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Unbinding of adhesive vesicles

Détachement des vésicules adhésives

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Abstract

We consider a vesicle, bound on one side to a pipette and sticking on the other side to a flat plate. When a pulling force f is applied to the pipette, the radius R_c of the contact patch decreases, and jumps to zero at a critical value of the force. We present here an extension of the Evans theory for these processes. Then we discuss the dynamics of separation for two distinct cases: (a) nonspecific adhesion; and (b) specific adhesion induced by mobile proteins. ***To cite this article: F. Brochard-Wyart, P.-G. de Gennes, C. R. Physique 4 (2003).***

Résumé

On considère une vésicule qui, aspirée par une pipette d'un côté, adhère de l'autre sur une surface plane. Lorsqu'on tire sur la pipette avec une force f, le rayon du contact adhésif décroît, et s'annule brusquement à une valeur critique de la force. On présente ici une extension de la théorie d'Evans pour interpréter ces processus de détachement. Puis l'on discute la dynamique de la séparation pour deux cas distincts : (a) adhésion non spécifique ; et (b) adhésion spécifique par des protéines mobiles. ***Pour citer cet article : F. Brochard-Wyart, P.-G. de Gennes, C. R. Physique 4 (2003).***

1. Introduction

A common way to test the strength of an adhesive contact of a cell on a substrate is to measure the force of detachment. The experiment is shown in Fig. 1 (here, the cell adheres to a plate, but it can also adhere on another cell). The experiment is performed as follows: the suction pressure ΔP is increased step by step. At each step, the pipette is pulled. If $\Delta P < \Delta P_c$, the contact is maintained, and the vesicle is extracted from the pipette: adhesion wins. At $\Delta P = \Delta P_c$, the cell separates from the substrate and remains attached to the pipette. The force $f_c = \Delta P_c \pi R_p^2$, where R_p is the pipette radius, is called the 'breaking force'.

We discuss here in Section 2 how f_c is related to the separation energy W. To model the cell, we consider a vesicle, which sticks on a substrate. The adhesion may be nonspecific (i.e., depletion forces [1,2], electrostatic [3], or van der Waals attraction), or specific [4–7]: a vesicle, decorated with mobile cellular adhesion proteins ('stickers'), is facing a wall grafted with the corresponding receptors.

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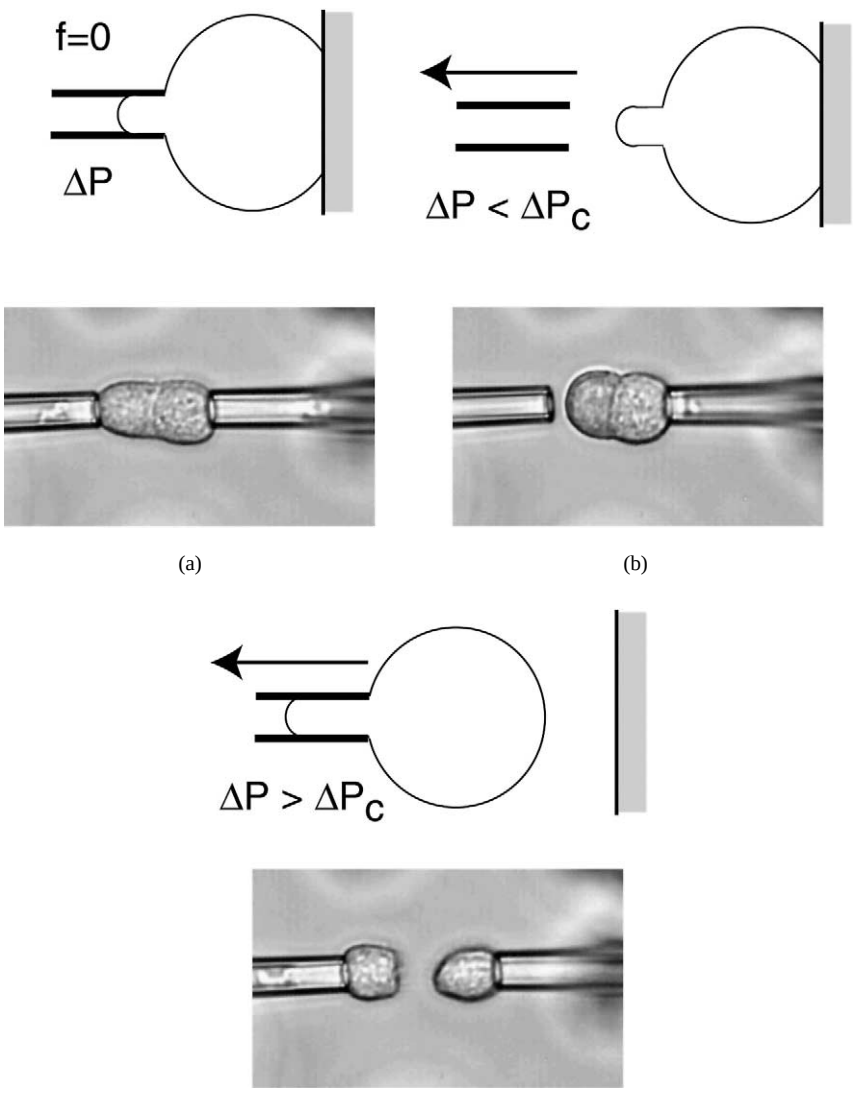


