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Permeation through Lipid Bilayers by Adhesion of Giant Vesicles on Decorated Surfaces

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We describe the dynamics of interaction of giant vesicles with properly designed decorated surfaces. The spreading of the vesicles leads to major deformations and is accompanied by the creation of transient macroscopic pores in the micrometer range. Different phenomena that have been observed by changing either the characteristic scale or the geometry of the decorated surfaces are reported and discussed.

Introduction

Controlling the transport of water-soluble solutes through lipid bilayers is a major issue in developing new approaches in drug targeting and delivery. Many strategies have already been designed to enhance the transmembrane passage of species as small as ions or as large as proteins. Chemical strategies generally make use of the addition of a suitable agent (detergent, carrier, channel, ...) whose conformation and favorable partition between the aqueous phase and the bilayer is responsible for the increase of permeability of the lipid barrier. In these strategies, transport is expected to occur in a steady state as long as the promoting factor is present in the system. Despite encouraging results obtained by acting upon the permanent endocytotic processes for transporting the largest solutes such as DNA,^{1,2} chemical strategies are most often restricted to the transport of small species.³ Physical strategies consist in applying an external perturbation (temperature rise,⁴ pulses of electrical field,⁵ ...) which leads to the formation of large permeating defects or pores in the bilayer. Unfortunately, together with often severe practical requirements, the physical approaches are intrinsically limited by the relaxation time of return to the nonpermeable equilibrium state via lipid migration or flip-flop processes for instance. Indeed, this characteristic relaxation time determines a kinetic window for permeation, in terms of both its extent and duration; the

permeation occurs under kinetic control in analogy with the kinetic/thermodynamic controls of wide use in chemistry.⁶ In the continuity of our previous studies devoted to the setup of chemical⁷ and physical^{8,9} strategies for inducing solute transport through lipid bilayers, we have been concerned with a new approach making use of a kinetic control that should lead to the transport of the largest solutes. Its basic principle lies in inducing the formation of transient pores in the membrane of giant vesicles^{10,11} adsorbed on suitably decorated surfaces interacting with the membrane.

In this work, the interaction between vesicles and substrates originates from the strong and *fast*¹² adhesion of a charged bilayer onto a surface of opposite charge. Anticipating that a contrast between alternating more or less adhesive elements of surface should facilitate permeation, attention has been focused on decorated surfaces with a surface fraction x_s of the more adhesive portions ($x_s = 0.5$, Figure 1). We have examined the significance of the scale and of the geometry of the surface patterns. In view of the bilayer thickness (a few nanometers) and of the typical radius of giant vesicles (10–100 μm), patterns with periodicities ranging from 1 to 50 μm have been chosen. Hence, the characteristic size of the pattern should be (i) large enough to promote large morphological changes of the vesicle and (ii) small enough to get a permeation

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(12) "fast" qualification deserves a comment. To be effective for permeation, the adhesion must occur faster than the relaxation of the bilayer tension. In contrast to more specific interactions requiring the appropriate positioning of interacting chemical groups, the electrostatic interaction arising between two surfaces of opposite charge leads to adhesion as soon as their relative distance drops below a characteristic screening length depending on the experimental conditions. See for instance: Northrup, S. H.; Boles, J. O.; Reynolds, J. C. L. *J. Phys. Chem.* **1987**, *91*, 5991.

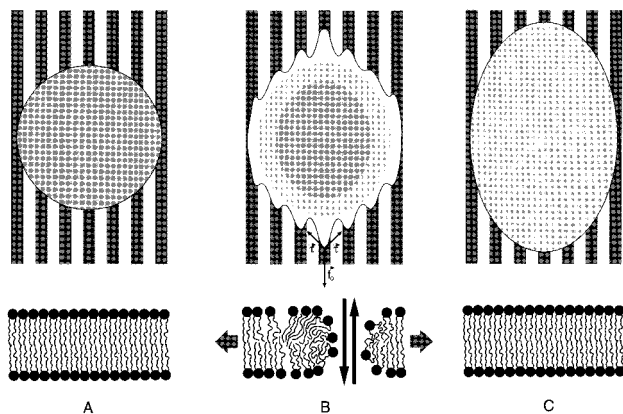


Figure 1. Forcing permeation through lipid bilayers by membrane adhesion on decorated attractive surfaces. Striped patterns have been chosen for the illustration with $x_s = 0.5$. From left to right: (A) nonadhesive regime, the vesicle stays just above the surface, the bilayer is not stretched and exhibits a low permeability toward water-soluble species; (B) dynamic regime of spreading, the vesicle spreads out onto the surface and adapts its shape to decrease the system energy, in this regime the bilayer becomes strained and its permeability toward hydrophilic solutes is increased as a consequence of the creation of microscopic defects or of macroscopic pores; (C) regime of equilibrium. Several mechanisms (lipid migration, lipid flip-flop, ...) lead to the partial relaxation of the membrane tension. The defect density drops and the pores close.

that does not depend on the size and position of the vesicle. Stripe, square, triangle, and herringbone geometrical shapes have been retained. In addition to promote permeation, we are also interested in highlighting (i) the dynamics of vesicle spreading onto decorated surfaces with respect to the results obtained on smooth surfaces.¹³ Despite the extensive use of vesicles for preparing supported bilayers and the numerous theoretical reports on the equilibrium morphology of vesicles interacting with surfaces,¹⁴ the dynamic aspect has been rarely studied,¹⁵ (ii) the most favorable patterns for promoting (or hindering) vesicle adhesion by changing the characteristic lengths and symmetries with regard to the thermal fluctuations of the vesicles,^{16,17} and (iii) any vesicle motility driven by Marangoni effect on suitably decorated surfaces.¹⁸

