Adhesive Emulsion Bilayers under an Electric Field: From Unzipping to Fusion

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Water-in-oil emulsion drops are formed and stabilized with phospholipids which can adhere and form a bilayer. Using microfluidics, adhesive drop pairs are then trapped and submitted to an ac electric field. We observe three distinct states as a function of the adhesion energy and the electric field intensity. The pair can be either stable, though slightly deformed, or unzip and separate, or coalesce. The frontiers between the different states directly reflect vesicle detachment forces and electroporation theories. The experimental approach that we propose for probing liquid interface wetting between monolayers allows us to finely tuned the tension in the bilayer and gives access to bilayer unzipping.

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Vesicle membranes have been actively studied for many years [1] because of their similarity with cell membranes. These membranes are commonly viewed as two adhesive phospholipid monolayers forming an unique bilayer. These bilayers are known to exhibit numerous behaviors depending on their composition, fluidity, and tension [2]. In addition, the use of an electric field can induce the formation of transient pores into the membrane, namely, electroporation [3,4]. By using water in oil drops stabilized by phospholipids and by carefully choosing the oil composition, drops may adhere and therefore create a bilayer which has *a priori* a similar structure than bilayer vesicles obtained with the same phospholipids [5]. Moreover, as previously shown, the bilayer tension can be finely tuned by adjusting the continuous phase composition. From this system, we can then explore some novel consequences related to the bilayer tension. First, we decide to revisit the existence of a threshold force for unbinding two adhesive monolayers as a function of their adhesion. This question was indeed theoretically tackled long ago [6,7] and then experimentally demonstrated for cells [8] but the unzipping of a single bilayer has never been addressed. We then consider how electroporation can turn into coalescence as a function of field strength and membrane tension.

In this Letter, we address these questions by using a microfluidic based technology that allows us to manipulate adhesive drop pairs [9,10]. We report the stability diagram of a drop pair subjected to an electric field as a function of the adhesive energy. By taking advantage of the multiparallelized approach offered by microfluidics, we are indeed able to map out the behavior of drop pairs within the adhesion energy-electric field intensity plane. We distinguish three zones separated by two distinct boundaries: the pair can be either stable, though slightly deformed, or unzip and separate, or simply coalesce. For the first time, we can experimentally demonstrate that the force required to completely unzip a single bilayer matches the theoretical threshold predicted for the detachment of adhesive vesicles [6,8]. We then show that the transition between transient electroporation and coalescence is perfectly accounted by the existence of an unique critical pore size above which the hole becomes unstable. Therefore, when adhesion is increased, corresponding to a decrease of the membrane tension, coalescence will occur at a larger field such that the net surface energy gained by opening a constant hole size remains the same.

Adhesive emulsions can be achieved in different ways [11–13]. Poulin and Bibette [5] formed water drops covered with phospholipids in diethyl ether oil (named good solvent), and found that the emulsion becomes adhesive when a bad solvent (silicone oil) for the surfactants was added to the continuous phase. Following the same footsteps, we replace diethyl ether by chloroform, which is less volatile, while keeping silicone oil (having a viscosity of 50 mPa · s) as the bad solvent and using dipalmitoylphosphatidylcholine (DPPC) as the surfactants. Although the continuous phase contains enough surfactants, we add 0.15 M of $MgSO_4$ in water drops [5] in order to stabilize the inverse emulsion [14]. Thus, for a sufficient proportion of bad solvent, two drops will form at their contact a DPPC bilayer, allowing us to investigate their behavior under an electric field by using microfluidics [10]. In Fig. 1, we show an adhesive drop pair that is trapped in a specific designed chamber where an electric field can be applied along the axis of the drop pair. This microfluidic device is

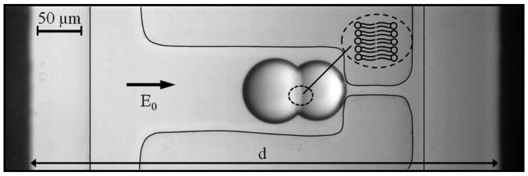

FIG. 1. View of a trapped adhesive pair forming a phospholipid bilayer. The water drops rest in a continuous phase composed by a mixture of silicon oil, chloroform and phospholipids. An electric field E_0 is generated by two parallel electrodes, seen in black on both extremities of the picture, which are separated by a distance $d \sim 540$ μm.

