Peeling Process in Living Cell Movement Under Shear Flow

Emmanuel Décavé,[1,2] Daniel Garrivier,[1] Yves Bréchet,[3] Franz Bruckert,[2] and Bertrand Fourcade[1]

[1]*CEA/Grenoble, DRFMC/SI3M, 17 rue des Martyrs, 38054 Grenoble cedex 9, France*
[2]*CEA/Grenoble, DBMS/BBSI, 17 rue des Martyrs, 38054 Grenoble cedex 9, France*
[3]*ENSEEG, LTPCM, 38042 Domaine Universitaire de Saint-Martin d'Hères, France*

(Received 27 November 2001; published 14 August 2002)

We present a direct optical observation of the behavior of the contact area between a living cell (*Dictyostelium discoideum*) and a solid substrate under shear flow. It is shown that the membrane is peeled off the substrate. The relationship between the peeling velocity and the applied force is obtained experimentally and explained from the behavior of individual adhesion bridges. The dissipation occurring during the peeling process is explicitly calculated in terms of out-of-equilibrium thermodynamics.

DOI: 10.1103/PhysRevLett.89.108101 PACS numbers: 87.10.+e, 05.70.Np, 68.35.Md, 68.35.Np

Since the publication of Bell's seminal paper in 1978 [1], many experimental [2,3] and theoretical works [4,5] have been dedicated to cell adhesion. From the biological point of view, cell-cell and cell-substrate adhesion is of central importance, since many adhesion proteins are also receptors, eliciting a cellular response upon ligand binding. From the physical point of view, the statistical nature of adhesion bridges is responsible for original and interesting behaviors. One common aim of biological and physical studies consists in determining the molecular properties of the adhesion bridges in situ, using both experiments and modeling [6,7]. Determining the strength of adhesive bridges requires applying external forces to the cells [8–10]. For example, [11] recently related the macroscopic adhesion energy to the properties of molecular bonds using an equilibrium statistical mechanics formulation. But both single molecule experiments and theoretical works [12–16] have emphasized that bond rupture is a nonequilibrium process revealing the importance of the kinetic properties of bond breakage.

The purpose of this paper is to report direct experimental observation of cell movement under shear flow at the scale of the individual cell. We will show that these observations are consistent with the peeling hypothesis introduced in [10] to describe first order kinetics cell detachment assays. An explicit calculation of the peeling velocity as a function of the external force is presented, and compared with experimental data. Orders of magnitude for molecular parameters will be deduced from this analysis. Moreover, it will be shown that this model can be interpreted as a phenomenon of fracture propagation, as in the layered materials [17]. The dissipation will indeed be related to the fracture velocity, and explicitly calculated in the high velocity limit.

The experiments were performed using the model unicellular organism *Dictyostelium discoideum* [18]. Forces are applied using a lateral flow chamber assay, described in [19]. Cells are spread on a glass plate and submitted to a net shear force generated by fluid flow in the space e between the glass plate and the upper surface. The force is determined by measuring the volumetric flow rate D, and the cell projected area S, using the classical Poiseuille-flow relation $F = 6D\eta S/le^2$ (η is the water viscosity and l is the width of the chamber). Cell preparation, glass treatment, and fluid composition are described in detail in [10]. The transparency of this setup allows microscopic observation during application of the flow. The contact area between the cell and the glass plate is imaged using reflection interference contrast microscopy (RICM) [9,20]. A first qualitative observation indicates that peeling is indeed the mechanism leading to cell detachment under shear flow, as shown by the reduction of cell-substrate contact area while progressive detachment proceeds (see Fig. 2). To be more quantitative, histograms of the instantaneous velocities at the cell's rear are plotted for different values of the local force F_l applied on the contact line. As schematized in Fig. 1, the region where the membrane leaves the

