Investigation of liquid droplet and liposome spreading on smooth and patterned substrates

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**INVESTIGATION OF LIQUID DROPLET AND
LIPOSOME SPREADING ON SMOOTH AND
PATTERNED SUBSTRATES**

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SCHOOL OF MECHANICAL AND AEROSPACE

ENGINEERING

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**INVESTIGATION OF LIQUID DROPLET AND
LIPOSOME SPREADING ON SMOOTH AND
PATTERNED SUBSTRATES**

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Abstract

In this project, the spreading of liquid droplets and giant liposomes is investigated experimentally and numerically within the scope of continuum physics. Five liquids of different properties in their surface tension and viscosity are used, including deionized (DI) water, SDS (at the critical micelle concentration (CMC)), SDS (10% CMC), glycerol, and water-glycerol mixture (volume ratio 1:1). Liposomes are prepared from DOPC and the mixture of DOPA and DOPC (1:9). Isotropically patterned substrates and anisotropically patterned substrates are prepared to study the effect of surface patterns on spreading. The substrates for droplet spreading are fabricated on shape memory polymer through wrinkling, while those for liposome spreading are on silicon wafers by photolithography and dry etching. The wetting and spreading of liquid droplets and giant liposomes is characterized by the equilibrium contact angles, the equilibrium droplet/liposome dimensions, the dynamic change of contact angles, and the dynamic evolution of droplet/liposome dimensions.

On the smooth substrates, liquid droplets and giant liposomes exhibit the shape of a spherical cap. The contact angles and the contact radii are the same along the contact line. Less viscous liquids spread faster than more viscous liquids. After adding surfactant into DI water, SDS (CMC) droplets need more time than DI water to reach the equilibrium states. The spreading speed decreases with the liquid viscosity and increases with the surface tension. On the isotropically patterned substrates, water droplets have similar equilibrium contact angles as on the smooth substrates, showing that the small surface roughness does not significantly affect droplet spreading. On the anisotropically patterned substrates,

contact angle difference is significant in the directions parallel and perpendicular to wrinkles/grooves. Liquid droplets and giant liposomes spread faster in the direction parallel to wrinkles/grooves than in the perpendicular direction, leading to droplet/liposome elongation along wrinkles/grooves. Wetting anisotropy is characterized, and pinning of the contact line is noticed, which implies the local effect of the surface patterns on spreading. Wrinkle wavelength is shown to affect the equilibrium contact angles and the droplet elongation.

A thermodynamic model is set up to simulate the dynamic spreading of liquid droplets and giant liposomes. The simulation results based on the thermodynamic model agree well with the experimental results. A system parameter, the mobility of the contact line, is introduced, whose value is obtained by fitting the simulation curves with the experimental curves. The results show that the mobility decreases with the liquid viscosity. On the anisotropically patterned substrates, the mobility for the droplet/liposome spreading in the direction parallel to wrinkles/grooves is larger than that in the perpendicular direction.

Nonlinear curve fitting of experimental results is conducted as well. The spreading speed is characterized by the critical velocity, a system constant, which depends on both the liquid viscosity and the surface tension. It decreases with the liquid viscosity and increases with the surface tension.

Keywords: liquid droplet, giant liposome, wetting and spreading, dynamic spreading, wetting anisotropy

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List of Publications

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Journal Papers and Book Chapters

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Zhang Y., Fan H., Huang W. M., and Chen Y. (2012) “Experimental investigation of the surface tension of lipid membranes.” *Analysis and Design of Biological Materials and Structures* by Springer, Germany.

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Papers in Preparation

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Nomenclature

A	Interfacial area, [m ²]
Ca	Capillary length [mm]
e	Elongation, [-]
E	Elastic modulus, [MPa]
f	Driving force, [mN/m]
f_c	Ratio of the contact surface area to the total horizontal surface area, [-]
G	Gibbs free energy, [J]
h	Height of liquid droplets or giant liposomes, [mm] or [μm]
h_c	Gold coating thickness, [nm]
L	Contact radius of liquid droplets or giant liposomes, [mm] or [μm]
M	Contact line mobility, [m ² /(N•s)]
r	Roughness factor, [-]
R_a	Average surface roughness, [μm]
R^*	Initial droplet radius of liquid droplets, [mm]
S	Spreading parameter, [mN/m]
t	Time, [s]
t_{lipo}	Characteristic spreading time of giant liposomes, [s]
T_g	Glass transition temperature, [°C]

v	Velocity (of the contact line), [mm/s]
v_c	Critical velocity of the contact line, [mm/s]
V	Volume, [μ l]

Superscripts

p	exponent
-----	----------

Subscripts

C	Cassie
e	Equilibrium state
f	Gold coating film
h	Horizontal grooves
L	Contact line
$lipo$	Liposome
s	Shape memory polymer (SMP)
SV, SL, LV	Interface between solid and vapor, solid and liquid, liquid and vapor in the liquid/vapor/solid system
$S1, S2$	Interface between solid and surrounding liquid, solid and membrane in the liquid/membrane/solid system
v	Vertical grooves
W	Wenzel

x, y	x -axis and y -axis in Cartesian coordinate systems
Y	Young

Greeks

γ	Surface/interfacial tension in the liquid/vapor/solid system, [mN/m]
δ	Differential
Δ	Difference
θ	Contact angle, [°]
θ_A	Contact angle with the layer of air, [°]
λ	Wrinkle wavelength, [μm]
μ	Liquid viscosity, [$\text{mN}\cdot\text{s}/\text{m}^2$]
ν	Poisson ratio
ρ	Liquid density, [kg/m^3]
σ	Surface/interfacial tension in the liquid/membrane/solid system, [mN/m]
τ	Line tension, [mN/m^2]

Abbreviations

AC	Alternating current
CCD	Charge-coupled device

CMC	Critical micelle concentration
DC	Direct current
DI	Deionized (water)
DOPA	1,2-dioleoyl- <i>sn</i> -glycero-3-phosphate
DOPC	1,2-dioleoyl- <i>sn</i> -glycero-3-phosphocholine
EWIF	Evanescent wave-induced fluorescence (method)
fps	Frame per second
ITO	Indium tin oxide
RICM	Reflection interference contrast microscopy
SDS	Sodium dodecyl sulfate
SMP	Shape memory polymer

Chapter 1 Introduction

1.1 Background

Wetting and spreading is a universal phenomenon in nature. It can be noticed from the drops of dew on leaves and the oil droplets floating on a pool of water. It is of significant importance in science and has wide applications in industry. Considering a liquid droplet on a solid substrate, there are three wetting states: complete wetting, partial wetting, and non-wetting. Surface wettability can be assessed by measuring the equilibrium contact angle that the liquid droplet forms with the solid surface. The equilibrium contact angle, in a three-phase system, is related to the interfacial tensions by the Young equation (Young 1805). However, the relationship in the Young equation is only applicable on ideally smooth and homogeneous solid surfaces. Real surfaces have roughness or micro-/nano-patterns which have effects on the equilibrium contact angles (Bico et al. 2002; Quéré 2008). It is important to study wetting and spreading on rough or patterned surfaces. Various surface patterns can be realized with the development of micro-/nano-fabrication technologies, and the wetting and spreading on surfaces with micro-/nano-patterns becomes tunable and controllable (Madou 2002). The control over wetting and spreading enables different applications which require specific wettability, such as inkjet printing and oil/water separation (Määttänen et al. 2010; Xue et al. 2011). Wetting and spreading of lubricants is an important factor to be considered in the applications of lubrication (Ma et al. 1999; Karis et al. 2005; Yang and Shivpuri 2006). In some other applications, such as spin coating (Wilson et al. 2000), the spreading speed is a determining factor and the dynamic spreading process needs to be investigated.