Experimental Section

Giant vesicles have been prepared by swelling a thin film obtained after evaporation of freshly prepared chloroform/methanol 9:1 solutions of EPC (egg phosphatidylcholine, Sigma-Aldrich) in 50 mM sucrose aqueous solutions in the presence of an alternating electrical field.¹⁹ To induce the sedimentation onto the surface, the vesicles are transferred at constant osmolarity into a less dense aqueous solution (50 mM glucose).²⁰ It has been experimentally observed that the trace amounts of negatively charged species resulting from EPC hydrolysis (evaluated at about 0.1% mol/mol²¹) are sufficient to promote vesicle adhesion

to the positively charged surfaces. The decorated surfaces have been obtained using the microcontact printing technique introduced by Whitesides.²² PDMS (poly(dimethylsiloxane)) patterned stamps impregnated with a 1 mM ethanol solution of dodecanethiol were applied onto ITO (indium tin oxide)-coated glass surfaces covered by a thin optically transparent gold layer. After removal of the stamp, the resulting surface was stepwise dipped for 1 min into a 1 mM water/ethanol 1:9 solution of sodium 3-mercaptopropionate sulfonate, then cautiously rinsed with Millipore water and finally immersed for 24 h in a 0.01% aqueous solution of positively charged poly-L-lysine (MW = 150000–300000, Sigma-Aldrich). The decorated surfaces are then immediately used after extensive washing with Millipore water. Selective etching processes and atomic force microscopy (AFM) imaging in friction mode have been used to characterize the decorated surfaces. They have shown that the patterns have been correctly transferred from the PDMS stamps to the gold surface, producing alternating hydrophilic and hydrophobic areas on the surface. The patterns have been also directly observed by reflection interference contrast microscopy (RICM).²³ The spreading of the giant vesicles onto the decorated surfaces as well as induced bilayer permeability have been examined using a custom-built microscope working in either phase contrast (to determine the vesicle radius) or evanescent wave induced fluorescence (EWIF).^{13,24} In the latter case, a suitable fluorescent water-soluble dye (calcein) was added to the external vesicular pool; it becomes thus possible to visualize the contact zone between the spreading vesicle and the decorated surfaces. EWIF microscopy also allows the determination of the shape of the adsorbed vesicle at the micrometer scale.^{13,24} In addition, one can evidence any permeability of the membrane toward the fluorescent probe by analyzing the contrast of the vesicle (dark) with regard to the background (bright). Complementary experiments have been performed with RICM or with epifluorescence microscopy using a lipophilic aminostyryl pyridinium dye (S-467, Molecular Probes). These techniques enable study of the topology of the bilayer with a high resolution.

Results

Figures 2, 3, 4, and 5 display the typical behavior of an EPC giant vesicle spreading onto surfaces patterned with stripes (Figure 2), herringbone (Figure 3), squares (Figure 4) and grids (Figure 5) exhibiting different attraction strengths for EPC vesicles. The initial time is taken as the moment when the vesicle sedimenting toward the decorated surfaces stops its descent. From this time, three different regimes have been evidenced. The longest regime (regime I; characteristic time τ_1 in the minute range) is associated with the formation of a first contact point between the bilayer and the surface. By comparison of the behavior on stripes of different widths (2.5, 5, and 12 μm), τ_1 is shown to increase when the surface fraction of attractive domains x_s is reduced. Nevertheless, τ_1 remains in the same range of values as observed for smooth surfaces under comparable conditions.¹³ By contrast to the case of smooth surfaces, the second regime reveals by EWIF microscopy a long sequential process of spreading (regime II, characteristic time τ_2 in the range of 10 s to be compared with 50 ms on smooth surfaces). The vesicle first undergoes a local spreading; for instance, it spreads rapidly along a stripe (Figure 2B). Then, one observes spreading along a neighboring attractive stripe (Figure 2C). This growth process occurs repeatedly until part of a homogeneous adhering surface of the vesicle is observed (Figures 2E, 3E, and 4B). This process looks analogous to the Haines jump of a liquid on a corrugated surface.²⁵ The adhered

