Cell adhesion: The effect of a surprising cohesive force

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When an experimentalist or a biological mechanism applies an external force onto a cell chemically sticking to its substrate, a reacting "suction" force, due to the slow penetration of the surrounding fluid between the cell and the substrate, opposes to the dissociation. This force can overcome other known adhesive forces when the process is sufficiently violent (typically 10^5 pN). Its maximal contribution to the total adhesive energy of the cell can then be estimated to 2×10^{-3} J/m^2. The physical origin of this effect is quite simple and it may be compared to that leaning a "suction cup" against a bathroom wall. We address the consequences of this effect on (i) the separation energy, (ii) the motion of the fluid surrounding the cell, and more especially on the pumping of the fluid by moving cells, and (iii) the inhibition of cell motion.

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Cell adhesion is fundamental in biology [1]. For instance, cell division, cell differentiation, cell migration, infections (adhesion of pathogenic agent), leucocytes-endothelium interaction, and colonization by the cells of a primitive cancerous tumor are partially regulated by the presence of sticky links between cells and their environment. An important stage for understanding these interactions has been investigated by Bell [2] in 1978 when he described their dissociation kinetics. His results stimulated a number of works on link properties and cell-substrate dissociation dynamics [3–10]. A powerful way for understanding sticky effects consists in studying the reaction of a cell to an external separating force [3,6]. In these conditions, it has been shown that the adhesion energy (separation energy when the extraction velocity is zero) is approximately equal [10] to 10^{-4} J/m^2 and the sticky-force strength for a bond [7] increases between 1 and 200 pN when the loading rate varies from 0.1 to 60 000 pN/s. On the other hand, the survival time for bond between ligands and receptors decreases between 60 and 10^{-3} s.

We show that an *additional* force, originating from the intercellular fluid viscosity, can play an important role in the cell-substrate separation dynamics. When an experimentalist or a biological mechanism applies abruptly an external separating force on the cell, a reacting "suction-cup" force opposes to the dissociation. This force can overcome other known adhesive forces when the process is sufficiently violent (typically 10^5 pN with 1.5×10^{-6} m cell-substrate initial contact radius). Its maximum contribution to the total adhesive energy of the cell can then be estimated to 2×10^{-3} J/m^2 in the context of Ref. [10]. The physical origin of this effect is quite simple and it may be compared to that of holding a suction cup against a bathroom wall. Thus, in contrast to similar hydrodynamic forces caused, for instance, by shear flow [8,9], the suction-cup force is purely attractive. Consequently, it regulates the intercellular fluid flow and, under extreme external conditions (e.g., shocks, tears, etc.), becomes the dominant cohesive factor of the cell assembly.

When a cell immersed in a liquid medium is pulled out from its substrate under external constraints, the pressure P_2 under the cell diminishes below the pressure P_1 of the surrounding fluid (Fig. 1). The pressure difference, ΔP, on the one hand yields a flow permitting the fluid to follow the cell motion and, on the other hand, pushes the cell against its substrate, hence generating the suction-cup force. Since both the suction-cup force and the fluid velocity (which is related to the cell extraction velocity V, and more precisely, to the extraction speed of the bottom surface cell) are proportional to ΔP, it follows immediately that this force is an increasing function of V. This connection between the fluid flow and the pushing force has the following remarkable effect. When the flow is inhibited under the cell, the suction-cup force increases. Indeed, the fluid motion is induced by the pressure difference, in such a way that slowing it artificially maintains ΔP strong and reinforces the suction-cup force. As a consequence, the suction-cup effect may be very efficient at the beginning of the separation process, i.e., when the presence of unbroken sticky links (for instance, ligand linked to a receptor by a flexible polymer) and the small size of the under-cell channels (where the fluid flows) strongly inhibit the fluid motion (inhibited pumping). Thus, a large energy barrier preventing the cell-substrate separation may be active during a short time at the beginning of the process.

Since large velocities induce large forces, two situations must be considered:

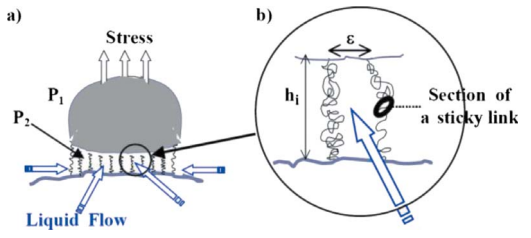

FIG. 1. (Color online) Schematic of a cell embedded in a liquid at pressure P_1. The cell sticks to its substrate by means of sticky links. (a) When the cell is stretched, the pressure P_2 at the cell-substrate interface decreases, which yields a pumping of the external fluid toward the cell-substrate contact zone. The liquid enters under the cell by "doors," one of which being represented in (b).

