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Giant liposome spreading on a silicon wall

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The spreading of a giant liposome on a vertical silicon wall can be realized when the gravity effect on the spreading is negligible. The dynamic spreading process is experimentally observed and recorded, while the evolution of the liposome shape and the change of the contact angle are quantitatively examined. Based on this, the spreading process, especially the contact line of the liposome on the silicon wall, is modeled by a non-equilibrium thermodynamics formulation. The driving force of the spreading process is the combination of the surface/interfacial tensions, and the mobility of the contact line determines the speed of spreading. The experimental data of liposome dimensions during the dynamic spreading process are closely fitted by the proposed model. © *2011 American Institute of Physics*. [doi:10.1063/1.3614497]

I. INTRODUCTION

Liposomes are artificially prepared vesicles made of lipid bilayers.[1] They have been widely studied in biological researches and employed in bioengineering applications due to their amphiphilic material composition and cell like properties. They are usually used for drug delivery,[2,3] gene transfection,[4,5] or as a convenient model system to study biomembrane properties and their functions.[6,7] Besides, they can also serve as biosensors[8,9] or bioreactors to produce some important biomaterials *in vivo*.[10,11] In aqueous environment, the inner and the outer liquids are separated by a lipid membrane, which is considered as a kind of fluid.[12,13] The physical properties of the lipid membrane have been extensively studied, especially via the shape transformation of the membrane from both molecular level[14–18] and continuum level.[19,20]

Among the various shape transformation behaviors of liposomes, the study of liposome adhesion and spreading is of great importance in understanding some cellular behaviors, such as the cell aggregation and proliferation. In some studies, numerical simulation results were reported, showing the adhesion phenomenon. And various methods, such as Monte Carlo method, were adopted to simulate the liposome adhesion or spreading process.[21,22] Experimental work was also reported, many of which were on small and large liposomes that could lead to the formation of planar lipid monolayer or bilayer on solid surfaces.[23,24]

It is known that, according to the size, there are small liposomes with diameters of several tens of nanometers, large liposomes with diameters of several hundreds of nanometers, and giant liposomes with diameters in several tens of micrometers. Due to the size of giant liposomes, the behaviors of their shape transformation can be directly observed under optical microscopes, which would facilitate the investigation of lipid membranes.[25–27] As for the adhesion process, in 1992, Rädler and Sackmann[28] proposed that the reflection interference contrast microscopy technique (RICM) could be utilized to study the interaction between giant liposomes and bilayer covered substrates. Later in 1998, they employed this technique to devise a method to measure the weak forces exerted on liposomes adhered to the solid surfaces.[29] In this method, the changes of the interference Newtonian rings are used to determine the distance between the liposome and the substrate. Besides this technique, evanescent wave-induced fluorescence method (EWIF) was reported and applied to study the strong adhesion of giant liposomes on both smooth and patterned solid surfaces.[30–32] The distance between the liposome and the substrate could also be determined via this method. However, neither of these two methods (RICM and EWIF) can directly show the shape changes of liposomes during dynamic spreading process. The contact angle of the lipid membrane on the solid surface cannot be measured because the observation is from the top of the liposome. In addition, special microscopes (RICM and EWIF) as used in their methods also add complexity and difficulty in operation and pose obstacles to many researchers.

Though the micropipette aspiration technique could be used to study the liposome shape transformation behaviors, it is more like a method to study the adhesion and de-adhesion phenomenon.[33] Although the liposome spreading process shares some common features with the adhesion process, and sometimes, the latter is the prerequisite of spreading, it is not suitable to study the whole spreading process with the micropipette aspiration method. When a liposome is aspirated, the external force exerted by the pipette participates in driving the spreading process and the membrane tension would be varied by tuning the aspiration pressure. However, the spontaneous spreading process is only driven by surface/interfacial tensions and other unrelated force should be avoided if possible.

Some experimental and analytical investigations on the pore opening phenomenon indicated that the surface tension, which predominantly controlled various shape transformation behaviors of liposomes, was variable during the deformation process.[34–36] However, in some cases, the surface tension of the lipid membrane could be simplified to be constant. Different from normal liquids, the number of lipid

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molecules is constant when a liposome forms, no more lipid molecules could come to the surface to fill the enlarged interspace during the shape transformation process. Thus, the membrane surface tension changes with the surface area. For pore opening process, only when the membrane is stretched to a large extent, membrane failure would occur. This is because the big change of the surface area leads to a correspondingly big increase in surface tension till it exceeds a critical value. If the change of the surface area is relatively small and no visible pore is detected, the surface tension could be simplified as constant.