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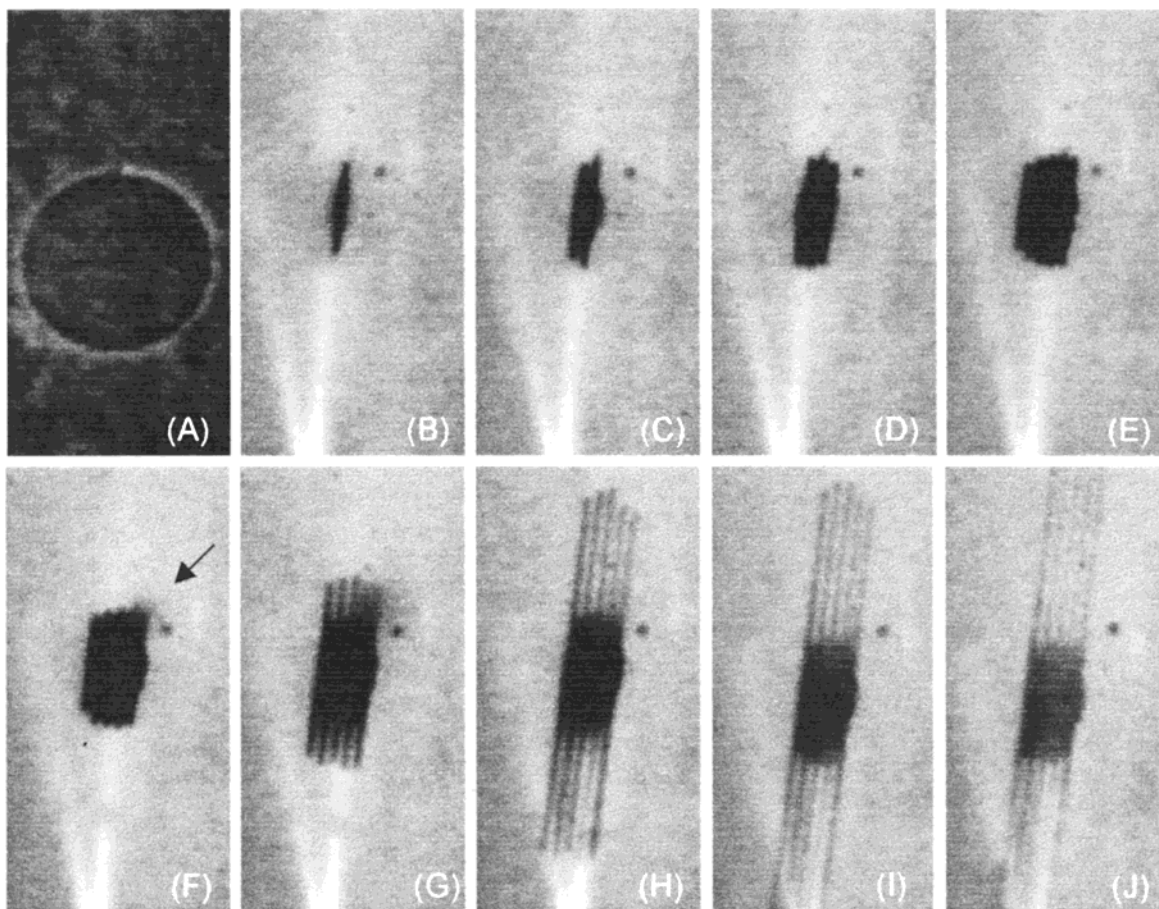


Figure 2. Spreading of an EPC vesicle on striped patterns (width = $2.5 \mu\text{m}$) as a function of time. Vesicle as observed in phase contrast microscopy at $t = 0$ (A). Vesicle as observed in EWIF microscopy (the depth of penetration of the evanescent wave is estimated between 600 nm and $1 \mu\text{m}$) at $t = 1$ (B), 3 (C), 4 (D), 15 (E), 67 (F), 68 (G), 73 (H), 84 (I), and 92 s (J). The arrow on photograph F emphasizes the formation of a macroscopic pore along the contact line.

part of the vesicle appears homogeneously dark in EWIF microscopy, and the intensity of the vesicle image does not evolve during the second regime. The vesicle shape remains circular as observed by phase-contrast microscopy. Then, the vesicle pauses for a period equal to a few seconds on square and grids patterns (Figures 4 and 5) or to 1 min on striped and herringbone patterns (Figures 2 and 3). The last regime (regime III, characteristic time τ_3 in the minute range) begins with the formation of a pore in the lipid bilayer along the contact line. In EWIF microscopy, the pore is evidenced by the emission of a diffuse dark cloud (see arrow in Figures 2F, 3F, 4C, and 5E). Finally one notices spreading of the lipid bilayer along the attractive zones at a constant velocity of about $20 \mu\text{m}\cdot\text{s}^{-1}$. Epifluorescence microscopy has been used to demonstrate that the vesicle parts spreading along attractive lines are not simply adsorbed bilayers but true tubular extensions (pseudopods) of the vesicle undergoing a Rayleigh-type instability (Figure 6A).²⁶ In view of the difference in luminosity between attractive and nonattractive stripes, EWIF microscopy confirms the preceding observation. Indeed, the tubes lower the fluorescence intensity above the attractive stripes in EWIF microscopy (Figure 6B). EWIF also shows that the vesicle/background optical contrast drops during regime III. At the end of the spreading process, the vesicle adopts a shape that depends on the surface pattern. In the case of the $2.5 \mu\text{m}$ wide striped pattern, phase contrast microscopy shows that the vesicle is elongated along the stripe axis (Figure 6C).

The spindle adopted morphology is confirmed by RICM (Figure 6D) and epifluorescence microscopy (Figure 6E). A typical contact angle of the vesicle along the in-plane perpendicular axis to the stripes equal to $15\text{--}20^\circ$ was extracted from the spacing between the interference fringes observed by RICM. Among the different regimes observed, regime III is the only one to exhibit scale- and geometry-dependent features. Within the series of striped patterns, regime III is only present for patterns with the $2.5 \mu\text{m}$ periodicity. For patterns made of larger stripes at the same value of x_s , (i) the spreading phenomenon stops at the end of regime II (regime III does not occur) and (ii) no significant solute permeation can be observed and the vesicular shape as observed by phase-contrast microscopy remains circular. Note that under the experimental conditions investigated, the vesicle never blows up on stripe patterns. In contrast, regime III was always present on the other decorated surfaces examined (triangles, squares, grids, herringbone) and led to solute permeation. Moreover, the spreading vesicles could not be observed anymore by phase contrast, and it is thus suggested that the latter geometries promote vesicle rupture.