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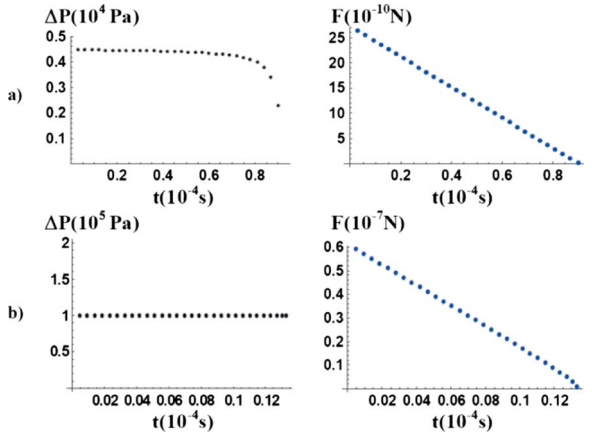

FIG. 2. (Color online) Suction-cup pressure and suction-cup force. (a) Suction-cup pressure ΔP and suction-cup force F in the undercritical regime ($V=10^{-3}$ ms^{-1}) just before the rupture of sticky links (i.e., when $h_i=h_c$) and (b) in the critical regime. In (a) and (b), the sticky links density is $4\times10^{14}/\mathrm{m}^2$ and the fluid dynamic viscosity is 10^{-3} kg s^{-1} m^{-1}.

(i) In the small-velocity (undercritical) regime, the pressure P_2 remains strictly positive [Fig. 2(a)]. This regime terminates at the critical velocity V_c for which P_2 vanishes (critical regime).

(ii) Above V_c (overcritical regime), the fluid fails to fill the growing under-cell cavity and P_2 remains locked to zero. An empty volume (i.e., a low-pressure gas volume) must then be created between the cell and the liquid. In this regime, the velocities of the top surface of the under-cell fluid and the suction-cup force are locked to their maximal values, V_c and P_1S, respectively (S is the under-cell surface area).

The efficiency of the suction-cup effect is thus maximal in the critical and overcritical regimes since ΔP and the cell-substrate separation energy barrier are then maximum [Fig. 2(b)]. In addition, since the separation time decreases obviously when V increases, the critical regime is the slowest among these efficient processes. In this paper, our approach is conceptually different to that of Ref. [6]. In effect, in Ref. [6], P_2 is taken equal to P_1 so that the suction-cup effect is not taken into account. However, the approach of Ref. [6] is perfectly justified in the regime of the very small velocity. Let us now focus attention on the critical regime.

In order to estimate the magnitude of the suction-cup effect (see the Appendix), one has to consider a more realistic scenario for the fluid penetration. We have previously introduced V and V_c as velocities of the interface considered as a rigid object. In reality, since the cell is deformable, they can take different values at different points of the interface. Moreover, they both vary with time during the separation process. Consequently, the suction-cup effect applies only in a small area neighboring the closed line ("contact line"), moving from the border of the cell toward its center, which separates the tackled (inside the line) and the already free (outside) parts of the cell (Fig. 3). The relevant parameter for the study of the cell adhesion is the cell-substrate separation energy [6], which is calculated below. Since the suction-cup pressure is constant in the critical regime, the corresponding suction-cup separation energy W is easily evaluated by using

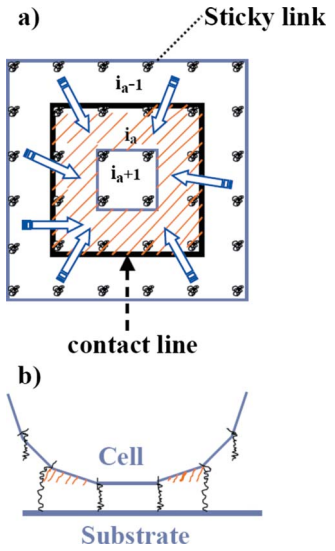

FIG. 3. (Color online) Network formed by the sticky links and zipper mechanism sketches. (a) Square network formed by the sticky links seen from above. It forms coronas which rise successively during the cell-substrate separation process. Only the corona denoted by i_a (hachured part) is suction-active (where the suction-cup force applies). (b) Zipper mechanism. The figure represents a section of the cell bottom during the raising process. Each couple of symmetric segments belongs to a single corona. In (a) and (b), the links in the central "corona at rest" are not yet stretched. The links in the i_a intermediate corona are stretched but not broken, whereas the links of the external "liberated corona" are already broken. During the zipper mechanism, i_a moves toward the center of the cell. The liquid (blue arrows) fills progressively the corresponding cavities while the volumes above external coronas fill up instantaneously.