manufactured with polydimethylsiloxane (PDMS) following the standard soft lithography techniques [15]. A homogeneous electric field throughout the 65 μm of the trap's height H is generated by electrodes with the same thickness [16]. Emulsion drops of various radii R_0, ranging from 40 μm to 50 μm, are produced using a flow-focusing device. The emulsion drops flow within a parallelized array of hundred traps, allowing to form pairs at rest. The residual drops are then pushed away from the circuit. After that, the electric field is turned on and the pair behavior is recorded with the help of a high speed camera mounted on a microscope. Since the dielectric constant of PDMS ε_d is 2.5 and the mean one of the oil mixture ε_c is 4, the electric field E_0 is about 80% of V/d, where d is the distance between electrodes at a potential difference V, and varies between 0.5 kV/cm and 30 kV/cm. The excitation mode is ac at a frequency f of 10 kHz. The electrical conductivity σ_w of the aqueous solution is approximately 1 S/m and leads to a relaxation time of the free charges $\varepsilon_w/\sigma_w \sim 10^{-9}$ s, which is much smaller than the period of the electric field equal to $1/f = 10^{-4}$ s. Therefore, the drops get polarized fast enough and we can only consider the average action of the electric field and thus use the rms value of V.

We first characterize the adhesive drop pairs as a function of the continuous phase composition in glass capillaries [17] for avoiding any evaporation of the solvents, as it occurs in PDMS, and without any electric field. The adhesion energy ΔF, that is an energy per surface area, can be deduced from the Young-Dupre equation: $\Delta F = 2\gamma_0(1 - \cos(\theta))$. The contact angle θ defined in Fig. 2 is given by $2\theta = \sin^{-1}(R_p/R_1) + \sin^{-1}(R_p/R_2)$, R_p and R denoting for patch's and drops' radii. From the pending drop technique, the surface tension γ_0 (Fig. 2) of water/chloroform-silicone oil interface in presence of DPPC is 1 mN/m for a chloroform-silicone oil mixture of 90/10. This value is almost constant when we add more of the bad solvent, since water/chloroform and water/silicone oil interfacial tensions are similar (27 mN/m and 22 mN/m, respectively). By changing the proportion of the bad solvent versus the good solvent, we measure the contact angles and determine the adhesion energy ΔF (Fig. 3). We note that such emulsion breaks down for a mass fraction φ of added silicone oil larger than 0.53 when the surfactant precipitates. The adhesive energy increases with the fraction of bad solvent and displays two distinct regions of lower rates of change in the ranges 0–0.2 and 0.4–0.5 of bad solvent, corresponding to adhesive energies close to 0.1 mN/m and 1.5 mN/m. As depicted in Fig. 2, the mechanical equilibrium at the Plateau border is given by $\gamma_m = \gamma_0 \cos(\theta)$, where γ_m is the surface tension of a monolayer forming the bilayer. Since $\gamma_m = \gamma_0 - \Pi$, where Π is the surface pressure acting on the monolayer, $\Pi = \Delta F/2$. Thus, the experimental plot of $\Delta F(\varphi)$ must also reflect the two-dimensional bilayer equation of state $\Pi(\Gamma(\varphi))$ where Γ is the phospholipid surface density which is naturally controlled in our experiment by solvent composition. In other words, DPPC surface density within the adhesive monolayer is governed by its bulk chemical potential which is directly linked to solvent composition. This differs from the Langmuir balance method for which the surface density is set by compressing the monolayer located at the interface; in that case, surfactants must be strictly non soluble such as phospholipids at the water-air interface. In our set up, an increase in surface pressure is directly linked to the recruitment of DPPC within the bulk oil phase under the driving action of adhesive interactions. We can then conclude that the regions of lower rate of change of ΔF displayed in Fig. 3 reflect the existence of two successive phase transitions undergone by adsorbed DPPC. We assume that these two transitions correspond to the two well accepted gas-liquid and liquid-gel transitions [18]. We remind that at room temperature dense DPPC monolayers are in gel phase.

When these pairs are subjected to an ac electric field, three different states are observed as reported in Fig. 4. These states are identified at a duration under field of 5 to 10 seconds since the behavior is not changing over longer period of time. For convenience, we take advantage of the

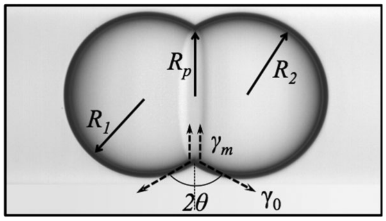

FIG. 2. Snapshot of two adhesive drops along with the main parameters of the drop pair: R_1 and R_2 are the adhesive drops' radii, R_p is the radius of the patch, θ is the contact angle, γ_0 is the bulk interfacial tension of water-chloroform-silicone oil mixture in presence of DPPC and γ_m is the surface tension of a monolayer building the bilayer.