No motion of the center of mass of vesicles has been ever noticed during experiments, even when a driving force was expected (large triangle and square patterns for example). But an oscillating behavior has been frequently observed when aged patterned stamps of $2.5 \mu\text{m}$ stripes were used instead of the new stamps that were used to obtain the results reported in the preceding paragraph. Under such conditions, EPC vesicles hardly spread during

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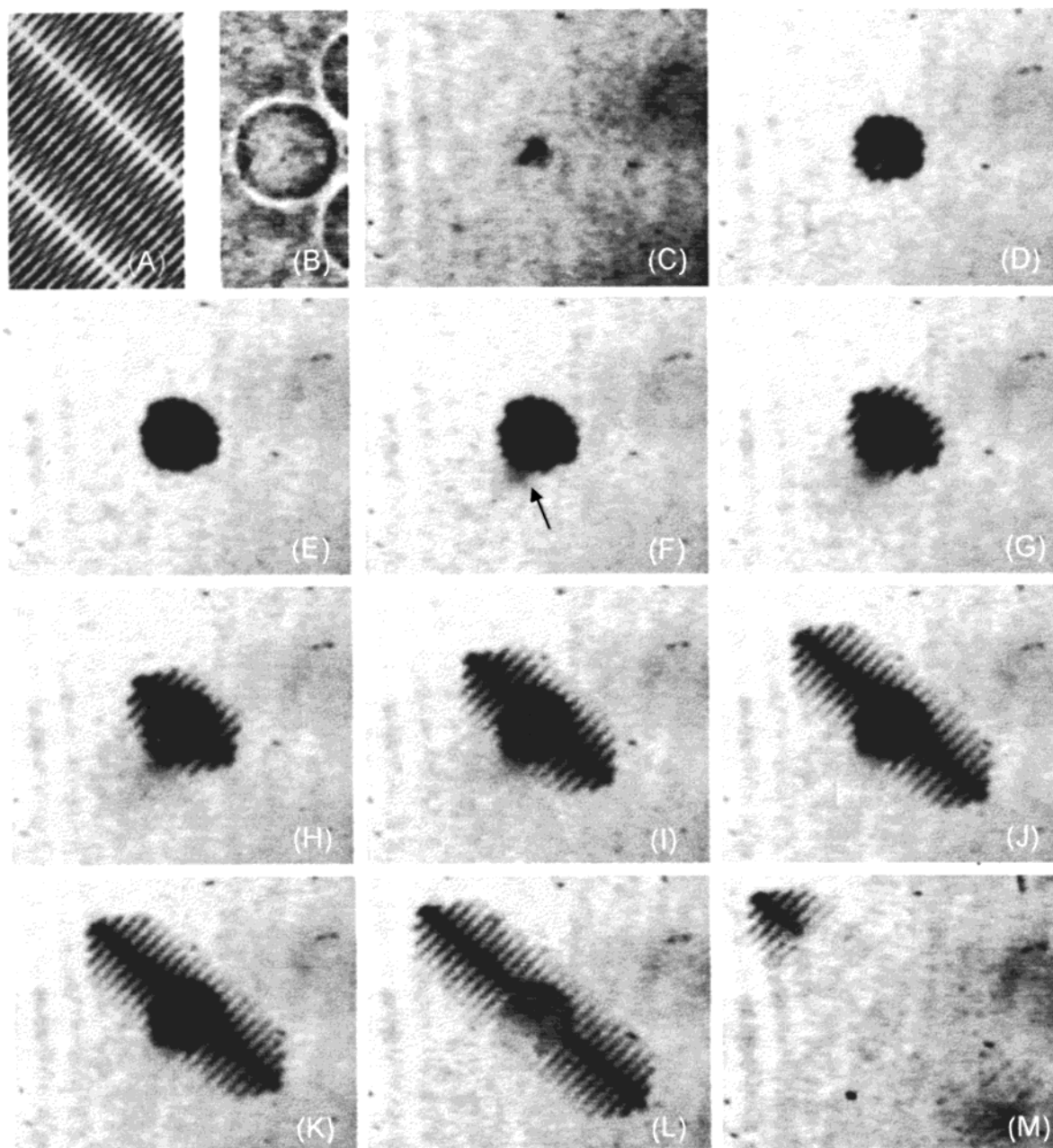


Figure 3. Spreading of an EPC vesicle on herringbone patterns (width = $50\ \mu\text{m}$) as a function of time. Patterning stamp as observed by phase contrast microscopy (A). Vesicle as observed in phase contrast microscopy at $t = 0$ (B). Vesicle as observed in EWIF microscopy (the depth of penetration of the evanescent wave is estimated between $600\ \text{nm}$ and $1\ \mu\text{m}$) at $t = 1$ (C), 6 (D), 31 (E), 31.3 (F), 32 (G), 33 (H), 35 (I), 37 (J), 39 (K), 40 (L), and 43 s (M). The arrow on picture F emphasizes the formation of a macroscopic pore along the contact line.

regime III but one observes the regular opening and closing of a pore formed on the contact line at the border between zones exhibiting different attractive interactions (Figure 7). At the end of this beating step, one notices a loss of vesicle contrast in EWIF microscopy which is evidence of the internalization of the fluorescent probe.

Discussion

Our observations emphasize the complexity of the spreading behavior of giant vesicles on adhesive surfaces. Similar to the case of smooth surfaces,¹³ a sequential dynamic process with three regimes has been observed (Figure 8).