The phenomenon of wetting and spreading is intrinsically about surfaces and interfaces. Besides normal fluids, biological systems are often involved in the interfacial phenomena. Biological systems at fluid interfaces are one focus in the emerging field of biomedical engineering, which attract much attention of researchers and engineers. The wetting of a biological fluid interface is often considered in the research of drug delivery, biomaterials processing, and tissue engineering (Hao et al. 2005; Safinia et al. 2007). Fluid interfaces, playing a crucial role in cellular or physiological behaviors, are formed by biological membranes which can be categorized as soft matter and regarded as two-dimensional solutions of proteins and lipids according to the fluid mosaic model (Singer and Nicolson 1972). The fluid mosaic model, consistent with the restrictions imposed by thermodynamics, can be applied to cell membranes and membranes of some organelles, such as mitochondria and chloroplasts. Lipid bilayers are the main constituent of cell membranes, whose structure and functions could also be explained by the fluid mosaic model.

Liposomes, also referred to as lipid vesicles, can be spontaneously formed from lipid bilayers. Bilayers in liposomes exist with the hydrophilic “head” facing aqueous solutions and the hydrophobic “tails” lining up away from water. It is the thermodynamically minimum energy state in aqueous environment. Liposomes not only serve as simplified models of real cells to study cell behaviors, but also are used as drug delivery vehicles and biomimetic reactors (Karlsson et al. 2004; Al-Jamal et al. 2008). Most of these applications are related to the adhesion and spreading of liposomes. Thus, it is of both scientific and practical significance to study the spreading of liposomes.

In general, both liquids and lipid bilayers are fluids in the viewpoint of thermodynamics. It is reasonable and possible to investigate the spreading of these two soft matters with similar methods.

1.2 Objectives and scopes

With the development of research in wetting and spreading, many experiments have been carried out to understand the spreading process and many theories have been established to describe the physics behind the phenomena. However, there are still problems to be solved, such as the effect of surface properties of the solid substrates, the characterization of spreading speed in dynamic spreading process, and the spreading of droplet-like liposomes. Recent developments in micro-/nano-technologies pave the way for studying the effect of surface patterns on wetting and spreading. The investigations need to be widened and deepened by employing more types of solid surfaces and focusing on more detailed features of spreading behaviors.

Liposomes and liquid droplets share similarities in their fluid nature and spherical shape. Though they are different in size, their spreading processes are dominated by surface/interfacial tensions. Thus, the spreading of giant liposomes and liquid droplets can be studied in a similar strategy, and comparison can be made between their spreading behaviors.

The first objective of this project is to study the static spreading of liquid droplets on smooth, isotropically patterned, and anisotropically patterned substrates, and to study the dynamic spreading on smooth and anisotropically patterned substrates.

The patterned substrates will be fabricated through wrinkling method with shape memory polymer. The effect of droplet size will be studied, and wetting anisotropy will be analyzed. A thermodynamic model will be set up for the dynamic spreading process. The spreading speed in the directions parallel and perpendicular to wrinkles will be characterized.

The second objective of this project is to study the spreading of giant liposomes on smooth and patterned substrates. In order to observe and measure the spreading of liposomes under normal optical microscopes, a spreading device will be designed and fabricated. The patterned substrates will be prepared on silicon wafers through microfabrication method. The theoretical model used for the spreading of liquid droplets will be applied to describe the spreading of giant liposomes. The evolution of liposome dimensions on the smooth and anisotropically patterned substrates will be studied experimentally and numerically. The spreading speed will be characterized.

1.3 Outline of the thesis

This thesis, containing seven chapters, is organized as follows.

The existing work and results related to the spreading of liquid droplets and giant liposomes are reviewed in Chapter 2. It includes experimental and theoretical studies on the spreading on rough and patterned solid surfaces, as well as wetting anisotropy and shape transformations of liposomes.

The spreading of liquid droplets is reported in Chapters 3 and 4. Chapter 3 reports the experimental results of the static spreading of water droplets with the emphasis on the effect of droplet size, while Chapter 4 reports the dynamic spreading of five different liquids, which is studied by experimental measurements and numerical simulations. The spreading speed is characterized, and the wetting anisotropy is quantified. The fabrication process of the wrinkled substrates is also introduced in these two chapters.

Chapters 5 and 6 focus on the adhesion and spreading of giant liposomes on smooth and anisotropically patterned substrates, respectively. It includes the preparation of giant liposomes, the fabrication of the spreading device, and the spreading experiments on vertically fixed solid substrates. Based on the thermodynamic model used for the spreading of liquid droplets, numerical simulations are performed and the comparison with the experimental results is made in these two chapters. The spreading speed is characterized.

Finally, this thesis is concluded in Chapter 7 with suggestions of future work in the relevant research fields.

Chapter 2 Literature Review

2.1 Wetting and spreading of liquid droplets

2.1.1 General concepts on wetting and spreading

The phenomenon of wetting and spreading has been investigated for more than a century (Bonn et al. 2009). Spreading is usually an outcome of droplet impact on a solid surface (Yarin 2006), and is of great interest to both scientists and engineers. Spreading has been widely investigated due to its ubiquity in daily life and potential applications in industries. It plays an important role in industries, such as soldering (Kim et al. 2008; Prabhu and Kumar 2010) and inkjet printing (Määttänen et al. 2010). Wetting is an indispensable factor to be considered in microfluidics techniques. By controlling the wettability of the substrates, discrete droplets can be manipulated individually, and different functions can be achieved, such as transporting, mixing, reaction, and analyses (Fair 2007). Numerous studies on wetting and spreading have been carried out, and the existing results show that the spreading of liquid droplets on solid substrates can be controlled by the material properties of the liquids and the solid substrates, and by the surface geometric properties of the solid substrates. The surface energies of the liquid and the solid together determine whether the liquid could wet the solid surface, and the influence of micro-/nano-pattern geometries on spreading can be described by analyzing the shape of the contact line. The solid surface wettability can be characterized by the equilibrium contact angles. For liquid droplets on ideally smooth and chemically homogeneous solid surfaces, the contact angles are related to surface/interfacial tensions through the Young equation (Young 1805) (Figure 2.1),

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_Y, \quad (2.1)$$

where γ_{SV} , γ_{SL} , and γ_{LV} are the interfacial tensions between solid and vapor, solid and liquid, and liquid and vapor, and θ_Y is the Young contact angle corresponding to the thermodynamic equilibrium state.

Figure 2.1 Illustration of the Young equation.