In the present work, the spreading process of giant liposomes is experimentally and analytically investigated within the scope of continuum physics. And the spreading experiment is carried out on a vertical silicon wall, which enables the real time observation of the shape evolution during the spreading process and the measurement of contact angles by image processing. This is realized without taking the gravity effect into consideration.

The structure of the paper is as follows. The experiments will be introduced in Sec. II. From the experimental observation, a theoretical model is proposed in Sec. III for the process of a giant liposome spreading on a solid substrate. The model is based on non-equilibrium thermodynamics formulation, and the concept of the mobility of the contact line is introduced. The solving algorithm will follow the model and be introduced in Sec. III. The experimental results will be fitted by simulation results to demonstrate the feasibility of the model in Sec. IV. And finally, the conclusions will be drawn in Sec. V.

II. EXPERIMENTS

A. Materials

Synthetic phospholipid, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), was purchased from Avanti Polar Lipids (USA). Sucrose and glucose of microscopy grade, chloroform, and poly-*L*-lysine ($M_w = 150\ 000 - 300\ 000$) were bought from Sigma-Aldrich (USA). De-ionized water (DI water) was purified by Milli-Q water system (Millipore, USA).

B. Formation of giant liposomes

Giant liposomes were prepared from neutral DOPC with the electroformation method[37–39] as reported in Ref. 40. In brief, the lipid solution of DOPC was diluted with chloroform to 1 mg/ml. Under nitrogen, the diluted lipid solution was deposited onto the conductive surface of one piece of ITO coated glass plate (ITO coating thickness: 1200–1600 Å, resistance: 5–15 Ω, Delta Technologies, USA). The plate was then put in a vacuum oven and maintained for at least 6 h to completely remove the organic solvent. Before electroformation, the glass plate with dry lipids on ITO coated surface was assembled together with another piece of glass plate to form a formation chamber. The two pieces of plates were separated by a silicone spacer (Sylgard 184 Silicone Elastomer Kit, Dow Corning, USA) with the ITO coated surfaces facing each other. The chamber was connected to a function generator (Thurlby Thandar Instruments, United Kingdom), which was used to generate and apply the ac electric field. After gently introducing the sucrose aqueous solution (100 mM) into the formation chamber and raising the voltage from 0.2 V to 2.0 V (peak-to-peak), liposomes formed as observed synchronously from the microscope eyepiece and the computer monitor (Microscope: BX51WI, Olympus, Japan; CCD camera: QICAM, QImaging, Canada; Image capturing and analysis software: Image Pro Express, MediaCybernetics, USA). The formation process was maintained at 10 Hz and the liposomes were detached by decreasing the frequency to 0.5 Hz after 2 h. The electro-formed liposomes were harvested into a plastic tube and stored in a refrigerator for future use.

C. Adhesion and spreading of liposomes

The spreading experiments were carried out with giant liposomes on a home-made spreading device.[40] The device was made from a piece of silicon sheet and two pieces of transparent polymer sheets by assembling them together in T shape. The silicon sheet, serving as the spreading substrate, was cut from a piece of commercially available silicon wafer (P type/boron doped, single side polished, Bonda Technology Pte Ltd, Singapore), and the polymer sheets were cut from cast acrylic sheet (Ying Kwang Acrylic Trading, Singapore). The setup is schematically shown in Fig. 1.

Firstly, the solution of liposomes was diluted with glucose aqueous solution (102 mM) at the volume ratio of 1 to 10 to enhance the image contrast. It is known that polylysine with high molecular weight could promote membrane adhesion on various solid surfaces.[41,42] In this work, the silicon substrate was pre-coated with polylysine aqueous solution (0.01% w/v) after DI water cleaning and plasma cleaning. It was emerged in the polylysine solution overnight. Before use, the polylysine pre-coated silicon substrate was thoroughly rinsed with DI water and assembled together with the polymer sheets to form the T-shape spreading device. The vertically fixed silicon wall served as the spreading substrate and the polished side would be the spreading surface. As studied by Tay *et al.*,[43] the surface roughness (R_q) of a well polished silicon wafer with an isotropic surface is about 20 nm, which is low enough to consider the surface as smooth. Thus, there would be no influence of the surface roughness on the spreading process.