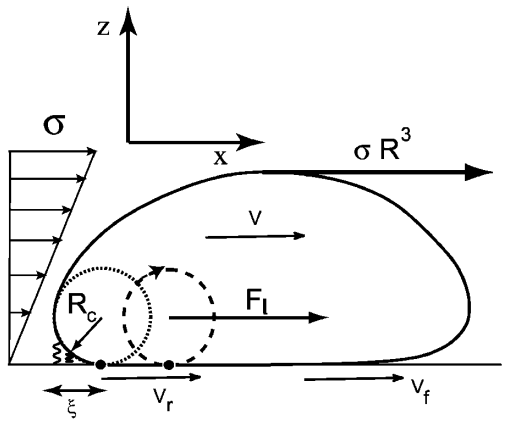

FIG. 1. A cell adhering on a solid substrate is submitted to a steady shear flow stress. Imaging the cell allows one to measure the velocities of the rear v_r, the front v_f, and the center of mass v. In the 1D peeling model, the margin of the cell is modeled as a circle of radius R_c. Under the action of the external force, the circle rolls and the adhesive bridges are stretched and detached. The width of the adhesive belt is ξ. σR^3 is the moment with respect to the contact line.

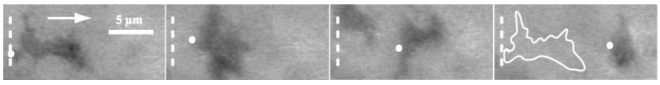

FIG. 2. RICM observation of a *Dictyostelium discoideum* cell under shear flow. The arrow indicates the direction of the flow. Time between images is 45 s (40× magnification). To evidence the peeling process, the contour of the cell-substrate contact area is reported on the right panel. The white dot represents the position of the rear front (the black spot in the upper left corner of the third picture is the image of an other cell moving in the direction of the flux). The advancement of the rear front has been determined by measuring the position of the outermost contact point.

substrate is curved, with radius of curvature R_c [9]. F_l is thus evaluated by requiring the equality of the moment of the hydrodynamic force and the moment of the local force with respect to the contact point O. This scaling argument gives $F_l R_c \simeq \zeta F R$, where we have introduced an effective ζ coefficient to account for the active response of the cell which is proportional to the external force in the low flux regime. For *Dictyostelium*, $R_c \simeq 1\ \mu$m [9] and $R \simeq 6.2\ \mu$m (see Fig. 2). Observation shows that the velocity of the rear contact line exhibits fluctuations with a well-defined mean value. Results for two values of the local force are given in Fig. 3. Moreover, in the peeling condition reported here, the front velocity v_f is very close to the rear velocity v_r. Consequently, the cells move over large distances (of the order of 50 μm) before leaving the substrate. This allows one to estimate the rear velocity v_r by that of their center of mass v, which can be measured more easily on a whole cell population, thus increasing the statistics of this velocity measurement. Figure 4 shows the position of the rear front for a single cell. The curve scales with time in contrast to the forefront which moves in a cyclic way by extending pseudopods. The nonlinear relation v versus F_l obtained using this method, when varying the external force on the cells, is presented in Fig. 5.

Figure 5 compares this result with the theoretical expression of v versus F_l derived in the frame of the peeling model presented below [21]. This model deals with the

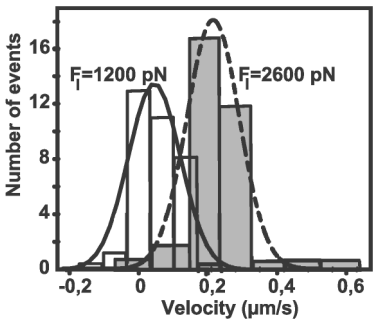

FIG. 3. Histograms of rear instantaneous velocities at two values of the applied local force F_l. The histograms for one cell are fitted with Gaussian distributions as a guide for the eye.