According to the value of the contact angle that the liquid droplet forms with the solid substrate, there are three wetting states: complete wetting, partial wetting, and non-wetting. Considering the Young contact angle, when $\theta_Y = 0^\circ$, the liquid completely wets the solid; when $0^\circ < \theta_Y < 180^\circ$, the liquid partially wets the solid; when $\theta_Y = 180^\circ$, the liquid cannot wet the solid. If the three surface/interfacial tensions (γ_{SV} , γ_{SL} , and γ_{LV}) are known, the wetting state could be predicted through the spreading parameter S , which is defined as

$$S \equiv \gamma_{SV} - (\gamma_{SL} + \gamma_{LV}). \quad (2.2)$$

In Eq. (2.2), $S = 0$ indicates complete wetting while $S < 0$ indicates partial wetting or non-wetting.

Although the Young contact angle as defined in the Young equation (Eq. (2.1)) could characterize the surface wettability, the Young equation is valid only on smooth and chemically homogeneous surfaces. Real surfaces are not ideally smooth or homogeneous; they are rough, heterogeneous, or with patterns on them. The surface properties affect the wetting and spreading process (Quéré 2008). Thus, it is of great significance to study the effect of roughness and pattern geometry on the wetting and spreading process.

2.1.2 Wetting and roughness

Flexible control over the wetting process has been the aim of many researchers. Among the various factors influencing wetting and spreading, surface properties of solid substrates are of great importance, and they are often adjusted with two strategies: chemical modification and morphological texturing (Prabhu et al. 2009; Jradi et al. 2011). Chemical modification is based on material compositions and interactions between functional groups. Morphological texturing is to create micro-/nano-patterns on solid surfaces regardless of the inherent chemical properties of the solid surfaces. In comparison, the morphological texturing methods can provide more flexible control over the solid surface properties than the chemical modification methods. The strategy of morphological texturing is adopted in this project, and the spreading on morphologically textured surfaces will be reviewed and introduced here.

When a liquid droplet spreads on a rough surface, the contact angle that it makes with the solid surface is not the Young contact angle any longer, but depends on the roughness of the solid surface. The contact angle on a rough surface can be

calculated through the Wenzel model (Wenzel 1936) or the Cassie-Baxter model (Cassie and Baxter 1944), see Figure 2.2. The Wenzel model is defined as

$$\cos \theta_w = r \cos \theta_Y, \quad (2.3)$$

where θ_w is the apparent (Wenzel) contact angle on the rough surface and r is the roughness factor, which is the ratio of the true solid area to the planar projection area. The Cassie-Baxter model is written as

$$\cos \theta_C = f_C \cos \theta_Y + (1 - f_C) \cos \theta_A, \quad (2.4)$$

where θ_C is the apparent (Cassie) contact angle on the rough surface, θ_A is the contact angle with the layer of air, and f_C is the ratio of the contact surface to the total horizontal surface. The Wenzel model is often applied to homogeneous rough surfaces while the Cassie-Baxter model is preferred in the case of heterogeneous or structured surfaces. When the Wenzel model is applicable, it is called the Wenzel state, while it is the Cassie state when the Cassie-Baxter model is applicable.

Figure 2.2 Illustration of (a) Young, (b) Wenzel, and (c) Cassie-Baxter models. In the Wenzel model, the liquid fills the space between surface protrusions, and the wetting tendency is amplified by the roughness. In the Cassie-Baxter model, the liquid only wets the tip of the protrusions, and leaves the air pockets underneath it.

At the Wenzel state, the liquid fills the space between surface protrusions, and the contact area is increased due to the protrusions. In contrast, at the Cassie state, the liquid only wets the tip of the protrusions, and air pockets form underneath the liquid droplet. In some wetting phenomena, the Wenzel state and the Cassie state could transit from one to the other by designing and tuning the surface patterns. Ran et al. (2008) achieved the transition between the Wenzel state and the Cassie state of water droplets by adjusting the size of the holes on the alumina surfaces, as shown in Figure 2.3.

Figure 2.3 (a-d) Scanning Electron Microscope (SEM) images of porous alumina with the nanometer scaled holes. The corresponding diameters of the holes are 85 nm, 180 nm, 290 nm, and 420 nm, respectively. (e) Contact angle of water droplets on the porous alumina surfaces as a function of the hole diameter (Ran et al. 2008).

Although the Wenzel model and the Cassie-Baxter model work well for the spreading on many rough surfaces, there are limitations in their applicability. The roughness factor (r in the Wenzel model) and the ratio of the contact surface (f_C in the Cassie-Baxter model) are global properties which are independent of the places where the droplets are deposited (McHale 2007). Thus, the above two models cannot be directly applied when local properties of the surface have influence on the spreading. In addition, the Wenzel model requires the dimension of the rough structures to be small compared with that of the liquid droplets.

During wetting and spreading, the contact angle measured from the liquid tending to advance is called the advancing contact angle (θ_A) while the contact angle measured from the liquid tending to recede is named the receding contact angle (θ_R). The advancing contact angle, θ_A , is usually larger than or equal to the receding contact angle, θ_R , and the difference between them ($\theta_A - \theta_R$) is called the contact angle hysteresis, as shown in Figure 2.4. The magnitude of the contact angle hysteresis can be as large as a few degrees, or even of the order of several tens of degrees. When a contact angle hysteresis exists, the real contact angle is within the range between the receding contact angle and the advancing contact angle (Drelich 1997). The contact angle hysteresis, as well as an apparent contact angle different from the Young contact angle, can be caused by surface roughness or heterogeneity (De Gennes 1985).

Figure 2.4 (a) Advancing contact angle; (b) receding contact angle; (c) contact angle hysteresis (McHale et al. 2004).

Besides static spreading, many researchers studied the dynamic process of spreading (Zosel 1993; Kumar and Deshpande 2006; Wang et al. 2009). Zosel's work indicated that the first stage of wetting was only determined by the wetting liquid and was independent of the solid surface (Zosel 1993). The influence of the solid surface was more remarkable at a later stage when the dynamic contact angle approached to the equilibrium contact angle than at the initial stage. Their findings imply that more attention should be paid to the late stage and the equilibrium state of spreading when investigating the effect of solid surface properties. Prabhu et al. (2009) experimentally studied the effect of surface roughness on the wetting and spreading of vegetable oils of different viscosities. Their results showed that contact angles changed with surface roughness and the trend was consistent with the prediction of the Wenzel model. The investigations on dynamic spreading showed that the viscosity of the oil had effect on the kinetics of the spreading. Xu et al. (2008) studied the dynamic spreading of silicone oil and characterized the spreading speed on rough steel surfaces. According to their results, a critical roughness exists below which the spreading speed is much higher than that on the surfaces with other roughness values.

Based on the experimental observations of spreading phenomenon, researchers have set up many theoretical models and have carried out numerical simulations on wetting and spreading (Gu and Li 1998; Park et al. 2011). Lunkad et al. (2007) used the volume of fluid (VOF) method to numerically investigate the droplet impact and spreading on both horizontal and inclined surfaces. They compared their simulation results with the experimental work of Sikalo et al. (Sikalo et al. 2005a, b), and showed that the spreading on less-wettable horizontal surfaces was easier to explain than the spreading on inclined surfaces. Their dynamic contact angle model does not work for all the spreading on inclined surfaces, but is always applicable for the spreading on the horizontal surfaces used in their experiments. Although many models have been set up and reported on the spreading, it is difficult to build a universal model for all the spreading phenomena.