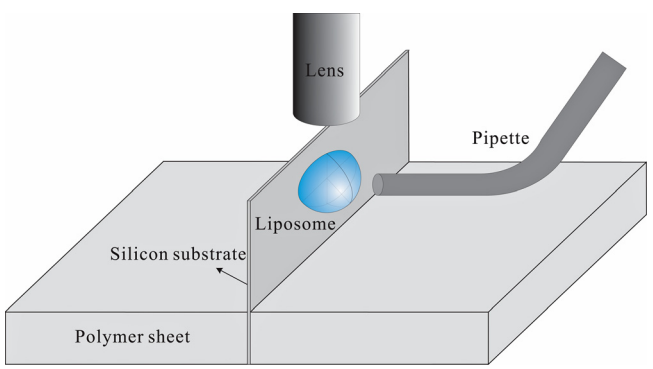

FIG. 1. (Color online) Experimental setup for liposome spreading on a silicon wall. The gravitational force is negligible due to the small size of liposomes, which enables the study of spreading on vertically fixed substrate.

Secondly, a drop of diluted liposome solution (about 30 μl) was added along the silicon wall under the microscope. A single liposome would be selected and aspirated by a glass micropipette (VacuTip, Eppendorf, Germany) whose translational movements were controlled by a micromanipulator (UM-3FC, Narishige, Japan) fixed on the microscope stage. A microinjector (IM-6, Narishige, Japan) was also used to generate the aspiration pressure by simply turning the operation handle.

Finally, the aspirated liposome was transferred to approach the silicon wall until it touched it. The micropipette was retracted immediately and the liposome spread on the silicon surface. Some liposomes could spread till it reached the equilibrium state. All the adhesion and spreading process was observed under the microscope and recorded by the CCD camera.

III. NON-EQUILIBRIUM THERMODYNAMICS FORMULATION OF A LIPOSOME SPREADING ON A SOLID SUBSTRATE

In this work, the analysis of the spreading process is limited to the continuum physics scope. And the theoretical modeling of the spreading of liposomes is based on the framework of non-equilibrium thermodynamics.[44] After a liposome touches the solid substrate, it experiences a rapid spreading at the initial stage. And then, it spreads at much slower speed until it reaches the equilibrium state. This evolution develops fast at initial stage and slows down with time. Without losing the key features of the problem, the shape of the liposome will be approximated by a spherical cap during the spreading process. In the following parts of this section, the energy and the driving force of the spreading process will be considered.

A. Energy and driving force in the spreading process

Considering a liposome spreading on a solid substrate, the lipid membrane, the solid substrate and the liquid surrounding the liposome constitute the liquid/membrane/solid system as shown in Fig. 2. Interfaces form between the membrane and the surrounding liquid, the liquid and the substrate, and the membrane and the substrate with the surface/interfacial tensions as σ, σ_{s1}, and σ_{s2}, respectively. From the experimental observations, it is found that there is no visible pore on the membrane and that the change of the membrane area is small, thus in this theoretical model, the surface tension of the membrane, σ, is regarded as constant. The liquid enclosed in the membrane has no contribution to the driving of spreading process.

Within the scope of continuum physics, for a condensed matter of macroscopic size, the volume related energy dominates in driving the dynamic spreading process,[45] while for a condensed matter of sub-millimeter scaled size, the surface associated energy becomes dominant.[46] If the size of the matter is further reduced to micrometer or sub-micrometer scale, the line tension effect will be considered to play an important role in driving the spreading process.[47] For the liquid/membrane/solid system studied in the present work, only the energy contribution from the interfaces is considered, while the volume contribution is negligible due to the small size of the liposomes. On the other hand, the line tension usually contributes only when there is pore or domain on lipid membranes, and is too small, compared with the surface tension, to be considered in the spreading process of giant liposomes with diameters in several tens of micrometers.[34,48] Therefore, this work will focus on the description and formulation of the membrane itself. Even though the matter inside of the liposome contributes to the liposome shape and the internal pressure, its associated energy is negligible.