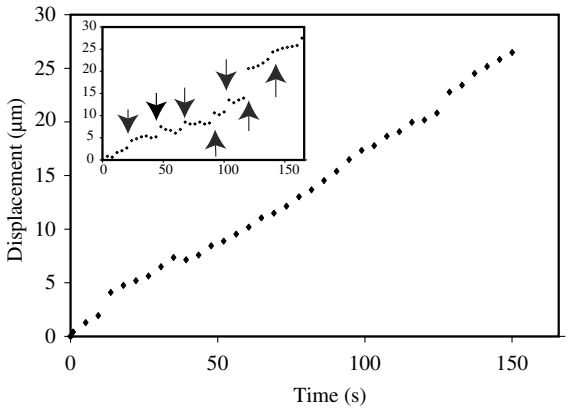

FIG. 4. Plot of the position of the rear front as a function of time. Note that the position scales almost linearly with time and that the cyclic component corresponding to the extension of pseudopods at the forefront is a small perturbation on the scale of the figure. Inset: Plot of the position of the forefront as a function of time. Each step indicated by an arrow corresponds to the extension of a pseudopod.

problem of determining the velocity v of the contact point O between the cell membrane and the substrate as a function of the applied force F_l (see Fig. 1). Since *D. discoidum* adhesion to glass depends on two adhesion molecules [22,23], the cell adhesion is modelized by discrete molecular bridges. The passing fluid exerts a net force on the cell membrane, which is transmitted to the extreme margin of the cell facing the flow. The region where the plasma membrane leaves the substrate, called the adhesive belt, is curved, as observed in [9]. Our simple 1D model consists in modeling the adhesive belt as a part of a circle, with constant radius of curvature R_c. The circle rolls under the action of F_l, peeling the membrane off the substrate. In the simplest approximation, an adhesive bridge is modeled by a spring of stiffness k, with a noncovalent bond at its

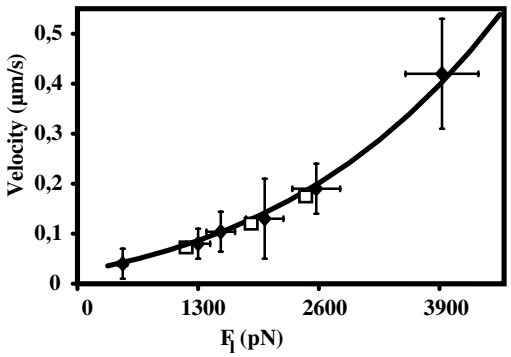

FIG. 5. Peeling front velocity versus local applied force. ◆ represent data obtained by the measurement of the mean velocity v of the center of mass of 20 cell panels, □ represent data obtained by velocity measurement of the cell's rear velocity v_r on three cells. The solid curve is obtained using Eq. (1). A good fit is obtained with $v_0 = 0.01\ \mu$m/s and $F_0 = 240$ pN.