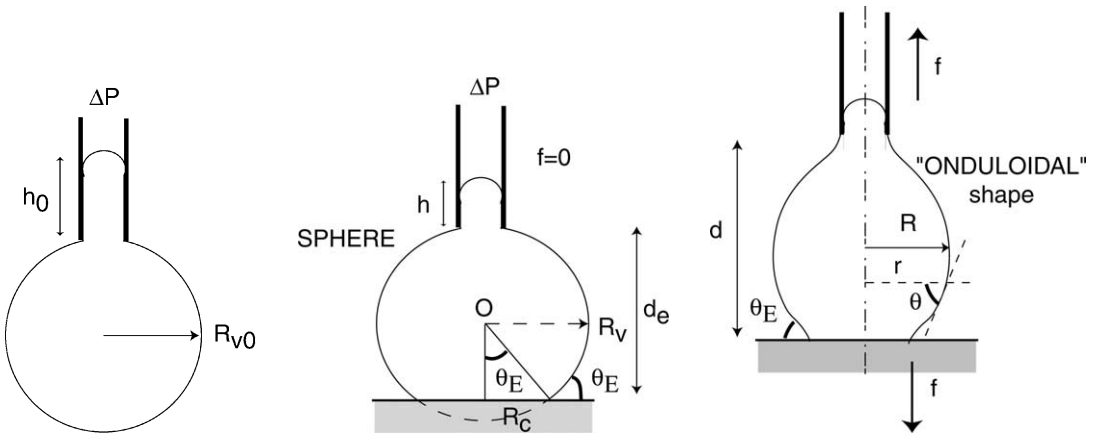
Fig. 1. (a) A vesicle, held to the pipette under an increasing aspiration pressure ΔP, sticks to a wall. As the pipette is moved outwards: (b) if $\Delta P < \Delta P_c$, the vesicle pipette junction becomes too weak and the finger is pulled out; or (c) if $\Delta P > \Delta P_c$, the junction is strong and the vesicle is ultimately detached from the wall. We show how this method is applied to break nonspecific bonds between cells (courtesy of Yeh-Shiu Chu and S. Dufour).

The paper is organised as follows: we first reconstruct the equilibrium state with no applied external force. Then, always assuming equilibrium, we see how the contact resists to an applied force, and suddenly breaks. This analysis is classical: the principles can be found in papers by E. Evans and coworkers [8,9]. However, we have attempted to present it in very simple terms. In the last sections, we describe the dynamics of detachment above f_c.