Regime I can be described as the approach regime. After the sedimentation process controlled by buoyant and viscous forces, the vesicle motion considerably slows down

as soon as the distance between vesicle and surface becomes small enough (comparable to the membrane fluctuations). The vesicle then appears immobile in phase contrast microscopy. The corresponding time scale τ_1 is the longest time encountered during the entire adhesion process. In the case of undecorated surfaces which sufficiently attract the lipid bilayer,¹³ spreading begins as soon as a contact between the membrane and the surface is established. In the case of decorated surfaces, one also has to take into account the probability of creating a nucleation point whose stabilizing energy exceeds the energy of thermal fluctuations ($k_B T$). In the range of scales investigated, this probability is governed by the surface fraction of attractive zones. Nevertheless, it is reasonable to anticipate that some more complicated dependence of τ_1 on the pattern geometry of the decorated surface may

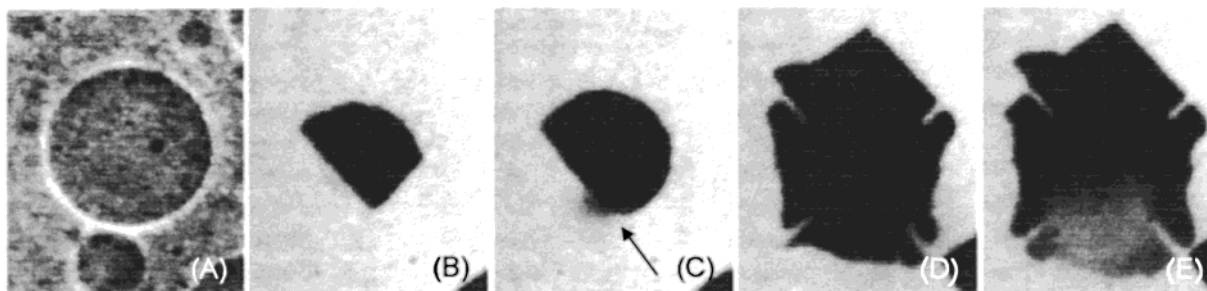


Figure 4. Spreading of an EPC vesicle on square patterns (width of the square grid, 5 μm ; side of the attractive square, 50 μm) as a function of time. Vesicle (diameter 50 μm) as observed in phase contrast microscopy at $t = 0$ (A). Vesicle as observed by EWIF microscopy (the depth of penetration of the evanescent wave is estimated between 600 nm and 1 μm) at $t = 5$ (B), 30 (C), 34 (D), and 35 s (E). The arrow on picture C emphasizes the formation of a macroscopic pore along the contact line.

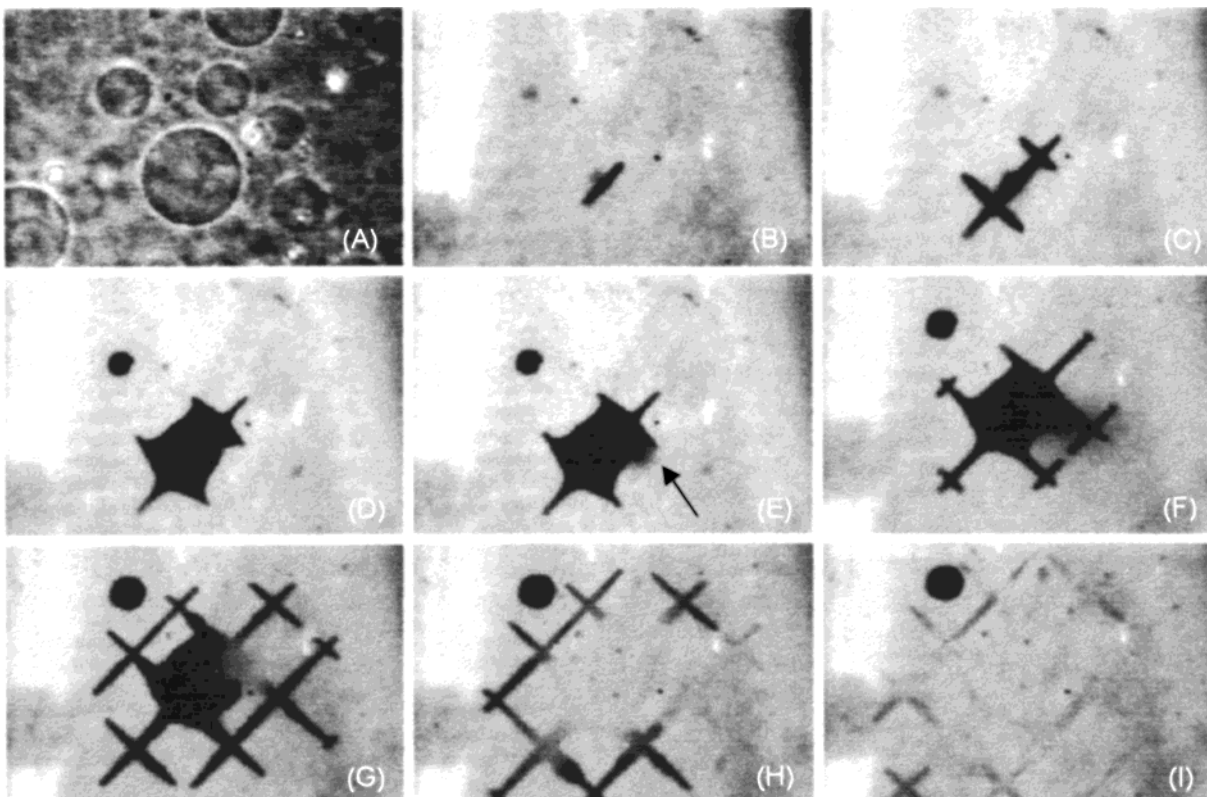


Figure 5. Spreading of an EPC vesicle on grid patterns (width of the attractive square grid, 5 μm ; side of the square, 50 μm) as a function of time. Vesicle (50 μm diameter) as observed by phase contrast microscopy at $t = 0$ (A). Vesicle as observed by EWIF microscopy (the depth of penetration of the evanescent wave is estimated between 600 nm and 1 μm) at $t = 0.3$ (B), 2.7 (C), 24.5 (D), 25.3 (E), 28.1 (F), 34.9 (G), 45.9 (H), and 47 s (I). The arrow on picture E emphasizes the formation of a macroscopic pore along the contact line.