end. The local force exerted on the molecular attachment is $f_b = kz$, where z is the extension of the spring. The interaction between this adhesive bridge and the substrate is modeled by a two states system, anchored and free, separated by an energy barrier. The kinetics of the adhesive bridge is governed by on and off rates, depending on the force f_b applied to the chemical bond [1], namely, $k_{\text{on}}(z) = k_{\text{on}}^0 \exp(-\Delta kz/k_BT)$ and $k_{\text{off}}(z) = k_{\text{off}}^0 \exp(\Delta kz/k_BT)$ [24], where $k_{\text{on,off}}^0 \propto \exp(-\Delta U_{\text{on,off}}/k_BT)$. $\Delta U_{\text{on,off}}$ are the values of the energy barriers seen, respectively, from the free (anchored) state, and Δ is the range of the interaction responsible for molecular adhesion. When the circle rolls under the action of F_l, adhesive bridges, which were previously under the cell in the contact zone, are progressively stretched in the adhesive belt. The number of adhesive bridges in the adhesive belt depends on F_l and v. Neglecting lateral diffusion, the kinetics equation for anchored bridges is $\partial_t n_b(z) = k_{\text{on}}(z)(n_0 - n_b) - k_{\text{off}}(z)n_b$, where the total density of adhesive bridges n_0 is defined by $n_0 = n_b + n_f$ and n_f is the density of free bridges. Neglecting the attachment because of the exponential falloff of $k_{\text{on}}(z)$ yields to $\partial_t n_b(z, v) = -k_{\text{off}}(z)n_b(z, v)$. Solving this equation [25], one obtains the expression of n_b in the large velocity limit $n_b(v, x) \simeq n_b^0 \exp[-\frac{v_0}{v}(\frac{\xi_0}{x})e^{(x/\xi_0)^2}]$, where $\xi_0[2(R_c k_B T/k\Delta)]^{1/2}$. In this formula, v_0 is a characteristic velocity given by $v_0 = k_{\text{off}}^0 \xi_0/2$. So, in the limit $v \gg v_0$, n_b has the shape of a step function, with a rapid falloff to 0 at a characteristic distance $\xi(v)$ which defines the width of the adhesive belt. From $n_b(v, x)$, the following autocoherent equation is obtained, defining the size of the adhesive belt: $v/v_0 = [\xi(v)/\xi_0]\exp[\xi(v)/\xi_0]^2$. In the large velocity limit, this leads to $\xi(v) \approx \xi_0[\ln(v/v_0)]^{1/2}$. ξ increases logarithmically with the peeling velocity v. Assuming that the circle rolls at a constant velocity, the balance of the moment of the restoring force due to the anchored bridges with the moment of the external local force F_l, with respect to the contact point O, gives $F_l = k\sqrt{2/R_c}\int_0^\infty n_b(v, z)z^{3/2}dz$. This equation, combined with the expression for $n_b(v, x)$, allows the determination of the relation $v(F_l)$. Approximating the distribution $n_b(x, v)$ by a step function between 0 and $\xi(v)$, the former equation leads to the expression of the size of the adhesive belt as a function of the external force $\xi = (8R_c^2/n_0 k)^{1/4} F_l^{1/4}$ and to the expression of v as a function of the external force in the thermoactivated regime:

$$v(F_l) \simeq v_0 \frac{\exp[(F_l/4F_0)^{1/2}]}{(F_l/4F_0)^{1/4}}, \tag{1}$$

with $F_0 = (n_0/8k)(k_BT/\Delta)^2$. Taking $\zeta \simeq 1$ is enough to get orders of magnitude and this gives $v_0 = 0.01\ \mu$m/s and $F_0 = 240$ pN (see Fig. 5). Typical values of the constants appearing in v_0 and F_0 are $R_c \approx 1\ \mu$m [9], $k \approx 10^{-3}$ N/m [21], $\Delta \approx 1$ Å [24], and give orders of magnitude for $k_{\text{off}}^0 \simeq 10^{-2}\ \text{s}^{-1}$ and $n_0 \simeq 10^7$–$10^8\ \text{m}^{-1}$ in agreement with previous literature [1,7]. Precisely, we show that measuring the velocity of the contact line of a single cell under external force as in Fig. 5 agrees with the results of macroscopic cell detachment assays which measure the first order kinetics of detachment for a whole population. This allows the measurement of the two important molecular parameters, namely, k_{off}^0 and n_0. It is noteworthy that the parameter F_0 corresponds precisely to the scaling stress $\sigma_0 \simeq (F_0/S)(R_c/R)$ ($S \simeq 120\ \mu\text{m}^2$ being the mean cell projected area), determined independently in *Dictyostelium discoideum* detachment experiments from glass (see [10]).

In this last section, we calculate the dissipation occurring during the peeling of the membrane, and interpret this phenomenon in the more general frame of fracture phenomena. The fracture's tip has a velocity v under the action of F_l. Let G_T be the work needed to extend the fracture over a unit area and $W_{\text{adh}} = n_0 \Delta G$ the reversible work required to separate the cell from the substrate reversibly without dissipation. Following de Gennes [17], the dissipation due to the movement of the contact line can be written, in terms of out of equilibrium thermodynamics, as $T\frac{dS}{dt} = (G_T - W_{\text{adh}})v$. $G_T - W_{\text{adh}}$ is interpreted as a generalized thermodynamics force and v as the corresponding generalized conjugate flux, which characterizes the response of the fracture to the force. In the low flux regime, a linear relationship between flux and force is generally postulated, as $G_T - W_{\text{adh}} = \eta v$. But as pointed out in [17], a more general nonlinear relationship has to be considered, such as

$$G_T - W_{\text{adh}} = \Phi(v), \tag{2}$$

where Φ is a nonlinear function of v.