2. Unstressed contact ($f = 0$)

The free vesicle (Fig. 2(a)) maintained with the pipette before attachment is composed of a 'finger' of length h_0 and a spherical part (radius R_{v0}). The tension γ is imposed by the suction pressure $\Delta P = P_0 - P_p$ [10]:

$$\Delta P = 2\gamma\left(\frac{1}{R_p} - \frac{1}{R_{v0}}\right) \cong 2\frac{\gamma}{R_p}. \tag{1}$$



(a) (b) (c)

Fig. 2. (a) Free vesicle under aspiration by a micropipette maintained at a prescribed membrane tension γ; (b) adhesion of the vesicle to a wall in the absence of external forces: the vesicle is spherical; (c) vesicle stretched under a force f: the shape (reminiscent of droplets hung on a fiber), is onduloidal.

The 'bound' vesicle (Fig. 2(b)) includes again a finger of length h plus a truncated sphere. The contact angle θ_E can be deduced from a capillary force balance

$$\gamma_{SV} - \gamma_{SL} = \gamma \cos\theta_E, \tag{2}$$

where γ_{ij} are the substrate/vesicle, the substrate/liquid and the vesicle surface tension. The separation energy $W = \gamma + \gamma_{SL} - \gamma_{SV}$ is directly related to θ_E from Eq. (2)

$$W = \gamma(1 - \cos\theta_E). \tag{3}$$

The contact radius is $R_c = R_v \sin\theta_E$ and the equilibrium distance between the plate and the pipette is $d_e = R_v(1 + \cos\theta_E)$.

2.1. Remarks on the free energy

The contribution of the finger to the volume balance is negligible. The finger provides a reservoir of area: in the presence of a finger, we may change the area A of the truncated sphere without any work performed in the finger (because of the pressure balance equation (1)). We may thus write the free energy (at constant volume) in the form:

$$F = \gamma A - \pi R_c^2 W + \text{constant}. \tag{4}$$

For small θ the area A of the truncated sphere differs from the area A_i without contact (and with the same volume) by

$$A = A_i\left(1 + \frac{\theta^4}{16}\right) = 4\pi R_{v0}^2\left(1 + \frac{\theta^4}{16}\right). \tag{5}$$

We also have $R_c = R_v\theta$. Inserting this into Eq. (4) we arrive at:

$$f = 4\pi\gamma R_{v0}^2 \frac{\theta^4}{16} - \pi W R_v^2\theta^2 + \text{const}. \tag{6}$$

Optimizing this with respect to θ gives

$$\frac{1}{2}\gamma\theta^2 = W \tag{7}$$

and this is the Young equation (3) (for small θ).

3. Unbinding = statics

The pipette is now used not only to create a finger, but also to stretch the vesicle and to unbind it. The pipette is pulled from the plate with a force f (Fig. 2(c)). The free part becomes elongated, with a length $d = d_e + \delta$. The extension $\delta(f)$ has

been calculated in detail in [8,9]. Using this calculation, red blood cells have been operated as soft spring to pull on one single molecular bond [8]. Very roughly $\delta \approx f/\gamma$ (omitting logarithmic factors).

Our aim here is to study how R_c decreases with the external force f. In [9], both the pipette radius R_p and the contact radius R_c were kept constant. For the sticking vesicle, what is maintained constant is the pipette radius R_p and the Young angle θ_E (Eq. (2)), imposed by a balance of forces.

The contour of the vesicle is a surface of constant curvature (because the pressure inside is uniform) and looks like the profile of a droplet deposited on a fiber [10,11]. The profile can be derived from a balance of forces. On any section of the vesicle (radius r, angle θ shown in Fig. 2(c)), the projection of the force along the symmetry axis is constant and equal to f. It contains a surface term and a bulk pressure term:

$$2\pi r\gamma \sin\theta - \pi r^2 \gamma C = f. \tag{8}$$

For $f = 0$, the solution is a sphere ($C = C_0 = 2/R_v$).