typical cell characteristics given in Refs. [10,11] $W/S=2\times10^{-3}$ J/m^2, where $S=6.4\times10^{-11}$ m^2 is the cell-substrate initial contact area. This value is to be compared to the adhesion energy needed to break the sticky links, the maximum of which being 8×10^{-5} J/m^2 in the context of Ref. [10]. One can see that the suction-cup energy barrier is one order of magnitude larger than this sticky barrier when $V\geq V_c$. Let us note that the value of the adhesion energy reported in Ref. [10] corresponds to a single point in the *separation energy–extraction velocity* diagram. For very small velocity ($v\approx10^{-7}\exp[\varphi10^{12}]$; see Ref. [10]), the rupture force φ for individual link [7,10] is equal to 5 pN. We can infer an approximate sticky energy value, $\Gamma\ \varphi\ h_B=5\times10^{-5}$ J/m^2 ($\Gamma=4\times10^{14}$ m^{-2} is the density of links and $h_B\approx2.5\times10^{-8}$ m is the maximal size of a sticky link, i.e., ligand linked to a receptor by a flexible polymer, before rupture) which does not take into account the suction-cup effect and is in good agreement with the value of 8×10^{-5} J/m^2. In this case, $V\approx1.5\times10^{-5}$ m/s is undercritical and the corresponding suction-cup energies are equal to 10^{-6} J/m^2 and 5×10^{-6} J/m^2 in water or extracellular liquid [12], respectively. For a constant high-speed extraction just below the minimum of V_c (V_c depends on time; see the Appendix) i.e., 4×10^{-3} m/s for water ($V_{c\,\mathrm{min}}=4.2\times10^{-3}$ m/s) or 6.9×10^{-4} m/s for extracellular liquid ($V_{c\,\mathrm{min}}=7\times10^{-4}$ m/s), we find $\varphi\approx10$ pN, the corresponding sticky energy value

becomes 10^{-4} J/m^2, whereas the suction-cup energies are equal to 2.3×10^{-4} J/m^2 and 2.5×10^{-4} J/m^2, respectively.

In order to estimate the efficiency of the suction-cup force, one needs to further evaluate the separation time $\Delta\tau^c$ in the critical regime (see the Appendix). Within the same context as in Ref. [10], $\Delta\tau^c\approx10^{-5}$ s (with water) or 10^{-4} s (with extracellular liquid [12]). These values are much shorter than typical separation times (from 10^{-3} to several seconds, which corresponds to $V\ll V_c$) usually reported in the literature for artificial as well as natural intercell motions [13]. This means that, for such small velocities, the dynamics are in fact under critical and the suction-cup energy barrier becomes of the same order of magnitude as the sticky one or smaller.