In order to obtain the expression of G_T, we consider the energy variations due to the steady-state movement of the contact line at a velocity v under the action of F_l. For an arbitrary displacement δx of the contact line along x, the work of the external force is $\delta W = F_l \delta x$. All of the bonds located between $\xi(v)$ and $\xi(v) - \delta x$ detach, absorbing an energy $\delta W_r = n_0 \Delta G \delta x$ and dissipating the elastic energy $\delta U_{el} = -2n_0 k z_{\max} \Delta \delta x$ (see [26]) to the leading order in δx. The maximum extension $z_{\max}$ of a bond corresponds to the extension of the most stretched bond, which scales as $z_{\max} \simeq \xi^2(v)/2R_c$. This leads to $\delta U_{el} = -n_0 k \Delta\{[\xi^2(v)]/R_c\}\delta x$. Moreover, the elastic bonds are stretched in this process. The corresponding energy cost is $\delta U_s = \int_0^{\xi(v)} n_b(x, v)\frac{1}{2}kz^2dx - \int_{-\delta x}^{\xi(v)-\delta x} n_b(x, v)\frac{1}{2}kz^2dx \simeq (n_0 k/8R_c^2)\xi^4(v)\delta x$ to the leading order in δx. The conservation of energy implies $\delta W = \delta U_s + \delta U_{el} + \delta W_r$ leading, for an arbitrary displacement δx, to $F_l - n_0 \Delta G = (n_0 k/8R_c^2)\xi^4(v) - n_0 k \Delta \xi^2(v)/R_c$. Replacing $\xi(v)$ with its expression in the high velocity limit, one gets $F_l - n_0 \Delta G = F_0(\ln v/v_0)^2 - n_0 k_B T(\ln v/v_0)$. As F_l is the force applied on the fracture per unit of fracture's length, the work done by the external force in a fracture's

displacement δx scales as $G_T \delta x \simeq F_l \delta x$ resulting in $G_T \simeq F_l$. We obtain the equivalent of Eq. (2):

$$G_T - W_{\mathrm{adh}} = 4F_0(\ln v/v_0)^2\left[1 - \frac{4k\Delta^2}{k_B T}\frac{1}{\ln v/v_0}\right]. \quad (3)$$

With the previous given values of the parameters, and taking $v \geq 10v_0$, one obtains $8k\Delta^2/[\ln(v/v_0)k_B T] \simeq 10^{-2}$ in typical experimental conditions [27].

It is interesting to compare the dissipation to the reversible work, by calculating $(G_T - W_{\mathrm{adh}})/W_{\mathrm{adh}} \simeq [(k_B T)^2/(8k\Delta^2 \Delta G)]\Phi(v)$. Taking $\Delta G \simeq k_B T$, one gets $(G_T - W_{\mathrm{adh}})/W_{\mathrm{adh}} \geq 10^2$, showing that, under our conditions, the dissipation is much larger than the reversible work. The stretching and rupture of the adhesive bonds in this special case of fracture propagation are thus responsible for a very strong dissipation during the peeling process.

In summary, we presented new direct observations of the forced movement of the contact line between a living cell (*Dictyostelium discoideum*) and a solid substrate, using reflective interference contrast microscopy. Cell detachment under these conditions occurs by peeling the membrane off the substrate, as shown by the reduction of the contact area with time. This observation supports the use of a peeling model to describe *Dictyostelium discoideum* cell detachment under flow [10,21]. The peeling model we present here points out the fundamental role of the adhesive belt under force. The nonlinear behavior of the contact line under external force has two origins: (i) the statistical behavior of the adhesive bridges in the adhesive belt leading to the strong dissipation during bond rupture, and (ii) the geometry of the adhesive belt, which fixes the relation $\xi(F)$ and, consequently, the value of the power in the exponential term in Eq. (1). It appears that the study of the kinetics of the contact line allows the determination of important molecular parameters ($k_{\mathrm{off}}^0 \simeq 10^{-2}$ s^{-1}, $n_0 \simeq 10^{16}$ m^{-2}).

