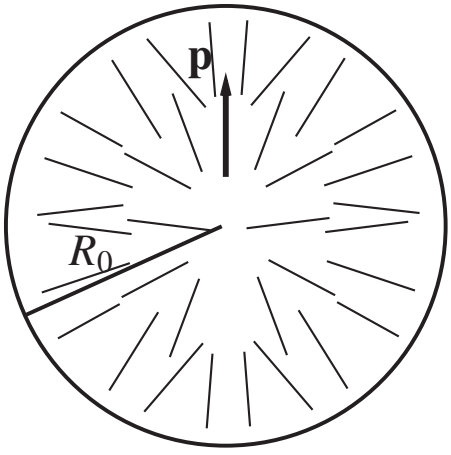

FIG. 1. Schematic drawing of actin cytoskeleton in the unperturbed, radial state of the fragment. The direction of average actin polarization is radial, and is indicated by $\mathbf{p} = \hat{\mathbf{r}}$.

that the thickness of the fragment is constant. This assumption may be appropriate to the study of cells or cell fragments in confined geometries [12]. We also assume we can lump the dynamics of the actin filaments, monomers, motors, and cytosol in the fragment into a single component theory. Furthermore, we treat the cytoskeleton as a viscous, incompressible liquid, ignoring the elastic response that occurs on times shorter than the viscoelastic relaxation time. Rheological experiments have shown that actin gels are only weakly compressible [13]. We discuss our neglect of the elastic response further below.

Actin polymerization is regulated by proteins such as those of the Wiskott-Aldrich syndrome family (WASP), which localize in the cell membrane at the fragment edge [14]. For the purposes of this work, it is sufficient to assume that actin polymerizes at the fragment edge and in the direction normal to the boundary. Newly polymerized actin flows away from the fragment edge by treadmilling, due the turnover of free actin monomers back to the edge for further polymerization that is enabled by depolymerization in the bulk. For simplicity, we assume that depolymerization is spatially uniform and occurs at a rate proportional to the filament density.

These simplifications imply that the actin flow in the unperturbed, circular state induced by localized polymerization and uniform depolymerization is imposed by the continuity equation $\nabla \cdot (\rho \mathbf{v}_0) = -k_d \rho$, where ρ is the actin filament density and k_d is the depolymerization rate. The assumption of incompressibility directly leads to the radially directed treadmilling speed $v_0 = -\frac{k_d}{2} r$. Note that in the stationary state continuity requires that $\frac{k_d}{2} R_0 = v_p$, where R_0 is the unperturbed fragment radius and v_p is the polymerization speed [15].

The cytoskeleton dynamics is described here using the hydrodynamic equations for single component, active polar gels of Ref. [16], which themselves are a generalization of the hydrodynamics of liquid crystals [17,18] modified to account for the coupling between stresses and motors as well as between actin polarization and motors. We will find below that the fragment instability is driven by actin treadmilling and friction with the substrate; at the level of linear stability analysis, it is reasonable that the polarization dynamics are not important and we assume throughout that the polarization field $\mathbf{p}$ is everywhere a unit vector pointing in the radial direction $\hat{\mathbf{r}}$. Ignoring the dynamics of the polarization field, the constitutive laws of Ref. [16] reduce to those for an isotropic, viscous fluid of viscosity η, augmented by an active term in the deviatory stress component σ_{rr} that reflects the myosin-mediated interaction between actin filaments that are nearly aligned: that is, $\underline{\underline{\sigma}} = 2\eta \underline{\underline{u}} - \zeta \Delta\mu \hat{\mathbf{r}}\hat{\mathbf{r}}$, where η is the actin viscosity; $\underline{\underline{u}}$ is the velocity gradient tensor; $\Delta\mu$ is the chemical potential difference between ATP and its hydrolysis products; and where for contractile motors the activity coefficient ζ is negative [19]. The constitutive laws are completed at low Reynolds numbers by the force balance $\nabla \cdot \underline{\underline{\sigma}} = \nabla P + \xi \mathbf{v}$, where ξ is the friction coefficient between the cytoskeletal filaments and the substrate. Scaling lengths by R_0, times by $1/k_d$, and stresses and pressures by ηk_d (keeping the same variable names for the new, dimensionless quantities), it follows that viscosity and friction affect the cytoskeletal dynamics through the dimensionless parameter $\lambda^2 = \frac{\eta}{\xi R_0^2}$. In the limit of large friction, $\lambda \to 0$, the leading term in the force balance is simply