For $f \neq 0$, we write Eq. (8) at both ends, and at the apex where the cross section radius is maximal ($r = R,\ \theta = \pi/2$).

$$f = 2\pi R_p \gamma \sin\theta_p - \pi R_p^2 C\gamma, \tag{9}$$

$$f = 2\pi R_c \gamma \sin\theta_E - \pi R_c^2 C\gamma, \tag{10}$$

$$f = 2\pi R\gamma - \pi R^2 \gamma C. \tag{11}$$

We set $\bar{f} = f/(2\pi R\gamma)$. Eq. (11) gives $C = \frac{2}{R}(1 - \bar{f})$ and Eq. (10) becomes:

$$\bar{f} = \frac{R_c}{R}\sin\theta_E - \frac{R_c^2}{R^2}(1 - \bar{f}) \cong \Psi\theta_E - \Psi^2(1 - \bar{f}),$$

where we put $\Psi = R_c/R$. The relation $\bar{f}(\Psi)$ is

$$\bar{f} = \frac{\Psi\theta_E - \Psi^2}{1 - \Psi^2}. \tag{12}$$

The result is plotted in Fig. 3(a). For small $\theta_E,\ \Psi$ is small and we may replace the denominator by unity $f(\Psi)$ as a maximum for: $\Psi \cong \theta_E/2$

$$f_{max} = \pi R \frac{1}{2}\gamma\theta_E^2 = \pi R W. \tag{13}$$

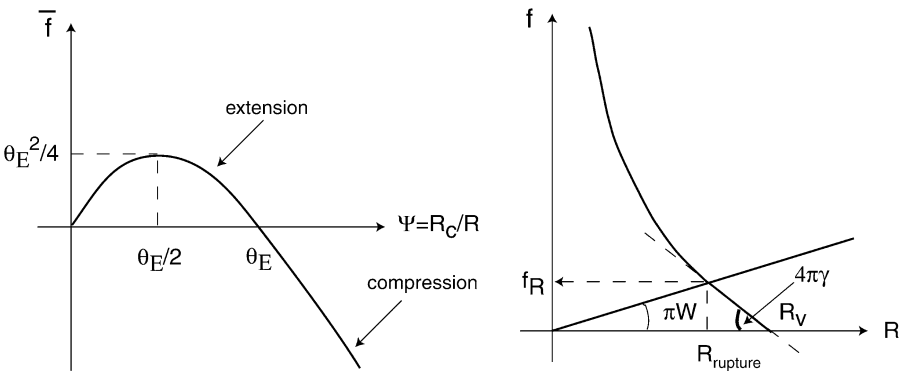
The maximal force is related to the maximal radius R (not R_c!) and to the adhesion energy W.

Eq. (15) defines the rupture force. It is the intersection of $f(R)$ by the line of slope $2\pi W$ (Fig. 3(b)).

(i) If $W \ll \gamma,\ \bar{f} = W/\gamma$ is very small and the profile is almost spherical. From [9], $R \cong R_v - f_{max}/(4\pi\gamma)$, i.e., $R/R_v \approx 1 - W/(2\gamma)$. This leads to:

$$f_{rupt} \cong \pi R_v W\left(1 - \frac{W}{2\gamma}\right) \cong \pi R_v W.$$

(ii) If $W \simeq \gamma$, the relation $R(f)$ must be calculated numerically.



(a) (b)

Fig. 3. (a) Plot of the force versus contact radius (in reduced units (Eq. (12))). Above $\bar{f}_{max} = \frac{1}{4}\theta_E^2$, the contact is destroyed abruptly; (b) the intersection of the curve relating the force to the apex radius R with the line of slope πW gives the rupture force f_R.

Detachment of contact/versus extraction from the pipette

As shown in Fig. 1, what is measured is not the force f acting on the pipette, but the critical pressure ΔP_c. If $\Delta P < \Delta P_c$, the vesicle is extruded because the aspiration force f_a

$$f_a = \pi R_p^2 \Delta P = 2\pi R_p \gamma$$

is smaller than the rupture force. On the other hand, as soon as $\Delta P \geqslant \Delta P_c$, the break arises at the wall. Thus $f_a(\Delta P_c) = \pi W R$ allows us to measure W.