Triggered by the nature, extreme wetting behaviors can be achieved by designing appropriate surface patterns. Some plant leaves, such as lotus leaves, have high water repellency effect which makes water droplets roll off the leaves. This natural phenomenon shows an extreme wetting behavior of superhydrophobicity. Superhydrophobic surfaces are those with contact angles larger than 150° and they are very important in the applications of self-cleaning and contamination prevention (Blossey 2003; Li et al. 2007).

The methods to prepare superhydrophobic surfaces can be categorized into two groups: (i) chemical modification and (ii) roughening or patterning of the solid surfaces. With chemical modification, a thin layer of material with super low surface energy is coated on the raw solid surfaces. This approach is relatively easy

to operate; however, it is limited by the availability and the effectiveness of coating materials. For example, the highest contact angle that can be achieved on smooth CF₃-terminated surface was 120° (Nishino et al. 1999; Chattopadhyay et al. 2010). In contrast, higher contact angles, such as 160°, could be achieved on surfaces with micro-/nano-patterns (Feng et al. 2011). The methods of roughening or patterning of the solid surfaces, with the development of micro-/nano-fabrication technologies, enable the fabrication of surface patterns of various shapes (Martines et al. 2005; Xia and Brueck 2008), as shown in Figure 2.5. Therefore, the surface wettability can be flexibly tuned with contact angles in a wide range, and either superhydrophobicity or superhydrophilicity could be realized (Kietzig et al. 2009; Xiao et al. 2009).

Inspired by the natural superhydrophobic surface property of lotus leaves that possess dual scale micro-nanostructures along with a waxy coating (Barthlott and Neinhuis 1997; Neinhuis and Barthlott 1997), hierarchically patterned surfaces were fabricated and the spreading was studied, as schematically shown in Figure 2.6 (Feng et al. 2002; Lin et al. 2011; Liu et al. 2011). In addition, tunable adhesion behavior could be achieved on a hybrid superhydrophobic surface fabricated on a single substrate, as reported by Dawood et al. (2011).

Figure 2.5 (a-b) SEM images of patterned surfaces (Yoshimitsu et al. 2002): (a) unidirectional groove structure and (b) square-shaped pillar like structure. (c-d): SEM images of PDMS trapezoids (Im et al. 2010): (c) cross-sectional view of a single trapezoid and (d) tilted view of a PDMS trapezoids array. (e) SEM image of a microstructured PDMS surface (Dufour et al. 2010).

Figure 2.6 Schematic concept of the roughness hierarchy: (a) intrinsic contact angle, θ_i , formed on a normal flat surface and single microroughness; (b) intrinsic contact angle amplified by nanoroughness and double micro-/nano-roughness (Kwon et al. 2009).

2.1.3 Wetting anisotropy

Existing experimental observations and theoretical predictions show that surface roughness has effect on the wetting and spreading behaviors of liquid droplets. The patterns of the surface structures greatly influence the spreading speed and the equilibrium droplet shape. In this section, wetting anisotropy induced by anisotropic patterns is introduced and many studies on this phenomenon are reviewed.

Compared with the spreading on smooth surfaces, the spreading of droplets on rough surfaces is complex because of contact angle hysteresis (Andrew and Anthony 2006; Herminghaus et al. 2008; Li et al. 2008), and the spreading on anisotropically patterned surfaces is even more complex. On anisotropically patterned surfaces, the spreading speed varies in different directions, leading to a non-spherical shape of the droplet. Another phenomenon of spreading on anisotropically patterned surfaces is pinning of the contact line which is the trap of liquid at discontinuous parts of a surface or at turnings in a channel. On patterned solid surfaces, these discontinuities are corners of a trench, sidewalls of a groove

or a squared pillar, and edges of a hole. Pinning can alter the local wetting properties by tremendously affecting the local contact angles and the shapes of the contact lines (Xia and Brueck 2008).

Sivakumar et al. (Sivakumar et al. 2005; Kannan and Sivakumar 2008a, b) investigated water droplets impact and the subsequent spreading on structured rough surfaces. They fabricated channel like microgrooves and square-shaped asperities on stainless steel plates and studied the effects of asperity height and the influence of Weber number on the spreading of water droplets. Their results revealed (i) solid surface topographies had a significant influence on the spreading pattern and (ii) surface textures governed the degree of hydrophobicity and the spreading process on grooved surfaces. Kusumaatmaja et al. (2008) reported interesting experimental observations on the spreading on anisotropically patterned substrates. Unlike the common phenomenon that liquid droplets elongate in groove direction, the droplets studied by them could elongate in either direction parallel to grooves or direction perpendicular to grooves. The droplet elongated along grooves when the contact line was advancing and it elongated perpendicular to grooves when the contact line was receding. These two behaviors correspond to wetting and de-wetting, respectively. Thus, the final droplet shape strongly depends on the path to achieve it.

To create anisotropic patterns on solid surfaces, many fabrication methods have been employed, such as chemical adsorption of patterned monolayers (Morita et al. 2004), laser interference lithography (Zhao et al. 2007; Wu et al. 2010; Xia et al. 2010), and photolithography (Andrew and Anthony 2006; Wu et al. 2011). Other

than these traditional methods, wrinkling of hard thin films on soft substrates has emerged and been proven to be effective in creating both regular and random patterns, as shown in Figure 2.7. Compared with the traditional methods, wrinkling is easier and more flexible, and requires no sophisticated equipment in the fabrication process.

Figure 2.7 Scanning force microscopy images (scanning area $11 \times 11 \mu\text{m}^2$) of wrinkled patterns prepared by evaporating a thin layer of platinum onto a thick PDMS substrate (Genzer and Groenewold 2006).

Many studies show that spreading on anisotropically patterned surfaces can be greatly enhanced in one direction while inhibited in another direction, enabling the liquid droplet to exhibit hydrophobicity in one direction while hydrophilicity in another direction. Superhydrophobicity can be achieved in a certain direction by designing appropriate pattern shapes and dimensions (Chen et al. 2005; Choi et al. 2006; Nosonovsky and Bhushan 2008). To control the wetting property in a certain direction leads to many applications in different research areas, such as in fluid mechanics and material science.

Theoretical modeling and numerical simulations were performed on wetting anisotropy. The software Surface Evolver has been employed by researchers to

predict droplet shapes in both two-dimensional and three-dimensional studies (Brandon et al. 1997; Brandon et al. 2003; Chen et al. 2005). Molecular dynamics simulation is another useful tool to study micro/nano-scale spreading process (Grest et al. 2006; Yong and Zhang 2009).

Although there have been numerous studies on static spreading and equilibrium contact angles, it is required to investigate the dynamic process of spreading in order to have a deeper understanding of the spreading phenomenon (Wilson et al. 2000). The driving force of spreading and the mechanics behind the wetting phenomenon can be known by studying the dynamic spreading. Though existing studies show that the spreading behaviors of liquid droplets can be tuned throughout the whole process (Kumar and Deshpande 2006; McHale et al. 2009), the evolution of droplet shapes needs to be monitored and the spreading speed should be characterized explicitly.