Suction-cup effect takes place even in systems in which the cell separation is not necessarily described by the zipper model. Consider, for instance, two cardiac cells glued together by sticky links (desmosomes) with contact area 10^{-12} m^2 and $h_B=10$ nm. The presence of desmosomes (sticky links assembled in rigid plates) between the cardiac cells prevents zipperlike separation and the cells are stretched without deforming the contact zone. Hence, the order of magnitude of the suction-cup force and energy in the critical regime can be estimated to 10^5 pN and 10^{-3} J/m^2, respectively. The suction-cup effect opposes to separation as well as to any cellular fluctuation. In fact, it dissipates a part of the metabolic energy produced by the cells for generating small movements around their equilibrium positions in biological tissues. At this point of view, the suction plays an active role of regulator. This regulation can be estimated when one knows the amplitude and the frequency of the cell motion. Unfortunately, these data are usually not known for *in vivo* cell vibrations. However, their order of magnitude can be deduced from data reported in Ref. [13], concerning cell wall oscillations in yeast cells with 5 μm diameters surrounded by air. The amplitude of the wall vibrations is 3 nm, with a mean velocity $V=2.6-4.9\times10^{-6}$ m/s. The maximum internal force and energy that the cell metabolism can generate are given by the authors of the reference: 10^{-8} N and 3×10^{-17} J during one-oscillation with 3 nm amplitude. Considering now the same cell linked [11] to a substrate permits us to estimate the energies dissipated by suction when the fluid is either air or water: (i) $W_S=3.7\times10^{-20}$ J and $W_S=2\times10^{-18}$ J, respectively, when $V=2.6\times10^{-6}$ m/s; (ii) 7×10^{-20} and 3.9×10^{-18} J, respectively, when $V=4.9\times10^{-6}$ m/s. One sees that, at these velocities, the suction-cup effect would use a negligible part of the metabolic energy. Nevertheless, the suction-cup effect might act as a regulator of the cells fluctuations to prevent large amplitudes or velocities. Indeed, with the previous amplitude in water the velocity of the wall cannot reach 3.8×10^{-5} m/s because the whole metabolic energy would be dissipated by suction. By the previous regulation effect, the suction-cup effect can participate to the restriction of the nutriments (or dangerous elements) pumped by the cell in its environment.

Lastly, let us note that the suction-cup effect could be considerably magnified if cavitation-type effects would take place in the under-cell liquid. Indeed, in this case, metastable negative pressures are possible [14] so that the critical regime appears at values of V/V_c larger than unity, which would lead to a significant increase of the maximal energy barrier. Although such effects are not likely with usual low-viscosity organic fluids, one expects very large suction-cup energies, even at low velocities (because, in addition, the viscosity diminishes V_c), when more viscous fluids are involved.

We have seen that in general, $\Delta\tau^c$ is shorter than typical separation times (from 10^{-3} to several seconds) reported in the literature for artificial as well as natural intercell motions. Quantitative measures that appear in the literature are often related to the undercritical dynamics and the suction-cup energy barrier is smaller than the sticky one. On the contrary, when considering violent processes, which can be obtained under extreme external conditions (e.g., shocks, tears, etc.), the suction-cup effect becomes the dominant cohesive factor of the cell assembly. Unfortunately, such phenomena have not yet been studied experimentally at the relevant time scales. Sharpened studies of violent processes over a short small time could reveal new and unexpected phenomena and could then give new insights into the organic system under extreme stress.

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APPENDIX

In order to simplify the estimation of the critical velocity V_c and of the separation time $\Delta\tau^c$, we assume that the sticky molecules are distributed on a square network with lattice spacing ε. The contact surface of the cell is approximated by a square with side lengths $n\varepsilon$ that we decompose into small rigid surfaces having the form of concentric square coronas. The corona i (the outside corona is labeled by $i=1$) has a perimeter $L_i=4(n-2i+2)\varepsilon$, an area $S_i=4(1-2i+n)\varepsilon^2$, and a height h_i above the substrate. When the cell is raised, the fluid enters under the cell from the outside toward the center. Because of the presence of the sticky molecules ($h_f\approx4$ nm) [13], the initial height h_f of the cell underside above the substrate is not strictly zero.

Two neighboring sticky links form, together with the surfaces of the cell underside and substrate, a "door" by which the fluid enters. More precisely, the door plays the role of a pipe parallel to the substrate with an almost elliptical section. The length l of one door is typically equal to the diameter of the link section. The difference of pressure ΔP between the front and the back of the door generates a flow of fluid q[m^3 s^{-1}] given by [15]

$$q=(\pi/64\eta l)[h_i^3/(\varepsilon^3h_i^2+\varepsilon^2)]\Delta P, \tag{A1}$$

where η is the dynamic viscosity. At the level of the corona i, the total flow Q_i and the critical velocity V_{ci} are given by

$$Q_i=qL_i/\varepsilon, \tag{A2}$$

$$V_{ci}=2Q_i(t)/S_i. \tag{A3}$$

When $h_i\ll\varepsilon$, the role of the links in the inhibition of the fluid motion is negligible and the flow Q_i in Eq. (A2) depends no

longer on ε and becomes proportional to h_i^3. This arises, for each corona, at the beginning of the raising process or when the density of sticky links is small. The fluid penetration is then slowed down only by the smallness of h_i and it increases strongly with the height of the cell. When $h_i \gg \varepsilon$, the barrier to the motion due to the doors width ε becomes efficient and the flow Q_i increases more slowly with time and varies only as $h_i\varepsilon^2$. At this step of the process, large densities of sticky links much inhibit the fluid flow and increase the efficiency of the suction effect.