4. Dynamics of unbinding: the case of nonspecific adhesion

4.1. The rate equation

If we pull on the pipette with a certain force $\bar{f}$ (in reduced units), we induce a contact angle $\theta(t)$ different from the equilibrium value θ_e. The radius of the contact patch $R_c(t) = R_v \Psi(t)$ decreases. The dissipation by viscous flow near the contact line creates a force opposing the noncompensated Young force F

$$F = \gamma(\cos\theta_e - \cos\theta) \cong \frac{1}{2}\gamma\left(\theta^2 - \theta_E^2\right). \tag{14}$$

The viscous force is of the form [11]

$$-k\frac{\eta}{\theta}\frac{dR_c}{dt} = -k\eta R_v \frac{1}{\theta}\frac{d\Psi}{dt}, \tag{15}$$

where the factor θ^{-1} describes the strong dissipation present for narrow wedges. η is the solution viscosity and k is a numerical constant (ignoring logs)

$$\frac{R_v}{V^*}\frac{d\Psi}{dt} = -\theta\left(\theta^2 - \theta_E^2\right), \tag{16}$$

where $V^* = \gamma/k\eta 2$.

We express θ in terms of ψ by the force equation (12), which can be expressed

$$\theta = \Psi + \bar{f}/\Psi. \tag{17}$$

4.2. The case of weak forces

If $\bar{f} < \theta_e^2/4 = \bar{f}_c$, there is an equilibrium patch. We can linearize Eq. (16) in the vicinity of the equilibrium conditions. We then find an exponential relaxation, with a relaxation time

$$\tau = \frac{R_v}{8V^*\theta_E}\left(\bar{f}_c - \bar{f}\right)^{-1/2} \quad \left(\bar{f} \to \bar{f}_c\right) \tag{18}$$

when $\bar{f} \lesssim 0.9\bar{f}_c$, this time is short (seconds). But when $\bar{f}$ becomes very close to $\bar{f}_c$, there is a critical slowing down.

4.3. The case of strong forces

We now assume that the pulling force is far beyond threshold $\bar{f} \gtrsim 2\bar{f}_c$. Then the patch shrinks to zero: we focus our attention on the late stages, where ψ is small, and Eq. (16) reduces to

$$-\frac{R_v}{V^*}\frac{d\Psi}{dt} = -\frac{\bar{f}^3}{\Psi^3}. \tag{19}$$

The law of decay in this regime is:

$$\Psi = \Psi_0\left(1 - \frac{t}{\tau_l}\right)^{1/4}, \tag{20}$$

where

$$\tau_l = \frac{1}{4}\frac{R_v}{V^*}\frac{\Psi_0^4}{\bar{f}^3} \approx \frac{1}{4}\frac{R_c}{V^*\theta_E^3}\left(\frac{f_c}{f}\right)^3 \tag{21}$$

since $\Psi_0 = \theta_E$.

Thus the whole process is completed in a finite time. For usual conditions, τ_l is equal to a few seconds.

5. Dynamics of unbinding with specific stickers

We now discuss the case of specific adhesion, where a population of 'stickers' have built a dense adhesive patch, with an internal concentration (number of stickers/unit area) Γ_i which is high and fixed. The adhesion energy is then large: to observe an unbinding, we must choose a high surface tension γ (through the aspiration pressure (Eq. (1))).

The stickers are torn out at the periphery of the adhesive contact. The gain of mechanical energy $f\,\mathrm{d}\delta/\mathrm{d}t$ is transferred into heat, when sites near the contact line are detached. On the other hand, the viscous dissipation due to the flow of surrounding water is now negligible.