In the viewpoint of thermodynamics, the change of the system's energy is the product of the driving force and its associated displacement. Figure 3 shows the shape evolution of the spreading liposome. For the above liquid/membrane/solid system, the energy change could be represented by the sum of the interfacial energy changes,

$$\delta G = \sigma \delta A + \sigma_{s1} \delta A_{s1} + \sigma_{s2} \delta A_{s2}, \quad (1)$$

where G is the Gibbs free energy, and A, A_{s1}, and A_{s2} are the areas of the interface between the membrane and the surrounding liquid, the liquid and the solid substrate, and the membrane and the substrate, respectively.

Since the solid substrate studied in this work is smooth and the lipid membrane is considered as homogeneous without any phase separation or domain, the contact line is a circle with radius of L, and the driving force is uniformly distributed along the contact line. The geometric relations among the contact radius L, the liposome height h, the sphere radius R, and the contact angle θ are shown in Fig. 2.

It is seen from Fig. 3 that the contact area between the liposome and the solid substrate increases while the area of the interface between the solid substrate and the surrounding

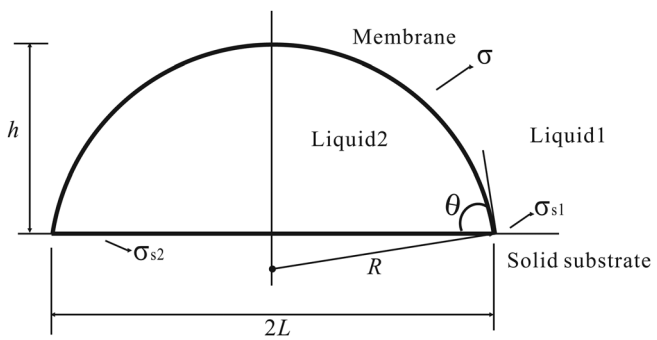

FIG. 2. Shape of the adhered liposome is approximated by a spherical cap.

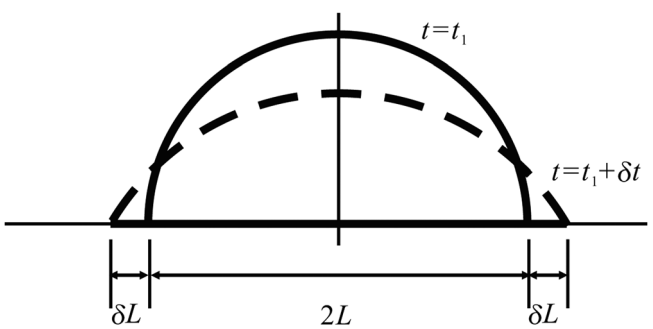

FIG. 3. Evolution of liposome shape during spreading process. The contact radius increases by δL when time passes by δt.

liquid decreases, and the increased area equals to the decreased one. If the contact radius is L and the increment of the contact radius is defined as δL, the area change of the interface between the membrane and the solid substrate could be written as

$$\delta A_{s2} = 2\pi L \delta L. \tag{2}$$

Similarly, the area change of the interface between the solid substrate and the surrounding liquid is

$$\delta A_{s1} = -2\pi L \delta L. \tag{3}$$

As for the spherical cap, the area change of the interface between the membrane and the surrounding liquid is

$$\delta A = \delta\left(\pi\left(L^2 + h^2\right)\right) = 2\pi L \delta L + 2\pi h \delta h. \tag{4}$$

The relationship between δL and δh can be found from the volume conservation of the spreading liposome. Since no visible pore or substance exchange is observed from our experiments, the volume of the spreading liposome, V, is constant with $\delta V = 0$. Under this condition, we have

$$\delta V = \delta\left(\frac{\pi}{6} h\left(3L^2 + h^2\right)\right) = 0, \tag{5}$$

which leads to

$$\delta h = -\frac{2Lh}{L^2 + h^2}\delta L. \tag{6}$$

Therefore, by substituting Eq. (6) into Eq. (4), the area change of the membrane cap as a function of δL can be obtained as

$$\delta A = \delta\left(\pi\left(L^2 + h^2\right)\right) = 2\pi L \delta L \frac{L^2 - h^2}{L^2 + h^2}. \tag{7}$$

Considering the spherical cap, the geometric relationship between the contact angle θ, and the liposome dimensions, L and h, is

$$\cos\theta = \frac{L^2 - h^2}{L^2 + h^2}. \tag{8}$$

Substituting Eq. (8) into Eq. (7), and considering together with equations 2 and 3, the energy change (shown in Eq. 1) becomes

$$\delta G = 2\pi L \delta L(\sigma \cos\theta + \sigma_{s2} - \sigma_{s1}). \tag{9}$$