$$\mathbf{v} \sim -\lambda^2 \nabla P. \tag{1}$$

The velocity satisfies a type of Darcy's law, as it does in a Hele-Shaw cell [20]. Based on available experimental results λ^2 is in fact quite small. Measurements of traction forces [21] and treadmilling [22] in keratocytes give $\xi \approx 10^5$ Pa $\cdot$ s$/\mu^2$; similar measurements in fibroblasts for tractions [23] and treadmilling [24] give $\xi \approx 10^7$ Pa $\cdot$ s$/\mu^2$. Furthermore, taking $\eta \simeq 10^4$ Pa $\cdot$ s [25] and $R_0 \simeq 1 - 10$ μm we find $\lambda^2 \simeq 10^{-1} - 10^{-5}$.

We now perturb the edge of the fragment, so that in terms of the polar angle θ the fragment edge is now at a position $R(\theta, t) = 1 + \delta R(\theta, t)$. For $\delta R(\theta, t) \ll 1$, we perform a linear stability analysis by writing $\delta R(\theta, t) = \sum_{m=1}^{\infty} \delta R_m(t) \cos(m\theta)$ and similarly for the two components of the perturbed velocity field, $\delta v_r = \sum_{m=1}^{\infty} \delta v_{r,m}(r, t) \cos(m\theta)$ and $\delta v_\theta = \sum_{m=1}^{\infty} \delta v_{\theta,m}(r, t) \times \sin(m\theta)$, and the pressure field $\delta P = \sum_{m=1}^{\infty} \delta P_m(r, t) \times \cos(m\theta)$. The $m = 0$ mode is stable because the density of actin filaments in the fragment is fixed and we have also assumed that the fragment thickness is fixed. Assuming that the depolymerization rate does not change as a result of the perturbation, it follows that $\nabla \cdot \delta\mathbf{v} = 0$. Applying Eq. (1) to the perturbed quantities $\delta\mathbf{v}$ and δP, we find that $\nabla^2 \delta P = 0$ and therefore $\delta P_m(r, t) \sim r^m$ and $\delta v_{r,m}(r, t) = -\delta v_{\theta,m}(r, t) = A_m(t) r^{m-1}$. The coefficient $A_m(t)$ can be found by imposing the force-free condition at the boundary, namely, $\delta P_m|_{r=1} + \delta R_m \frac{dP_0}{dr}|_{r=1} = 0$, leading to $A_m(t) = \frac{m}{2} \delta R_m(t)$. The growth rate of the perturbation modes $\delta R_m(t)$ is obtained by noting that, to linear order in δR,

$$\frac{d\delta R_m}{dt} \approx \delta v_{r,m}(R_0) + \delta R_m \frac{dv_0}{dr}, \tag{2}$$

which, using the expression for $A_m(t)$, gives $d\delta R_m/dt = \omega_m \delta R_m$, where the leading order growth rate, in units of k_d, is

$$\omega_m \sim \frac{m-1}{2} + O(\lambda^2). \tag{3}$$

Note that the mode $m = 1$, corresponding to an infinitesimal translation of the circular fragment, is marginally stable, as required by translational symmetry.

The linear dispersion relation, ω_m, is a common feature to a number of Laplacian growth problems, for example,

the viscous fingering instability that occurs at an interface between two immiscible liquids in a Hele-Shaw cell [26]. The physics of the instability is understood as follows. The pressure gradient at the edge is $\frac{dP_0}{dr}|_{r=1} > 0$, and a perturbation with $\delta R < 0$, for example, requires a perturbed pressure $\delta P|_{r=1} > 0$ to keep the boundary force-free, to leading order in λ^2. An excess pressure at the edge relative to the fragment center ($\delta P|_{r=0} = 0$ for $m > 0$) drives an inward-directed flow, thus amplifying the initial negative perturbation.