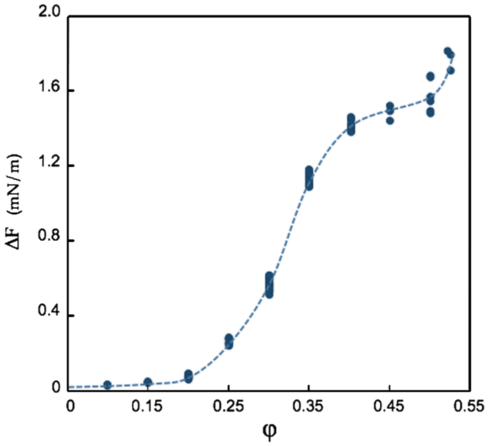

FIG. 3 (color online). Adhesion energy ΔF as a function of the mass fraction φ of silicone oil referred as the bad solvent.

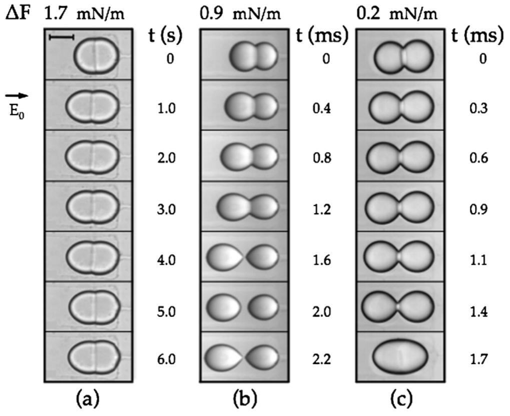

FIG. 4. Time sequences showing three different behaviors of an adhesive drop pair under an ac electric field (the amplitude of the applied electric field, 5 kV/cm, is the same for the three cases). (a) drops slightly deform but still adhere; (b) drops unzip and then repel each other; (c) drops coalesce while unzipping. The scale bar represents 60 μm.

PDMS permeability to organic phase, which is higher for chloroform, to continuously tune adhesive conditions on-chip. Indeed, when a slight constant flow of the continuous phase is maintained, a concentration gradient of oil composition is building up along the micro-channel. Therefore, there is a steady gradient of adhesion energy along the traps which is inferred from the contact angle. The first state corresponds to a stable situation where drops slightly deform [Fig. 4(a)]. The second state is characterized by the fast unzipping of the bilayer, drops then repel under field [Fig. 4(b)]. The third state corresponds to drop coalescence [Fig. 4(c)]. By exploring how the transition between these three states depends on both electrical field intensity and adhesive energy, we must be able to assess how the membrane tension directs those transitions.

When a conductive drop of radius R_0 is subjected to an electric field E_0, an electrostatic stress $\sigma_e \sim \varepsilon_0 \varepsilon_c E_0^2$ is built up at the interface [19]. Balancing the electrostatic stress σ_e by the capillary pressure $p_c \sim \gamma_0/R_0$, the drop shape can be described by an ellipsoid [20], the major axis being aligned along the field E_0 where σ_e is maximum. An adhesive drop pair is thus stretched along the electric field direction (Fig. 4). The stability diagram in the $\sigma_e - \Delta F$ plane is reported in Fig. 5. The different states previously discussed and shown in Fig. 4 correspond to three zones in this diagram. These three zones are delimited by straight lines, denoted A and B. Below frontier A, drops are stable although they can be slightly elongated. Between A and B, drops unzip and repel. Above frontier B they fuse. This diagram is raising the following points: (i) the linearity of frontiers A and B and (ii) the intercept of the two frontiers at $\Delta F \sim 0.25$ mN/m.

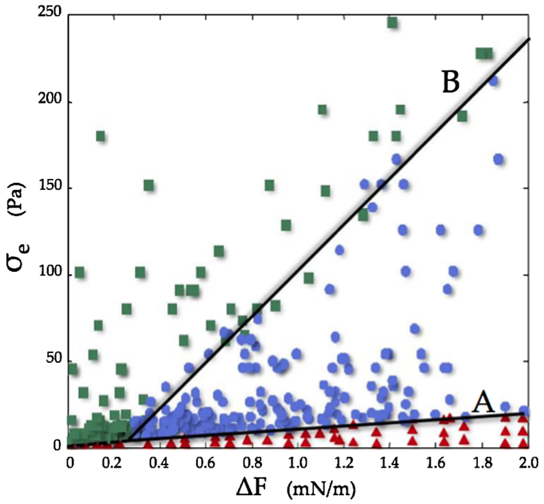

FIG. 5 (color online). Stability diagram of an adhesive emulsion drop pair under an ac electric field represented in the electrical stress (σ_e)-adhesion energy (ΔF) plane. A and B outline the frontiers between three regimes: (▲) drops adhere, (●) drops unzip, (■) drops coalesce.