The system energy is lowered by the thermodynamics driving force. Here, the driving force in this liquid/membrane/solid system is on the plane of the spreading surface directing normal to the contact line, and the displacement is the area that the liposome spreads over the solid substrate, which equals to δA_{s2}. Then, the change of the energy could also be written as

$$\delta G = -f_L \times 2\pi L \delta L, \tag{10}$$

where f_L is the "thermodynamics driving force" and the energy should decrease under the rule of non-equilibrium thermodynamics. By comparing Eq. (9) and Eq. (10), the driving force can be expressed as

$$f_L = \sigma_{s1} - \sigma_{s2} - \sigma \cos\theta, \tag{11}$$

and the above thermodynamic force has the unit of surface tension (force/length).

B. Motion equation and equilibrium condition

Let δL be the motion of the contact line. Under the scope of the thermodynamics, the motion is proportional to the driving force, i.e.,

$$\delta L = M_L f_L \delta t, \tag{12}$$

where M_L is called the mobility of the contact line and is used as the phenomenological parameter of the system. Equation (12) is the kinetic law for the contact line.

The combination of equations (11) and (12) leads to the expression for the motion of the contact line,

$$\delta L = M_L(\sigma_{s1} - \sigma_{s2} - \sigma \cos\theta)\delta t. \tag{13}$$

The above equation governs the motion of the contact line during the spreading process

At the end of the spreading process, the system reaches an equilibrium state with the contact angle to be at its equilibrium value, θ_e. The driving force vanishes to zero, and the contact line stops to move. By setting f_L to be zero in Eq. (11), the equilibrium condition for the contact line could be obtained as

$$\sigma_{s1} - \sigma_{s2} - \sigma \cos\theta_e = 0, \tag{14}$$

which is the famous Young equation.

C. Numerical simulation of a liposome spreading on a silicon wall

From the non-equilibrium thermodynamics formulation of the liposome spreading process, it is known that the liposome spreading is mainly controlled by the mobility of the contact line. To demonstrate the feasibility of our analytical model, numerical simulation was carried out.

From the above model, the increase of the contact radius of the liposome in contact with the silicon wall is given by

$$\delta L = \sigma M_L(\cos\theta_e - \cos\theta)\delta t. \tag{15}$$

By normalizing δL with the contact radius at the equilibrium state, L_e, Eq. (15) becomes

$$\frac{\delta L}{L_e} = \frac{\sigma M_L}{L_e}(\cos\theta_e - \cos\theta)\delta t. \tag{16}$$

With the dimensionless form of the equation of evolution, Eq. (16), the characteristic spreading time could be defined as

$$t_s = \frac{L_e}{\sigma M_L}. \tag{17}$$

The combination of equations (16) and (17) leads to

$$\frac{\delta L}{L_e} = (\cos\theta_e - \cos\theta)\delta\left(\frac{t}{t_s}\right), \tag{18}$$

by which the evolution of the system could be simulated.

The numerical simulation of Eq. (18) was carried out by a simple iteration. If the contact radius of the liposome at the ith state $\left((t/t_s)_i\right)$ is known and an increment of dimensionless time is set as $\Delta(t/t_s)_i$, the contact radius change is given by

$$\Delta(L/L_e)_{i+1} = (\cos\theta_e - \cos\theta_i)\Delta(t/t_s)_i. \tag{19}$$

Then, the contact radius at the $(i+1)$th state is calculated as

$$(L/L_e)_{i+1} = (L/L_e)_i + \Delta(L/L_e)_{i+1}. \tag{20}$$

According to Eq. (6), the increment of the liposome height is

$$(\Delta h)_{i+1} = \left(-\frac{2Lh}{L^2+h^2}\right)_i (\Delta L)_{i+1}, \tag{21}$$

and the height at the $(i+1)$th state becomes

$$h_{i+1} = h_i + (\Delta h)_{i+1}. \tag{22}$$

The contact angle could be calculated from the contact radius L and the liposome height h as

$$\cos\theta_{i+1} = \left(\frac{L^2-h^2}{L^2+h^2}\right)_{i+1}. \tag{23}$$

The initial condition of the iteration is set as $L_0 = 0, h_0 = 2\times(3V/4\pi)^{1/3}$ with V to be the known liposome volume, and $\theta_0 = 180°$. The time step for iteration $\Delta(t/t_s)_i = 0.001$ was found by several trials until the solution did not change even if further decreasing the time step. For a given equilibrium contact angle and the contact radius at the equilibrium state, the iteration could fully describe the evolution of the whole spreading process.