2.2 Adhesion and spreading of giant liposomes

Viewing the droplet spreading phenomenon from the liquid/vapor/solid system, it is a phenomenon taking place on interfaces. The interfacial tensions play a crucial role in governing the droplet spreading and the shape evolution. It is essentially a behavior of soft matter on solid surfaces. Liposomes, considered as another soft matter, can also exhibit shape transformations on solid surfaces. The difference between liposomes and liquid droplets is that the surface and the inner part of a liposome are of different materials. Aqueous solution is entrapped by lipid bilayer membranes which can exhibit the behaviors of spreading and other shape transformations. From both the perspective of physics and the practical viewpoint,

the spreading of liposomes is of comparable importance with that of liquid droplets.

2.2.1 Preparation of liposomes

Liposomes are artificially made of lipid bilayers (Jesorka and Orwar 2008) (Figure 2.8). They can be classified into three groups according to their size: small liposomes with diameters of several tens of nanometers, large liposomes with diameters of several hundreds of nanometers, and giant liposomes with diameters larger than ten micrometers. According to the number of bilayers, they are known as unilamellar liposomes or multilamellar liposomes which contain one or several bilayers of lipid molecules, respectively. Giant unilamellar liposomes attract much attention in the past decade due to their similar size with real cells and the convenience to visualize them directly under optical microscopes. They can be used to study the membrane curvature and elasticity (Evans and Rawicz 1990), or serve as bioreactors to produce important biomaterials *in vivo* (Tsumoto et al. 2001; Michel et al. 2004).

Several methods have been applied to prepare liposomes, including gentle hydration of lipids (Mueller et al. 1983; Akashi et al. 1996), evaporation of solvents (Moscho et al. 1996), and formation under electric field (Angelova and Dimitrov 1986; Estes and Mayer 2005).

Figure 2.8 Schematic illustration of a phospholipid bilayer and the cross section of a liposome. Various types of liposomes are shown.

Due to the amphiphilic characteristic, phospholipids could form spherical liposomes spontaneously when appropriate aqueous solution is added onto the dry lipids. However, for a long time, only solutions without salts or containing low concentrations of salts can be used for the preparation of giant liposomes. Akashi et al. (1996) reported an improved gentle hydration method to prepare giant liposomes under physiological conditions with relatively high concentrations of

salts. Initially, the lipids were dissolved in organic solvents, such as the mixture of chloroform and methanol. Then the solvents were removed by rotary evaporation. After complete drying under vacuum, aqueous solution containing salts were added to hydrate the lipid film. Giant unilamellar liposomes formed in this process. However, not only were giant unilamellar liposomes produced, small liposomes, multilamellar liposomes and myelin also appeared in the suspension. Evaporation is another method which has been widely adopted by many researchers. For example, Kim et al. (1985) proposed an evaporation method to particularly prepare multilamellar liposomes, and Moscho et al. (1996) reported a rapid evaporation method to produce giant unilamellar liposomes.

Besides the conventional hydration and evaporation methods, Buboltz and Feigenson (1999) developed a rapid solvent exchange method to prepare homogeneous membranes composed of lipid mixtures. In their rapid solvent exchange method, there was no artifactual demixing on the lipid membranes made of the mixture of phospholipids and cholesterol. Wang et al. (2006) adopted a freeze-drying method to produce unilamellar liposomes with the size below 200 nm. The liposomes produced in their method had relatively high encapsulation efficiency, which may be suitable for the delivery of therapeutic agents in biomedical applications. Sugiura et al. (2008) reported a “lipid-coated ice droplet hydration method” to produce homogeneous giant liposomes. The size of the resulted liposomes could be well controlled within the range between 4 μm and 20 μm , but the preparation procedure is complex.

In recent years, novel preparation methods have been proposed for some special purposes, such as to prepare asymmetric liposomes to investigate the biological asymmetric functions of lipid bilayers. In an asymmetric liposome, the inner layer and the outer layer of the lipid membrane are of different compositions (Hamada et al. 2008; Chiantia et al. 2011), as shown in Figure 2.9. Liposomes can also be conjugated with polymers to improve the performance of the resulted materials in biomedical applications (Nam et al. 2010; Quemeneur et al. 2010). Polyelectrolytes like chitosan are often used, which itself is an important biomaterial.

Figure 2.9 Typical fluorescent images of asymmetric liposomes (Hamada et al. 2008). (a) The outer leaflet is tagged with rhodamine-PE which appears red; (b) the inner leaflet is tagged with NBD-PE which looks green; (c) the inner and outer leaflets are simultaneously tagged with rhodamine-PE and NBD-PE, respectively. Cross section profiles of the respective microscopic images are shown at the immediate bottom of the fluorescence images. F.I.=fluorescence intensity.

Among the existing preparation methods, formation under electric field that was first proposed by Angelova and Dimitrov (1986) is often used to prepare giant unilamellar liposomes. Similar to the gentle hydration method, water or aqueous solution is added onto dry lipid films. The difference is that an electric field is

applied simultaneously. In Angelova and Dimitrov's seminal experiments, DC voltage was applied to the parallel cylindrical platinum electrodes where the lipids were deposited. The effects of the number of dried bilayers were studied for two lipid compositions and the optimum thickness of bilayers was found out. Their results show that the formation and the structure of the dry lipid film significantly influence the formation of giant liposomes (Figure 2.10).

After this pioneer work, Dimitrov and Angelova (1987) expanded their research scope to more lipids and started to use AC electric field instead of DC electric field. They identified the effects of the lipid charge and the influence of the electric field frequency on the formation process. They emphasized that bilayer separation and bending were prerequisites for liposome formation via hydration, and that electric field could facilitate these processes. The factors influencing the separation and bending of bilayers would have effect on the formation and the size of liposomes.

Based on the effects of AC electric field, Angelova et al. (1992) proposed a method to prepare fluctuating, isolated giant liposomes for the measurement of membrane mechanical properties. They used several lipid compositions and proved that the method worked well with pure phosphatidylcholines (PC), such as egg PC (EPC) and the mixtures of EPC and cholesterol (up to 50% cholesterol). They produced giant liposomes under electric field from the lipid compositions from which giant liposomes could not form with hydration method, and found that the size of liposomes depended on the amount of EPC.

In most studies of electroformation, neutral lipids, such as PC, are used to produce giant liposomes, and negatively charged lipids are added at a very small amount in some protocols (Estes and Mayer 2005; Rodriguez et al. 2005; Kuribayashi et al. 2006). With the development of liposome preparation technology, especially the electroformation method, liposomes of various compositions can be effectively and efficiently produced for the study of various membrane behaviors.

Figure 2.10 (a) A plane-parallel model of a hydrated lipid layer; (b)-(d) possible mechanisms of liposome formation (Angelova and Dimitrov 1986). h is the intermembrane separation, l is the layer thickness, and C_i and C_o are solute concentrations inside and outside the liposome, respectively.