As observed in Fig. 5, σ_e seems to linearly increase with ΔF at the unzipping frontier. The force f_e exerted by the electric field, which is responsible for detachment, can be derived from the integration of σ_e over the drops' surface. In our experiments, the drops have a pancake like shape, i.e. $R > H/2$ where R is the mean radius of the adhesive drop pair. Therefore, this force can be estimated to vary like $f_e \sim \sigma_e RH$. The force f_d of vesicle detachment has been calculated to be $f_d \sim v^{1/3}\Delta F$ [6–8], v being the volume of the adhering vesicle. In our case, $v \sim R^2 H$. Thus, by equating f_e with f_d, one finds $\sigma_e R^{1/3} H^{2/3} \sim \Delta F$. For $R = 50$ μm and $\Delta F = 1$ mN/m, the critical electrical stress is predicted to be 17 Pa, as observed in Fig. 5. In our experiments, H is kept constant and the polydispersity of R is around 20%. Therefore, following our hypothesis, σ_e must be proportional to ΔF at the unzipping threshold, i.e., at frontier A. The experimental data on the bilayer unzipping and the theoretical prediction are in good agreement like previously demonstrated for the unbinding of two cells [8]. Moreover, it is clear that monitoring electrical stress in inverse emulsion systems constitutes an appropriate method able to probe surface interaction.

As previously mentioned, the drops repel each other after unzipping under field. Then, we also note that they can attract each other when the electric field is turned off. It is known that bilayers can nucleate transient pores under field, a phenomenon known as electroporation [3]. For adhesive drop pairs, pores allow charge transport across the membrane during the unzipping step. This charge exchange between the drops therefore leads to oppositely charged drops which then naturally separate under field and attract one another in the absence of field.

The creation of a pore of radius r in a membrane of thickness h is associated to a free energy $\Delta W = 2\pi\Gamma r - 2\pi\gamma_m r^2 - 0.5\pi C_m h^2 E_m^2 r^2$ [3]. The first term is the energy increase due to the line tension Γ which is almost constant as it depends essentially on the membrane's thickness [21]. The second term represents the energy decrease due to a reduction of the surface area. The last term of ΔW comes from the accumulated energy of the membrane which behaves as a capacitor. In the same mindset, C_m is the capacitance of the pored membrane. The local electric field E_m is reasonably assumed to be proportional to E_0 [3,22], i.e., $E_m = \alpha E_0$. Indeed, the electric field at the facing poles of two conductive spheres subjected to an electric field E_0 is $E_m = 0.6R/hE_0$ for a thin gap [23], i.e., when $h/R < 10^{-3}$. From the minimization of the free energy, it results that the rupture of the bilayer (or drop fusion) occurs when a pore of radius $r^* = \Gamma/(2\gamma_m + 0.5\pi C_m h^2 \alpha^2 E_0^2)$ or larger is nucleated. Unzipping and repelling can occur when $r < r^*$. The associated free energy ΔW^* of the pore is $\Delta W^* = \pi\Gamma^2/(2\gamma_m + 0.5\pi C_m h^2 \alpha^2 E_0^2)$. The linearity of the frontier B in Fig. 5 suggests that the free energy ΔW^* of the pores under electric field that leads to the bilayer rupture is constant. Indeed, by assuming a constant bilayer thickness h, and therefore a constant line tension Γ and capacitance C_m and by recalling that $\gamma_m = \gamma_0 - \Delta F/2$ and $\sigma_e \sim E_0^2$, the denominator in ΔW^* is $a + b\sigma_e - \Delta F$, where a and b are constants. The formation of pores can be described via a thermally activated mechanism [3,14] where the activation energy is given by the free energy of the pore ΔW. According to this mechanism, the electric field thus decreases the energy barrier down to a value close to the thermal energy kT in order to observe a rupture of the bilayer during the time course of the experiment which is around 10 seconds. From the stability diagram reported in Fig. 5, the frontiers A and B meet at $\Delta F \sim 0.25$ mN/m. Unzipping does not occur below this critical value that is close to the gas-fluid like phase transition in the bilayer (Fig. 3). Below this point, in the gas phase, the bilayer is poor in phospholipids. Thus, the electric field easily leads to the appearance of pores of radius above r^* in the membrane, which entails drop coalescence. This process is probably occurring first, before drops are able to detach.

In this letter, by using microfluidics to form and manipulate inverse adhesive emulsions, we have investigated the behavior of adhesive drop pairs separated by a phospholipid bilayer and subjected to an electric field. The experimental approach that we propose for probing liquid interface wetting between monolayers is so far the only one able to access bilayer unzipping which is shown to be well described by the theory of vesicle detachment. Moreover, electroporation of bilayers, a microscopic process, is directly correlated with the coalescence of drops, which is easier to detect. A natural follow-up is to investigate the properties of more complex membranes by incorporating cholesterol and proteins to mimic cellular wall [24], and thus an adequate strategy to do so has to be designed. Finally, we believe that the stability diagram will be of great interest for emulsions themselves and their numerous applications where adhesive films, particularly for inverse emulsions, are well spread.

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