Viscosity, surface tension, and motor activity affect the growth rate, ω_m, at $O(\lambda^2)$. In short, one obtains from the radial component of the force balance a fourth order ordinary differential equation in r for $u \equiv r\delta v_{r,m}$

$$\lambda^2\Big[u^{(4)} + \frac{2}{r}u^{(3)} - \frac{(2m^2+1)}{r^2}u'' + \frac{(2m^2+1)}{r^3}u' + \frac{m^2(m^2-4)}{r^4}u\Big] - \Big[u'' + \frac{1}{r}u' - \frac{m^2}{r^2}u\Big] = 0, \quad (4)$$

where the primes indicate differentiation with respect to r. The four boundary conditions required are that $\delta \mathbf{v}|_{r=0} = 0$; that the edge of the perturbed fragment is force-free, namely, $\delta\sigma_{nn}|_{r=1} = 0$, where the subscript n refers to direction normal to the cell fragment; and that, neglecting the viscosity of the fragment's surroundings compared with the viscosity of the cytoskeleton, the tangential shear satisfies $\delta\sigma_{tn}|_{r=1} = 0$, where t refers to the direction tangent to the perturbed fragment.

In the limit $\lambda \to 0$, Eq. (4) together with the four boundary conditions is a singular perturbation problem. Following the boundary layer techniques of Ref. [27], a uniformly convergent approximation to $\delta v_{r,m}$ on the interval $0 \le r \le 1$, valid to $O(\lambda^2)$, is

$$\delta v_{r,m}(r,t) = r^{m-1}\{B_0(t) + \lambda^2[B_2(t) + C_2(t)e^{((r-1)/\lambda)}]\}, \quad (5)$$

where $B_0(t) = \frac{m}{2}\delta R_m(t)$ is the unperturbed growth rate $A_m(t)$ calculated above, which is obtained from the boundary condition $\delta\sigma_{nn}|_{r=1} = 0$ at leading order $[O(\lambda^{-2})]$. The two other constants $B_2(t)$ and $C_2(t)$ are found, respectively, by solving $\delta\sigma_{nn}|_{r=1} = 0$ and $\delta\sigma_{tn}|_{r=1} = 0$ at next-to-leading order $[O(\lambda^0)]$, giving, by way of Eq. (2), a growth rate

$$\omega_m \sim (m-1)\Big\{\frac{1}{2} + m\lambda^2\Big[\frac{\zeta\Delta\mu}{\eta k_d} - 2m\Big]\Big\} + O(\lambda^3). \quad (6)$$

Equation (6) shows that the stabilizing effect of viscosity is proportional to m^3 for large m. It can be easily seen that the stabilizing effect of the plasma membrane tension also scales as m^3 (as does the effect of interface tension in the viscous fingering instability in a Hele-Shaw cell [26]): including membrane tension, the normal stress at the boundary satisfies a two-dimensional Laplace law $\delta\sigma_{nn}|_{r=1} = -\frac{\gamma}{\eta k_d R_0}\delta H$, where γ is the membrane tension and where the mth mode perturbation in the membrane curvature in the (r, θ) plane (ignoring changes in curvature in the thickness direction) is $\delta H = (m^2-1)\delta R_m \cos(m\theta)$. The contribution of the membrane tension to $A_2(t)$ is $-\lambda^2\frac{\gamma}{\eta k_d R_0}m(m^2-1)$. Taking $\gamma \simeq 10^{-4}$ N/m [28] as an estimate for the membrane tension and $k_d \simeq 0.2$ s^{-1} [1] it is clear that the stabilizing effect of membrane tension is negligible compared to that of actin viscosity.

Equation (6) further shows that the contractile effect of the motors is to stabilize the growth of perturbations, proportional to m^2. This result depends strongly on the assumption, made for simplicity, that the filament polarization in the perturbed fragment remains everywhere radial and is not a dynamical quantity in the problem. Equation (6) shows that the relative contribution of myosins to ω_m is proportional to $\frac{\zeta\Delta\mu}{\eta k_d}$. Taking $\zeta\Delta\mu \simeq -10^3$ Pa [8], $\frac{\zeta\Delta\mu}{\eta k_d} \simeq -0.5$, and therefore small compared to the viscous contribution at $O(\lambda^2)$.