2.2.2 Shape transformations of liposomes

Cells, the basic constituent unit of most living creatures, function at certain shapes. To study cell shapes and to relate them to biological functions is of potential value. Some biological functions of cells are strongly dependent on the properties of lipid membranes, such as the sensitivity of the shape to the environment (Hotani et al. 1999). An easy and practical way to understand cell behaviors is to study the shape transformations of liposomes, especially giant unilamellar liposomes that often serve as cell models due to their phospholipid composition and comparable cell size (Hotani et al. 1999; Nomura et al. 2003; Shohda and Sugawara 2006). In addition, to study shape transformations of giant liposomes assists in understanding the physics involved in the phenomena. Thus, it is important to study the shape transformations of lipid membranes subjected to different conditions.

Giant liposomes exhibit a spherical shape with aqueous solutions inside and outside of lipid membranes. If the liquids inside and outside of a lipid membrane are different, giant liposomes could undergo morphological transformations through different pathways characterized by liposome shapes (Hotani 1984). Morphological transformations could also be caused by microtubule growth and F-actin growth, which is based on the biological notion that cellular morphology is determined and maintained by cytoskeletal networks of microtubules and F-actins (Hotani et al. 1999).

Liposomes can be deformed by the electric stress imposed on the lipid membrane when they are under an electric field. The type and the degree of liposome

deformation depends on the properties of the aqueous solutions and the electric field (Dimova et al. 2007). If a DC electric field is applied in the presence of salt, liposomes are flattened. The overall shape of giant liposomes is dependent on the ratio between the internal and the external conductivities (Dimova et al. 2007). Under an AC electric field, the influencing factors include the strength and the frequency of the electric field and the conductivities of the aqueous solutions. When the conductivities of the solutions inside and outside of the lipid membrane are different, liposomes exhibit distinct shapes at different frequencies and undergo shape changes with the increase in frequency, as shown in Figure 2.11. When the internal conductivity is higher than the external conductivity, the liposome only exhibits the shape transformation from prolate to sphere with the increase in the frequency of the electric field. When the internal conductivity is lower than the external one, the liposome exhibits the transitions from prolate, oblate, to sphere with increasing the frequency.

Figure 2.11 Two giant liposomes in different conductivity conditions subjected to AC electric field of 0.2 kV/cm and various field frequencies as indicated above the images (Dimova et al. 2007). The liposome radii are 21.5 μm for the liposome in (a-c), and 15.2 μm for the liposome in (d-f).

In addition to simple shape transformations caused by variations in surrounding environments, liposomes can exhibit characteristic behaviors, such as poration, fusion, and budding, which are related to cellular functions.

Under a strong electric pulse, macropores could be formed on the membrane of a giant liposome (Riske and Dimova 2005; Haluska et al. 2006), as shown in Figure 2.12. The pores have the size of 0–5 μm . Because poration happens quite fast, only high speed cameras can capture the phenomenon. Riske and Dimova (2005) recorded the dynamic process at a time resolution of 30 μs , and observed the lifetime of a pore to be as short as 10 ms. The shape of the giant liposome underwent a sphere-prolate-sphere change with the formation of macropores.

Figure 2.12 A snapshot sequence of a liposome subjected to a pulse, $E = 2 \text{ kV/cm}$, $t_p = 200 \text{ } \mu\text{s}$ (Riske and Dimova 2005). The image acquisition rate was 50000 fps. Macropores are first visualized in the third frame ($t = 125 \text{ } \mu\text{s}$). The electrode's polarity is indicated with a plus (+) and a minus (-) sign on the first snapshot.

Without an electric pulse, pores could be formed on mechanical stretched liposomes as reported by Sandre et al. (1999). When a liposome strongly adheres onto a substrate, macropores may appear and lead to liposome spreading and rupturing eventually. In contrast, when a liposome is only weakly stretched or intensely illuminated, transient pores will close again. The main difference of their

work from the others is that glycerol was added to the solution outside of lipid membranes to increase the external liquid viscosity. In such a condition, leaking of the internal liquid was so slow that pores could grow to a large size (5–10 μm). Their experimental observations showed that shape transformations of giant liposomes could be tuned within a wide range by adjusting the external conditions, which would potentially lead to applications based on the membrane shape transformations.

Membrane poration is a necessary step for the liposomes in contact to fuse with each other (Haluska et al. 2006). If a pore does not close, it will eventually lead to fusion of liposomes in contact under appropriate conditions, such as under an electric field. Thus, poration and fusion are often observed consecutively. Fusion can be caused either by chemical specific binding or by external stimulation such as electric pulse. By pre-introducing specific binding sites into liposome membranes, fusion could happen after certain chemicals are added to the contact zone. In the absence of such binding sites, poration and fusion can be stimulated by electric pulse, namely electrofusion. Haluska et al. (2006) provided two protocols based on these two mechanisms and they compared the dynamic process using the two protocols. Their results indicate that after rescaling, fusion neck diameter exhibits a similar dependence on time for ligand-mediated fusion and electrofusion.

Due to the symmetry of a lipid bilayer on a micron-size scale, the spontaneous curvature of giant unilamellar liposomes is close to zero. When amphiphilic molecules or ions adsorb onto or incorporate into the external leaflet of the bilayer,

the spontaneous curvature will change if the membrane chooses to accommodate the foreign molecules (Rumiana et al. 2006). As a result of the increased spontaneous curvature, budding happens with the overall volume and area being conserved. The complete budding event takes place within about 5 s (Figure 2.13).

Figure 2.13 Snapshots of a budding liposome (Rumiana et al. 2006). The liposome has been subjected to a solution of amphiphilic molecules which insert in the external leaflet of the membrane and induce a drastic increase in the spontaneous curvature. The liposome volume is osmotically stabilized and remains constant.

The above mentioned morphological transformations are important in explaining some cellular events, leading to bioengineering applications, and deeply understanding the biophysics behind the phenomena. Liposome fusion could mimic cell fusion which involves many protein reactions *in vivo*. Reactions would happen when two liposomes containing bioactive materials contact and fuse into one bigger liposome. This feature enables liposomes to serve as microreactors (for giant liposomes) or nanoreactors (for small liposomes) to study complex cellular chemistry. Liposomes can be used as targeting drug delivery carriers if liposomes with drug inside could target and adhere onto or fuse with cancer cells. Leak-out of interior materials may appear when a liposome adheres and spreads on a substrate, which is relevant to the transport of cellular metabolic products. Although many

bioengineering applications can be expected and some have already been under study, there is a long way to go and more research work is demanded.

2.2.3 Adhesion and spreading of liposomes on solid surfaces

For a liquid droplet touching a solid surface, spreading happens as long as the surface chemistry and the surface topography are favorable for this process. When replacing the liquid droplet with a droplet-like giant liposome, similar phenomenon can be observed. Liposomes, after touching the solid substrate, will spread on the substrate surface. Studies on adhesion and spreading of liposomes could be employed to understand cell adhesion behavior which is of great significance in cell-extracellular matrix (ECM) interactions. ECM interactions are mainly mediated by ligand-receptor interactions, and the spreading governed by the ligand-receptor interactions is classified as the first type of adhesion (Tordeux et al. 2002). The other type is nonspecific adhesion between the membrane and the substrate controlled by universal interactions, such as Van der Waals force. The latter case is like the spreading of liquid droplets and can be studied by following the same strategy with the spreading of liquid droplets. To study the spreading of liposomes, direct observation approach is demanded due to the small size of liposomes.