exist but at a smaller length scale than that investigated in the present study. A crude estimate of the typical length scale at which such effects should manifest themselves can be obtained as follows. In the preceding model used to derive τ_1 , the assumption of a planar bilayer has been used. In fact, a fluctuating vesicle at a distance h of a planar surface can be described as the sum of independent elements whose characteristic lengths are $\xi_{\parallel} = (\kappa_c/kTh)^{1/2}$ and $\xi_{\perp} \approx h$, where κ_c designates the Helfrich bending modulus.²⁷ If the surface is made of zones in the ξ_{\parallel} scale range (about 600 nm in the present case) and whose symmetry is more or less commensurate to the bilayer excursions, one should be able to observe geometry-dependent effects.

Once the nucleation point is created, fast spreading of the vesicle onto the surface indicates the onset of regime

II. It occurs at constant surface and volume of the vesicle. In the case of anisotropic patterns such as stripes, the spreading develops faster along the stripe axis than along the corresponding in-plane perpendicular axis. Indeed, although spreading is limited in both cases by the drainage of a thin aqueous film, a zip process takes place along the stripe axis whereas jumps are required along the perpendicular direction.^{25,28,29} Despite the expected difference of kinetics and in marked contrast with regime III, the shape of the vesicle remains spherical at the end of regime II. It is also interesting to note that in the time scale investigated in regime II, the absence of optical contrast in EWIF microscopy between the stripes of different attractiveness for the bilayer underlines the absence of

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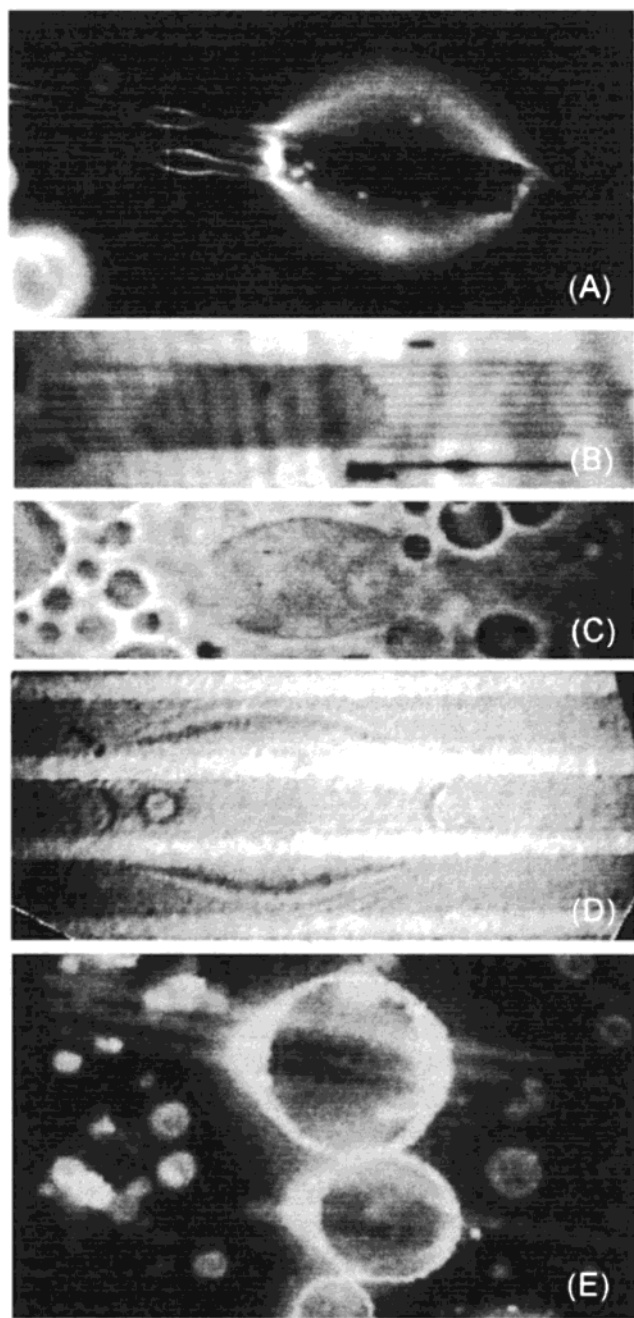


Figure 6. Typical shape of an EPC vesicle spreading on striped patterns ($2.5 \mu\text{m}$ width) of contrasted attraction as observed in epifluorescence microscopy (A), EWIF microscopy (B), phase contrast microscopy (C), RICM (D); projection of 30 fluorescence cross sections in epifluorescence microscopy; please note the curvature of the membrane induced by the pattern (E).

differences in the thicknesses of the aqueous film located between the surface and the bilayer. Due to the strong adhesion energy, a rather large tension is exerted on the bilayer at the end of regime II.³⁰

(30) In the case of smooth surfaces, the adhesion energy W has been evaluated to $(1-5) \times 10^{-3} \text{ mJ/m}^2$ from the consideration of the charge densities of the lipid bilayer and of the surface. Such a value is generally considered to correspond to a strong adhesion. The corresponding surface tension Σ is related to W and to the contact angle of the vesicle on the surface, θ , by the Young-Dupré equation (see: Seifert, U.; Lipowsky, R. *Phys. Rev. A* **1990**, *42*, 4768. Nardi, J.; Bruinsma, R.; Sackmann, E. *Phys. Rev. E* **1998**, *58*, 6340.): $W = \Sigma(1 - \cos \theta)$. In the present system, the bilayer surface tension at the end of regime II is evaluated to $5 \times 10^{-3} \text{ mN/m}$ from both experimental observations and theoretical predictions. See ref 13.