For each binder/receptor beginning to be separated by a vertical distance z, we expect a rate equation of the form [12]

$$\frac{\mathrm{d}z}{\mathrm{d}t} = V_0\,\mathrm{e}^{-(B-\varphi a)\kappa T} = V_1\,\mathrm{e}^{\varphi a/\kappa T} \tag{22}$$

where V_0 is a typical thermal velocity (of order 10 $\mathrm{m\cdot s^{-1}}$), B the barrier energy ($B \approx 10$ kT), φ is the pull out force on one binder and 'a' molecular length. Eq. (22) can be rewritten in the form

$$a\varphi = kT \ln \frac{V_z}{V_1}, \tag{23}$$

where the vertical velocity $V_z = V\frac{\mathrm{d}z}{\mathrm{d}x} = -\frac{\mathrm{d}R}{\mathrm{d}t}\frac{\mathrm{d}z}{\mathrm{d}x}$. Following [12], we can construct the entropy loss due to the retraction of the patch as an integral over all sites near the line that are partially detached. Per unit length,

$$\mathrm{T}\dot{S} = \gamma(1-\cos\theta)V = \Gamma_i \int \mathrm{d}x\,\varphi \mathrm{V}_z. \tag{24}$$

Omitting coefficients of order unity, and again assuming (for simplicity) that θ is small, the balance of force can be written as:

$$\frac{z_\mathrm{m}}{a} kT\,\Gamma_i\,\ell n\frac{V}{V_1} = \frac{1}{2}\gamma\theta^2, \tag{25}$$

where z_m is the maximum length of a bonded pair, and a is a molecular diameter.

We extract θ from Eq. (12). For small ψ, this reduces to $\theta = \bar{f}/\psi$. The rate equation is then

$$-\frac{R_\mathrm{v}}{V_1}\frac{\mathrm{d}\Psi}{\mathrm{d}t} = \exp\left(\frac{\bar{f}^2}{\varepsilon\Psi^2}\right), \tag{26}$$

where $\varepsilon = 2kT\,\Gamma_i z_\mathrm{m}/\gamma a$ is a parameter of order unity (since γ is large)

We set $u = \bar{f}^2/2\varepsilon\psi^2$ and $\tilde{t} = t/\tau$, with $\tau = (R_\mathrm{v}/V_1)(\bar{f}/(\varepsilon^{1/2})$.

The solution of Eq. (31) (neglecting logarithmic corrections) is:

$$\frac{\bar{f}^2}{\varepsilon}\left(\frac{1}{\psi_i^2} - \frac{1}{\psi^2}\right) = \ln\left(1 - \frac{t}{\tau_\mathrm{s}}\right), \tag{27}$$

where

$$\tau_\mathrm{s} = \frac{f}{2\pi\gamma V_1 \varepsilon^{1/2}}\,\mathrm{e}^{-f^2/4\pi^2\gamma^2 R_\mathrm{ci}^2\varepsilon}.$$

Here also, the time for detachment is finite. τ_s is maximal for $f \approx f_\mathrm{c}\varepsilon/\theta_\mathrm{E}$.

Remark 1. Eq. (26) can be understood if one assumes that the force f is distributed on the stickers at the periphery of the contact, on a band of width $\ell_\mathrm{s} \approx a/\theta$, proportional to f^{-1}.

Remark 2. The tear out process controls the dynamics of unbinding if the specific time τ_s is larger than the hydrodynamic time τ_ℓ. One must compare the 'wetting' velocity W/η to V_1: viscous dissipation is dominant if $W/\eta < V_1$, i.e., for weak adhesion or small energy barriers.

6. Conclusions

(i) It would be most useful to monitor not only the aspiration pressure but also the force f on the pipette. The threshold force f_c for separation is:

$$f_\mathrm{c} = \pi R_\mathrm{v} W$$

and allows for a direct measurement of W.

(ii) For nonspecific adhesion, or weak links (small activation energy), if we impose a force f significantly larger than f_c, we predict a separation time τ_ℓ controlled by hydrodynamic friction (Eq. (21)).

(iii) For strong specific adhesion, the separation time τ_s should be extremely sensitive to the pulling force (Eq. (27)). This regime is expected if $\tau_s > \tau_\ell$, i.e., for a 'wetting' velocity $W/\eta > V_1 = V_0\, e^{-B/kT}$, where B is an activation energy.

Tear out processes limit the rupture if the activation energy is large, while hydrodynamic losses are dominant for small adhesion or viscous solutions.

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