IV. MATCHING EXPERIMENTAL MEASUREMENT WITH NUMERICAL SIMULATION

The experimental results of the shape evolution are shown in Fig. 4. After touching the silicon wall, the liposome immediately adheres onto the silicon surface and starts to spread. At the early stage of spreading, the liposome adhesion area increases dramatically with the contact radius increasing as well. After a short period, the spreading slows down and finally reaches an equilibrium state.

As shown in Fig. 4, the experimental results can only provide us with the information of liposome spreading at the late stage. This is due to the fact that the evolution process at the early stage is so fast that our CCD camera is unable to capture the details of the shape evolution. To fully record the details at the early stage, CCD camera of much higher speed is needed. Though there is such obstacle in experiments, fortunately, our model can solve this problem without any difficulty and provides us with the information at the early stage.

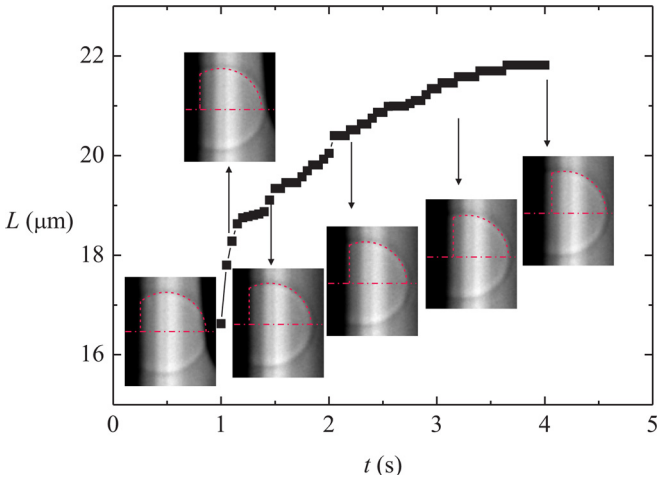

FIG. 4. (Color online) Evolution of the contact radius with time and the microscopic images obtained during liposome spreading process. Half of the liposome profile is highlighted in dashed lines.

To include the early stage of the liposome spreading process, in the numerical simulation, the initial contact angle of the liposome is $\theta_0 = 180°$, which implies a liposome, just touching the wall, with a full spherical shape. The final equilibrium configuration is set based on our experimental results of DOPC liposome spreading on smooth silicon substrate, i.e., $\theta_e = 107.5°$ and $L_e = 21.8$ μm for a liposome of the initial radius of about 20.5 μm. The simulated results are shown in Fig. 5 with a complete spreading process. Initially, once the liposome touches the wall, the dimensionless contact radius is 0 and the dimensionless height is 1.05, exhibiting a spherical shape. Because of the large difference of the contact angle from the equilibrium value, the driving force, $(\cos\theta_e - \cos\theta_0)\,\sigma$, is extremely large. The large driving force results in a high speed of the contact line. Therefore, the dimensionless contact radius dramatically increases from 0. With a conserved volume of the liposome, the dimensionless liposome height decreases correspondingly. With the increase of the contact radius and the decrease of the liposome height, the contact angle of the liposome decreases as shown in Fig. 5(b). As the contact angle, θ_t, becomes smaller, the driving force, $(\cos\theta_e - \cos\theta_t)\,\sigma$, also becomes smaller. And consequently, the speed of the contact line is reduced. Therefore, the variation rates of the contact radius and the liposome height decreases as time passes. The driving force vanishes when the contact angle finally reaches the equilibrium contact angle θ_e. Without force to drive the liposome to spread on the wall, the shape of the liposome reaches the equilibrium state, and the spreading process completes.