This work was supported by a grant ''adhésion cellule-matériaux'' from the Institut National de la Santé et de la Recherche Médicale and the CNRS-Matériaux, and a grant ''Reconnaissance et adhérence cellulaire'' from the I.P.M.C. (Grenoble).

[1] G. Bell, Science **200**, 618 (1978).
[2] C. Cozens-Roberts, J. Quinn, and D. Lauffenburger, Biophys. J. **58**, 107 (1990).
[3] S. Kuo and D. Lauffenburger, Biophys. J. **65**, 2191 (1993).
[4] E. Evans, Biophys. J. **30**, 265 (1980).
[5] M. Dembo, D. Torney, M. Saxman, and D. Hammer, Proc. R. Soc. London **234**, 1988 (1988).
[6] P. Bongrand, Rep. Prog. Phys. **62**, 921 (1999).
[7] E. Evans, in *Handbook of Biological Physics*, edited by R. Lipowsky and E. Sackmann (Elsevier Science B.V., New York, 1995), Vol. 1, p. 723.
[8] E. Evans, D. Berk, and A. Leung, Biophys. J. **59**, 838 (1991).
[9] R. Simson, E. Wallraff, J. Faix, J. Niewoehner, G. Gerisch, and E. Sackmann, Biophys. J. **74**, 514 (1998).
[10] E. Décavé, D. Garrivier, B. Fourcade, Y. Bréchet, and F. Bruckert, Biophys. J. **82**, 2383 (2002).
[11] F. Pincet, E. Perez, J. Loudet, and L. Lebeau, Phys. Rev. Lett. **87**, 178101 (2001).
[12] E. Evans, Faraday Discuss. **111**, 1 (1998).
[13] E. Evans and K. Ritchie, Biophys. J. **72**, 1541 (1997).
[14] D. Simson, M. Strigl, M. Hohenadl, and R. Merkel, Phys. Rev. Lett. **83**, 652 (1999).
[15] C. Zhu, J. Biomech. **33**, 23 (2000).
[16] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, and E. Evans, Nature (London) **397**, 50 (1999).
[17] P. D. Gennes, in *Adsorption et Adhésion* (Les Editions de Physique, Paris, 1983), pp. 1–10.
[18] Cells from AX-2 axenic strain were used in the vegetative phase.
[19] M. Wattenbarger, D. Graves, and D. Lauffenburger, Biophys. J. **57**, 765 (1990).
[20] J. Bereiter-Hahn and P. Vesely, in *Cell Biology, A Laboratory Handbook*, edited by J. Celis (Academic, New York, 1995), Vol. 3, pp. 54–63.
[21] D. Garrivier, E. Décavé, Y. Bréchet, F. Bruckert, and B. Fourcade, Eur. J. Phys. E 8, 79 (2002).
[22] S. Cornillon, E. Pech, M. Benghezal, K. Ravanel, E. Gaynor, F. Letourneur, F. Bruckert, and P. Cosson, J. Biol. Chem. **275**, 34287 (2000).
[23] C. P. Chia, Exp. Cell. Res. **227**, 182 (1996).
[24] S. Chen and T. Springer, Proc. Natl. Acad. Sci. **98**, 950 (2001).
[25] S. Izrailev, S. Stepaniants, M. Balsera, Y. Oono, and K. Schulten, Biophys. J. **72**, 1568 (1997).
[26] The expressions of k_{on} and k_{off} allow one to compute the change in the energy difference between bound and unbound states as a function of the restoring force exerted on the bond as $2kz\Delta$. This leads to this expression of the dissipated energy for the $n_0 \delta x$ bridges detached between $\xi(v)$ and $\xi(v) - \delta x$.
[27] Taking into account nonspecific interactions proportional to the contact area adds subdominant corrections. See also [5].