Regime III starts with the release of the corresponding tension by the formation of a macroscopic pore with a radius r that can be estimated as follows. The volume of the nonfluorescent solution expelled from the vesicle between $t = 67 \text{ s}$ and $t = 68 \text{ s}$ (panels F and G of Figure 2) corresponds to a volumic flow J of about $10^4 \mu\text{m}^3 \cdot \text{s}^{-1}$ (assuming a spherical dispersion of the vesicle contents). The Laplace pressure drop responsible for this flow J is

$$\Delta P = 2\Sigma/R \quad (1)$$

where Σ is the membrane tension and R , the vesicle radius that can be deduced from Figure 2A. If we consider that the flow J through the pore is limited by the viscous dissipation in the aqueous medium (this is justified by an a posteriori Reynolds number that gives $\text{Re} \approx 10^{-3}$), we have

$$J \approx \Delta P r^3 / \eta \quad (2)$$

where η is the solution viscosity. We have assumed that the pressure drop ΔP relaxes on a length r . Taking $\Sigma = 5 \times 10^{-3} \text{ mN/m}$,¹³ $R = 40 \mu\text{m}$ (Figure 2A) and $\eta = 10^{-3} \text{ Pa}\cdot\text{s}$, we obtain a pore radius $r \approx 5 \mu\text{m}$. This estimate compares with the repeat distance of the decorated pattern and is larger than the recent observed radii of the membrane pores formed under different conditions.³¹ Because of the leakage caused by the pore formation, an excess of area becomes available. Buds form and tubular pseudopods now spread.³² In the case of the $2.5 \mu\text{m}$ periodic stripe pattern, the spreading stops once enough tension is released. In contrast, for some other patterns (triangles, squares and herringbone), the spreading vesicles blow up before releasing enough tension to close the pores.

To propose a model for the dynamics of vesicle spreading during the regime III on all the decorated surfaces tested experimentally is out of the scope of the present paper. Nevertheless, we bring semiquantitative arguments for the stripped pattern. In ref 33, the authors study the profile of an adhering vesicle on a solid plate in the vicinity of a pinning center.³³ The latter exerts locally a force f_0 on the bilayer. To make the analogy with the adhesive stripes, we write $f_0 = W/a$, where W is the adhesion energy per unit area and a is the stripe width. The membrane resists against the deformation with an effective line tension τ given by the relation (see Figure 1)

$$\tau = \frac{1}{2} \frac{\kappa_c}{R_c^2} \lambda \quad (3)$$

where κ_c is the bending modulus, R_c is the radius of curvature close to the adhesion area, and λ is the angular correlation length that was first introduced in ref 34. It arises from the requirement for the membrane to merge smoothly with the surface (in contrast with a liquid droplet that makes a sharp wedge). This length is written as $\lambda = (\kappa_c/\Sigma)^{1/2}$ and can be measured by RICM.³⁵ By estimating

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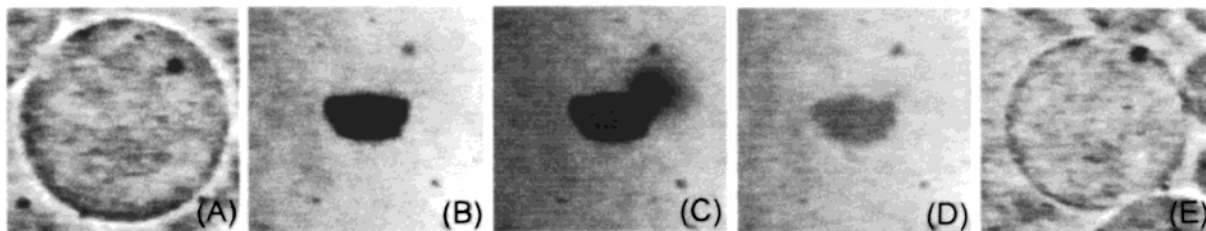


Figure 7. EPC vesicle exhibiting a periodic opening–closing process of a pore during spreading on aged $2.5 \mu\text{m}$ stripe patterns. Vesicle observed in phase contrast microscopy before adhesion (A). Same vesicle observed in EWIF microscopy after adhesion (B, C). The periodic opening–closing process (0.3 s duration, 3 s periodicity) allows the leakage of the fluorescent inner medium of the vesicle (D). Vesicle observed in phase contrast microscopy at the end of the phenomenon (E).