Integrating the balance equation $Q_i = qL_i/\varepsilon = d\Omega_i/dt$ [where $\Omega_i = 1/2(h_i - h_f)S_i$ is the volume of the fluid penetrating at the level of the corona i] yields

$$(4\eta l/\varepsilon^2 \pi \Delta P L_i) 8[S_i \log(h_i S_i) - \varepsilon^2 S_i^5 h_i^2/2] + A = t, \quad \text{(A4)}$$

where A is a constant of integration. The penetration time of the fluid at the level of the corona i is

$$\Delta\tau_i^c = t(h_i = \infty) - t(h_i = h_f), \quad \text{(A5)}$$

where $t(h_i)$ is given by the left-hand side of Eq. (A4). (From Ref. [10], above $h_i > 2.4 \times 10^{-8}$ m, the fluid penetration becomes so fast, from 0.5 m/s to several m/s, that it can be regarded as almost instantaneous. We denote by h_c this value 2.4×10^{-8} m.)

When h_{j-1} reaches a critical value h_M, the following corona j begins to rise on its turn. Figure 3 shows the profile of the cell during the raising process. It may be seen that the profile evolves with time like a "zipper" [9,16]. This is due to the fact that the membrane cannot be bent to an angle larger than a critical value characteristic of the local elasticity of the cell membrane (roughly speaking, the maximum angle value α_M (Fig. 3) above which the internal segment raises is related to the equilibrium contact angle (typically 45°) [10]: $\alpha_M = 180° - 45°$ then $h_M - h_f = \varepsilon \tan(\alpha_M) = 5 \times 10^{-8}$ m. The time before the cell separates completely from the substrate is therefore given by $\Delta\tau^c = \Sigma_{i=1}^N \Delta\tau_i^c$, where $N = (n+1)/2$ for n odd and $N = n/2$ for n even.

h_c being approximately equal to the (h_B) maximal size of a sticky molecule before it breaks ($h_B \approx 2.5 \times 10^{-8}$ m in the case of a ligand-receptor mediated by a flexible polymer and studied in Ref. [10]), then $h_M > h_B$ and there are only a few (typically, one) simultaneously raising coronas with unbroken links. The suction-cup force is non-negligible only on this "active" corona because ΔP decreases very quickly in the external ones.

In the critical regime, the suction-cup force depends simply on time. Indeed, the pressure exerted on the active corona, P_1, is constant so that the force depends only on the decreasing area of the corona when i increases. Then, the suction-cup force amounts $4(1 - 2i_a + n)\varepsilon^2 P_1$, where i_a is the index of the active corona. It decreases when i_a increases, i.e., when t increases, between a maximum value, $4(n-1)\varepsilon^2 P_1$, and zero during the time $\Delta\tau^c$ [Fig. 2(b)].

In this regime, the suction-cup pressure varies with time since $P_2(t)$ is no longer locked to zero. For each corona, $\Delta P_i(t)$ results from the equations $Q_i(t) = V_i(t)S_i/2 = q(t)L_i/\varepsilon$ and $h_i(t) = h_f + \int_0^t V_i(t')dt'$,

$$\Delta P_i(t) = (32\varepsilon\eta l/\pi)(S_i/L_i)\{[h_i(t)^2 + \varepsilon^2]/\varepsilon^3 h_i(t)^3\} V_i(t). \quad \text{(A6)}$$

If $V_i(t)$ is constant, then the suction-cup pressure on the corona i decreases with time. The suction-cup force and the pressure are plotted in Fig. 2(a) [between $h_i = h_f$ and $h_i = h_c$, each $\Delta P_i(t)$ decreases in its turn and, consequently, $\Delta P(t)$ oscillates with time].

The cell is free when all the sticky links are broken [10], i.e., when they are stretched to a length h_B. Thus, the suction-cup energy barrier is given by

$$W_S = \sum_{i=1}^{N} S_i \int_{hf}^{hB} \Delta P[h_i(t)] dh_i. \quad \text{(A7)}$$

In the critical regime, W_S is calculated by replacing $\Delta P(t)$ by P_1 in Eq. (A7) and in the undercritical regime by inserting Eq. (A6) into Eq. (A7).

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