Reflection interference contrast microscopy (RICM) technique facilitates both qualitative observation and quantitative measurement of the interactions between the lipid membrane and the substrate during the adhesion and spreading process. The principle of the RICM method is depicted in Figure 2.14. The interference pattern resulted from the lights reflected from the liposome membrane and the

substrate is used to calculate the membrane thickness and its height above the substrate (Feder et al. 1995). This method yields a pattern analogous to Newton rings for a spherical liposome under an RIC microscope.

Figure 2.14 Principle of the RICM technique (Stuart and Hlady 1999). (A) The incident beam is partially reflected at the coverslip-buffer interface, I_{12} , and partially transmitted through the buffer to be reflected by the probe, I_{23} . The constructive or destructive interference pattern formed by the superposition of the object beam, I_{23} , and the reference beam, I_{12} , is observed through the objective as in (B) as circular interference fringes, which are a function of vertical distance between the spherical probe and the surface.

In addition to RICM technique, Bernard's group proposed to apply the evanescent wave-induced fluorescence method (EWIF) to investigate the adhesion of giant liposomes. The setup of EWIF microscope is schematically shown in Figure 2.15.

The evanescent wave excites the fluorescence of a dye only present outside of the liposome, which makes it possible to distinguish between a spherical liposome sitting on the substrate and a flattened liposome (Bernard et al. 1999).

Figure 2.15 Schematic diagram of EWIF microscope (Bernard et al. 1999).

Bernard and co-workers studied the liposome adhesion and spreading on both flat and structured surfaces by using EWIF microscope (Bernard et al. 2000a; Bernard et al. 2000b). They prepared giant liposomes through electroformation method and made the adhesion surfaces on indium tin oxide (ITO)-coated slides covered with a thin layer of gold. The coated slides were used as flat surfaces directly and as structured surfaces by introducing a striped pattern. On both flat and structured surfaces, three regimes were characterized by time and radius of contact area, as shown in Figure 2.16. The liposome was considered to remain spherical at the end of the second regime though the spreading went faster in the direction along stripes than in the direction perpendicular to stripes when on structured surfaces. After the whole spreading process, the liposome exhibited an elongated shape.

In 1990s, the development of vision techniques greatly speeded the investigation of cell or liposome behaviors. In the meanwhile, the appearance and the application of some auxiliary instruments made it possible to manipulate a single cell or a single liposome. They are optical tweezers, micromanipulators, microinjectors and micropipettes (Figure 2.17).

Figure 2.16 Spreading of an EPC liposome on striped patterns ($2.5\ \mu\text{m}$ wide) (Bernard et al. 2000b). (1) Regime I, approaching; (2, 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 liposome shape, the macroscopic pore is closed. (A) Liposome image in phase contrast microscopy; (B) liposome image by EWIF microscopy; (C) liposome profile.

Figure 2.17 I: Schematic representation (top view) of the optimal conditions for microinjection experiments, showing the sector angle (θ) and the direction of microneedle approach (Bucher et al. 1998). Pt: platinum wire; M: microneedle tip. II: Two micropipettes in a chamber (Hochmuth 2000). A pneumatic micromanipulator controls the movement of a micropipette along three orthogonal axes. (a) A spherical cell being aspirated into a micropipette with a suction pressure ΔP ; (b) an attached cell being aspirated into a pipette; (c) a closely fitting cell or bead moving freely in a pipette like a piston in a cylinder. When static, the suction pressure times the cross-sectional area of the pipette equals the attachment force F .

An all-optical method was reported to manipulate and fuse giant liposomes (Kulin et al. 2003), and the micropipette aspiration of living cells was reviewed by Hochmuth (2000). The microinjection techniques were employed to enable giant liposomes to serve as biochemical compartments for chemical and enzymatic reactions (Bucher et al. 1998). Many studies show that micropipette is a useful tool to manipulate a single liposome and can help investigate the mechanical properties of lipid membranes, such as the change of membrane tensions with aspiration pressures and the compressibility modulus (Kwok and Evans 1981). Micropipette

can also serve as a force meter to estimate the adhesion energies (Colbert et al. 2009).

Microplate is another tool to manipulate individual cells or liposomes to study membrane transformations, as shown in Figure 2.18. The compression of cells between two flat surfaces was realized by using microplates, and the adhesion and spreading of cells could be promoted or inhibited by modifying the surface properties of the microplates (Thoumine and Ott 1997; Thoumine et al. 1999). Interactions between liposomes or cells and specific biomolecules can be studied to characterize the cell mechanics (Losey et al. 2009).

Figure 2.18 Diagrams of microplate-based manipulations (Thoumine et al. 1999). Cells are shown in gray. Thick bars represent rigid microplates, and thin bars represent flexible microplates, whose equilibrium positions are shown by the dashed lines.

The advancement of experimental investigations promotes the development of modeling of liposome adhesion and spreading. Gruhn and Lipowsky (2005) reported their Monte Carlo simulation results for adhesion behaviors of liposomes. They took the fluid characteristic of liposomes into account and found the temperature dependence of adhesion behavior in the absence of an osmotic

pressure. Gruhn et al. (2007) obtained the adhesion strength from the equilibrium shape of an adhering liposome with Monte Carlo simulations, with the assumption of the liposome shape to be a spherical cap which was similar to that for liquid droplets on smooth surface.

Capovilla and Guven (2002) studied the liposome adhesion from a geometrical point of view without any assumption of the symmetry of liposomes. They analyzed the discontinuities at the boundary of the contact region as well as the strong bonding limit in which the discontinuities were not smoothed. Their method is of significance in studying the liposomes of non-axially symmetric shapes. Xiao et al. (2004) briefly reviewed the existing theories for describing the geometry of adhering liposomes or cells and proposed their own model by making assumptions based on continuum mechanics which was proven to be applicable to characterize lipid membranes (Mukhopadhyay et al. 2002). Their model involves both membrane and curvature deformations as well as pressure difference. Different from cell membranes, there is no cytoskeleton in liposome membranes, which will lead to zero shear modulus and simplify the modeling work.

2.3 Summary

Spreading of liquid droplets and droplet-like liposomes has been reviewed in this chapter. Some relevant topics, such as wetting and spreading and the preparation of liposomes, have been introduced.

As revealed from the existing work, various phenomena may happen when a liquid droplet impacts a solid substrate, and spreading is one of them. Similar to liquid

droplets, giant liposomes spread and have shape transformations when they are placed onto solid substrates. Surface roughness and pattern structures have been demonstrated to play an important role in modifying and tuning the wetting and spreading of liquid droplets and the adhesion and spreading of giant liposomes. Various experimental studies have been carried out, and theoretical modeling and simulation work have been performed to predict or supplement the experimental results. However, the existing work on droplet spreading is mainly on the influence of solid surface roughness and the static spreading, and most of the studies on liposome spreading focus on the theoretical modeling and numerical simulations. Most solid substrates for the study of droplet spreading are fabricated through complicated and time-consuming methods. Flexible and effective method is demanded for the preparation of reproducible patterned substrates for the study of wetting and spreading phenomenon. To deepen the understanding in the particular interfacial phenomenon, spreading of liquid droplets and giant liposomes, more experimental and theoretical work are needed.