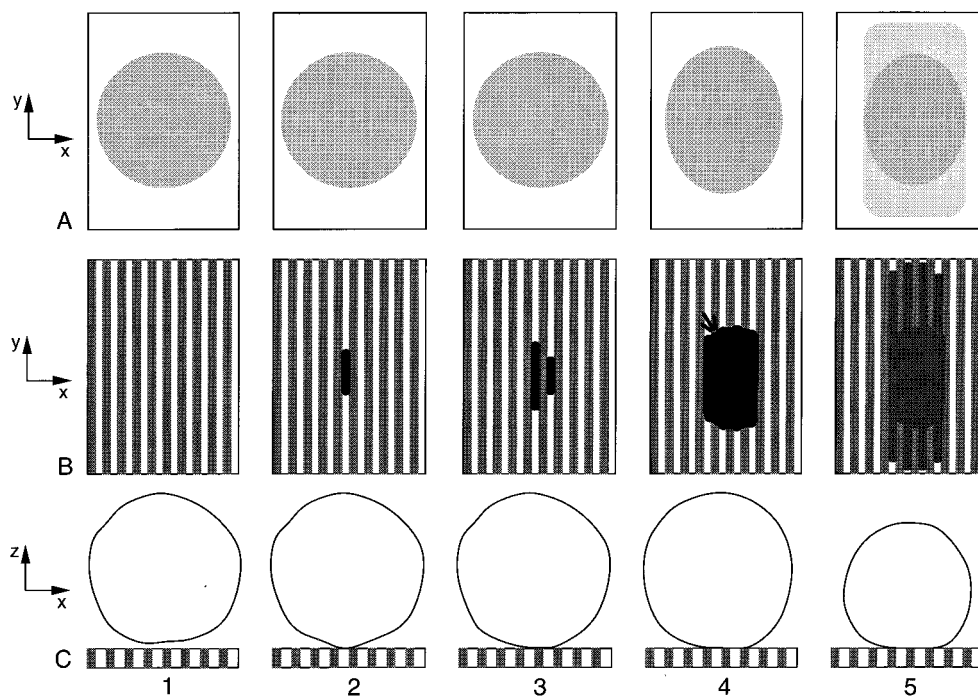


Figure 8. Spreading/permeation mechanism of an EPC vesicle on striped patterns ($2.5 \mu\text{m}$ width) as a function of time (from left to right): (1) regime I, approach; (2 and 3) regime II, spreading at constant lipid surface but increased contact area and constant volume, no permeation; (4) regime III, formation of a macroscopic pore, spreading at constant lipid surface, the internal volume decreases; (5) equilibrium vesicular shape, the macroscopic pore is closed. (A) Vesicle image in phase contrast microscopy. (B) Vesicle image by EWIF microscopy. (C) Vesicle profile.

also $R_c \approx \lambda/\theta_c$, where θ_c is the contact angle, one obtains

$$\tau = \frac{1}{2} \Sigma \theta_c^2 \lambda \quad (4)$$

As sketched in Figure 1, the bilayer adopts locally a triangular shape as long as $2\tau > f_0$. In contrast, if the bilayer cannot oppose a sufficient line tension to the applied force, an instability was predicted:³³ a tether is pulled from the vesicle. In our experiments, $\Sigma \approx 5 \times 10^{-6} \text{ N}\cdot\text{m}^{-1}$,³⁰ $\theta_c \approx 15^\circ$, $W = \Sigma(1 - \cos \theta_c) \approx 10^{-7} \text{ N}\cdot\text{m}^{-1}$, $\kappa_c \approx 4 \times 10^{-20} \text{ J}$,³⁶ $\lambda \approx 10^{-7} \text{ m}$, and $2\tau = 2 \times 10^{-14} \text{ N}$. The latter estimate is 1 order of magnitude smaller than the pulling force $f_0 = 3 \times 10^{-13} \text{ N}$. Thus we conclude that the growth of cylindrical protrusions from a vesicle can effectively be driven by spreading of the bilayer onto the charged stripes of the substrate. Since the initially floppy vesicle becomes tense and adopts a spherical cap shape at the end of regime II, the growth of the tethers during regime III would lead to dramatically increase the membrane tension Σ due to the corresponding increase of the contact area. Hence if there was no mechanism to release the membrane tension, the tether instability would stop immediately. In fact the

process of vesicle deflation through experimentally observed pores keeps long enough a low surface tension to create long protrusions.

In ref 31, only vesicles that slowly release a highly viscous solution can bear transient pores as large as those estimated in the present paper (in a low viscosity medium, both the outflow and the tension release occur too fast to let the pores grow). A possible explanation for the present observations is that the pores nucleate at a larger initial radius r_c on the decorated surface, where the pattern provides germs above $1 \mu\text{m}$. The nucleation radius is also strongly dependent on the line energy of the bilayer ($\gamma \approx 10^{-11} \text{ N}$ for an EPC bilayer) since $r_c = \gamma/\Sigma$.³⁷ We have invoked the presence of minor amounts of some hydrolysis products in our phospholipidic vesicles to account for their strong adhesion on oppositely charged surfaces.¹³ Among them, the water-soluble lysolecithins with a single hydrocarbon tail decrease γ by a factor of 2 at concentrations as low as $3 \times 10^{-4} \text{ mg/mL}$.³⁸ Thus $r_c \approx 1 \mu\text{m}$ could be

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expected. Then, the chemical heterogeneities of the solid surface could easily nucleate pores.

Conclusion

The present work demonstrates that the adhesion of giant vesicles onto decorated surfaces can lead to the permeation of large hydrophilic solutes through lipid bilayers. The observations made by EWIF microscopy on giant vesicles directly demonstrate both the internalization of calcein (size ≈ 1 nm) and the creation of a transient macroscopic pore (diameter ≈ 1 μ m) as a result of the spreading process. In addition to these new and interesting

features, some selectivity for transport might be expected. In fact, the size and the lifetime of the pore could probably be tuned by conveniently tailoring the size and the geometry of the patterns as well as the relative surface fractions x_s of the decorated surfaces.

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