To determine the characteristic spreading time, t_s, the experimental results were fitted with simulation results using the least square method. And after normalizing the experimental time by the characteristic spreading time, the simulation results agree well with the experimental results as shown in Fig. 5, which demonstrates that our analytical model is reliable. The mobility of the contact line could be obtained from Eq. (17) as

$$M_L = \frac{L_e}{\sigma t_s}. \tag{24}$$

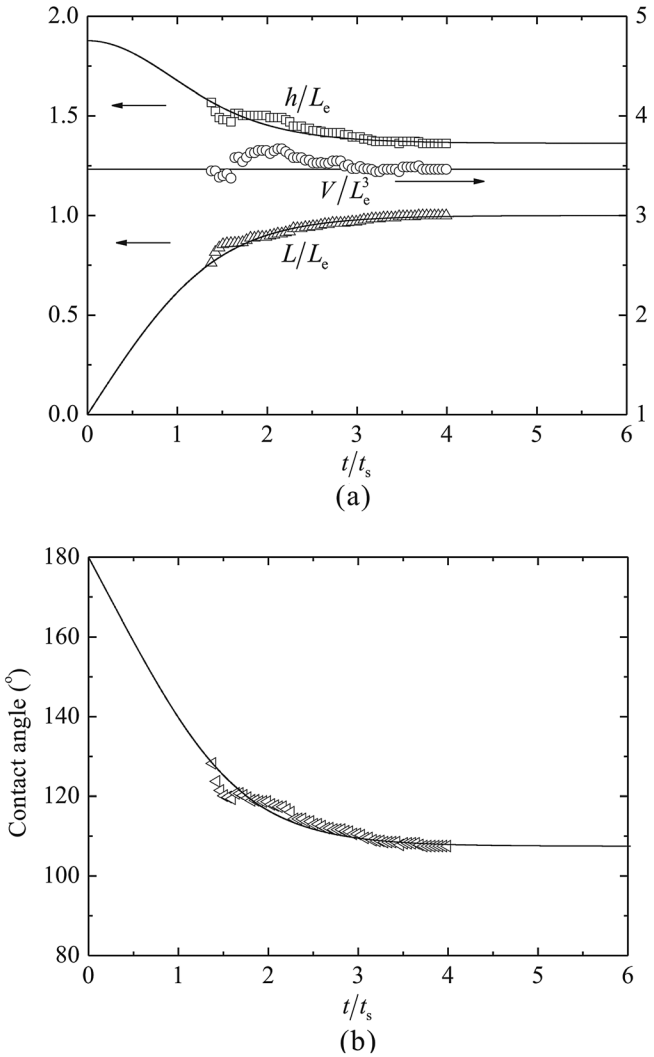

FIG. 5. (a) Dynamic evolution of the liposome shape with time (dimensionless contact radius L/L_e, dimensionless liposome height h/L_e, and dimensionless volume V/L_e^3). (b) The change of contact angle with time. The scattered symbols represent the experimental results, while the solid lines represent the simulation results. The comparison between the experimental and the simulation results shows good agreement.

From Eq. (24), we can find out that the mobility of the contact line depends on the membrane surface tension, but does not depend on the process parameters, such as the initial contact angle. In order to determine the mobility of the contact line, the surface tension of the lipid membrane needs to be known. Though the membrane surface tension is not experimentally accessible,[49] it could be estimated from some theoretical models, such as the polymer brush model proposed by Rawicz *et al.*[50] In this model, a lipid monolayer was modeled as an idealized polymer brush and the surface tension of a monolayer is predicted to be $K_A/6$, where K_A is the direct elastic stretch modulus. Based on this model, we borrow the data of the surface tension of a DOPC bilayer from their work,[50] where $\sigma = 88$ mN/m. Substituting it into Eq. (24), together with the equilibrium contact radius $L_e = 21.8$ μm and the characteristic spreading time $t_s = 1.15$ s, the mobility of the contact line is estimated to be 2.16×10^{-4} $m^2/(N \cdot s)$ for a giant liposome spreading on a silicon surface. This value is 1 order lower than the mobility of the contact line of a silicone oil droplet spreading on stainless-steel plates.[51]

V. CONCLUDING REMARKS

We know from the continuum physics theory that the spreading of micrometer-sized liposome should be controlled by the surface related energy of the system. In this work, we experimentally recorded the dynamic spreading process of giant liposomes. Based on the observation, a theoretical model was built under a framework of non-equilibrium thermodynamics. From a numerical simulation, we could visualize the evolution of the liposome shape during the whole spreading process.

By matching the numerical simulation curve with the experimental data, we demonstrate the validity and the feasibility of our analytical model. Also, for the liposome spreading on a silicon substrate, we estimated the value of the mobility of the contact line, which was much lower than the mobility of the contact line of a silicone oil droplet spreading on a stainless-steel plate.

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