Diffusion of free actin monomers also limits the instability growth; however, we may consider for now that the diffusion constant, D, is such that $D \gg k_d R_0^2$, so that perturbations that are area-preserving at first order in δR do not affect the essentially spatially uniform monomer density, and hence the polymerization rate, v_p. We also note that the time scale for the instability growth is set by the depolymerization rate k_d. Since the viscous relaxation time τ of actin cytoskeleton is on the order of a few seconds [25], on the one hand the criterion for ignoring the fragment's elastic response, $k_d\tau \ll 1$, might not be satisfied for fragments. On the other hand, the ratio of the elastic and friction terms of the stress will be roughly $\lambda^2/(k_d\tau)$, which will not affect the zeroth order instability growth, Eq. (3). A proper accounting of monomer diffusion and viscoelasticity will be considered in future work.

We can also estimate the most unstable mode in the high friction limit by maximizing ω_m in Eq. (6) with respect to m. Ignoring the effect of motors, we find that for $\lambda^2 = 0.01$ $m_{\max} \simeq 3$. The most unstable mode depends, however, quite strongly on λ^2. To know how the linear instability of the most unstable mode affects the fragment shape at later times would require a full nonlinear analysis, including the dynamics of the polarization field.

Finally, it might be experimentally useful to have an estimate of the critical value of friction, ξ_c, for which shape perturbations of a cell fragment become unstable. This critical value is defined such that for $\xi < \xi_c$, $\omega_m < 0$ and for $\xi > \xi_c$, $\omega_m > 0$. It is conceivable that one could observe the onset of growing shape perturbations by plating cell fragments on surfaces of varying degrees of adhesiveness or by culturing fragments from cells that have been mutated to weaken or strengthen the binding of integrins to the surface [29,30]. Equation (4) can be solved numerically for different mode numbers m to find the critical value ξ_c as a function of motor strength, $|\zeta|\Delta\mu/\eta k_d$; see Fig. 2.

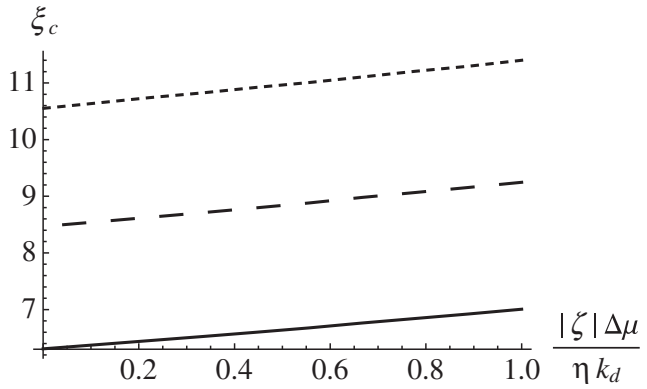

FIG. 2. Critical value of friction, ξ_c, in units of η/R_0^2, versus $|\zeta|\Delta\mu/\eta k_d$ for $m = 2$ (solid line), $m = 3$ (long dashed line), and $m = 4$ (short dashed line). Surface tension, $\gamma/\eta k_d R_0$, is taken to be zero.

The numerical estimates of ξ_c given in Fig. 2 are qualitatively consistent with the value obtained by setting $\omega_m = 0$ in the asymptotic growth rate, Eq. (6): lower modes are less stable as a function of increasing friction and motor activity has a weak effect on the growth of shape perturbations.

In summary, we have found that large substrate friction and the pressure field created by treadmilling in an initially circular cell fragment render it linearly unstable. This instability has the potential to be relevant to the related biophysical problems of cell shape change and cell motility, given that it presents a fundamentally hydrodynamic means for cell dynamics, independent of biochemical signaling and, significantly, of the presence or absence of molecular motors. In this work we have ignored some physics which may be relevant to actual cell fragments and cells: namely, the dynamics of the actin filament polarization, the elastic response on short time scales, fragment height variations, and filament density variations. In a more detailed calculation, we could include the effect of height variations by considering an effective two-dimensional compressibility, in a perturbative manner. We feel, nonetheless, that the underlying simplicity of this mechanism, that it is driven by actin polymerization, depolymerization, and substrate friction, might make the instability general enough and likely present when considering a more realistic model of shape change.

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