Chapter 3 Spreading of Liquid Droplets on Wrinkled

Substrates: Static State^{*}

3.1 Introduction

To study the spreading on patterned substrates will deepen the understanding in the spreading phenomenon and provide valuable information on the control of wetting and spreading. Among the factors influencing the spreading of a liquid droplet on a solid substrate, surface pattern of the substrate is an important but complex factor that deserves extensive studies. In this study, the static state of the spreading of water droplets is investigated on both smooth and patterned substrates. The patterned substrates are fabricated through wrinkling on shape memory polymers, and wrinkling is demonstrated to be an appropriate method to prepare substrates with isotropic and anisotropic patterns. The effect of surface patterns on the equilibrium shapes of liquid droplets is analyzed.

In the viewpoint of continuum physics, the total energy of a condensed matter could be divided into two parts: the energy associated with volume and the energy associated with surfaces. For a liquid droplet (such as deionized (DI) water used in this experiment), the spreading process is primarily driven by the conversion among surface energies, while the volume energy, such as the gravitational potential energy, is negligible because the initial droplet radii are smaller than the capillary length of DI water (Fan 2006; Xu et al. 2008). Thus, the effect of droplet

^{*}This part of work has been published in Zhang, et al. (2010). *Proceedings of the Institution of Mechanical Engineers, Part C: Journal of Mechanical Engineering Science* **224**(11): 2459-2467.

size on the spreading of droplets on smooth substrates is negligible. However, on patterned substrates, droplet size may influence spreading and its outcomes (Brandon et al. 2003). In this study, the effect of droplet size on the static spreading will be considered.

3.2 Experimental setup and procedure

3.2.1 Substrate preparation and characterization

Three types of substrates, smooth, isotropically patterned, and anisotropically patterned, were prepared for the study of spreading. Shape memory polymer (SMP) was used to fabricate these substrates. Isotropic and anisotropic patterns were realized through wrinkling which had been proven to be an appropriate method in forming highly ordered patterns (Volynskii et al. 2000; Genzer and Groenewold 2006). These two types of substrates are referred to as “isotropically wrinkled substrates” and “anisotropically wrinkled substrates”, respectively.

The SMP used in this study was thermoresponsive polystyrene from Cornerstone Research Group, USA. It was obtained by bulk random copolymerization. The as-received material was in sheet form with a thickness of about 3.5 mm, and no phase separation was found. The glass transition temperature (T_g) of the SMP material was about 65.5°C as provided by the supplier and further verified by differential scanning calorimetry tests (Liu et al. 2008).

To fabricate the substrates for spreading experiments, the SMP samples were cut into rectangular and dumbbell shapes and heated to 150°C before use to remove

any possible residual stress or deformation during polymer processing. Subsequently, the samples were polished gently using Micropolish alumina compound (0.3 μm and 0.05 μm ; Buehler, USA) on DP-Nap cloth (Struers, Denmark). The average surface roughness (R_a) of the resulted samples was measured to be smaller than 20 nm using a PL μ confocal imaging profiler (Sensofar-Tech, Spain).

To prepare smooth substrates, rectangular samples were coated with a thin layer of gold (20–60 nm) using a sputtering coater (SC7640 gold coater, Quorum Technologies, UK). To obtain different coating thicknesses, various coating time was applied. Empirically, the thickness of the gold film was approximately proportional to the coating time.

To prepare isotropically wrinkled substrates, firstly, the rectangular samples were coated with a thin layer of gold (20–60 nm). Secondly, they were heated at 150°C for 20 minutes and then cooled down to room temperature (22°C). After these procedures, isotropic wrinkles were created on the substrate surfaces, as shown in Figure 3.1. By depositing gold films of different thicknesses, isotropically wrinkled substrates with different surface roughness can be prepared.

To prepare anisotropically wrinkled substrates, dumbbell-shaped SMP samples were first stretched to a certain tensile strain (5–8%) in a hot chamber at a temperature a bit higher than its T_g (95–105°C). After extension, the samples were kept clamped on the stretching machine (Instron 5569, USA) to maintain the temporary shape when cooling down to room temperature. The typical loading and

unloading process is shown in Figure 3.2. After being removed from the stretching machine, the samples were coated with thin layers of gold and heated at 120°C for 20 minutes. After cooling down to room temperature, the samples returned to their original lengths, and wrinkles were formed perpendicular to the stretching direction. In such a way, anisotropic wrinkles were realized on the substrate surfaces. Figure 3.3 shows typical images of the anisotropic wrinkles. Anisotropically wrinkled substrates with various surface roughness and wrinkle wavelengths can be obtained by varying the conditions in which the SMP samples were processed, such as the temperature at which the SMP samples were stretched and the thickness of the coating film.

Figure 3.1 Typical images of isotropically wrinkled substrates: (a) two-dimensional surface micrograph, and (b) zoomed three-dimensional topography.

Figure 3.2 Typical stress versus strain relationship in uniaxial stretching of the SMP sample.

Figure 3.3 Typical images of anisotropically wrinkled substrates: (a) two-dimensional surface micrograph, and (b) zoomed three-dimensional topography.

The surface features of the prepared substrates were characterized by a PLμ confocal imaging profiler. Both surface roughness (R_a) and wrinkle wavelength (λ) were measured. For the anisotropically wrinkled substrates, the wrinkle wavelength is the average distance between two neighboring wrinkles. It characterizes the density of the wrinkles on anisotropically wrinkled substrates.

3.2.2 Spreading of liquid droplets and measurement of equilibrium contact angles

DI water was used as the spreading liquid in this study and it was purified by Milli-Q water system (Millipore, USA). To study the wettability of the smooth and wrinkled substrates, water droplets were gently placed onto the substrates using micropipettes, and front view images were taken using an FTA200 setup (First Ten Angstroms, USA) after several minutes when the water droplets reached their equilibrium states. The equilibrium contact angles were measured at four points on each substrate and in two orthogonal directions. As for the smooth and the isotropically wrinkled substrates, as the values obtained from the two directions were similar, the four results were averaged to get the equilibrium contact angle θ_e . As for the anisotropically wrinkled substrates, the two orthogonal directions were perpendicular (x -direction) and parallel (y -direction) to wrinkles, respectively. As shown in Figure 3.4, contact angles θ_{ex} and θ_{ey} were the average values of the two angles measured at the contact points along the x - and y -directions, respectively. Besides, top view images of the droplets were captured by a CCD camera (Leica DFC290, Germany) or a digital camera (Ricoh R6, Japan) to

show the droplet shapes after spreading. The spreading experiments were carried out at the temperature of 24°C and a humidity of 50%.

Figure 3.4 Schematic drawing of droplet shape after spreading on anisotropically wrinkled substrates. The wrinkles are along the y-direction.

To investigate the effect of droplet size on the equilibrium contact angles, water droplets with various volumes were used. The volumes (V) and the corresponding initial droplet radii are 0.5 μl ($R^* = 0.49 \text{ mm}$), 1 μl ($R^* = 0.62 \text{ mm}$), 3 μl ($R^* = 0.89 \text{ mm}$), 6 μl ($R^* = 1.13 \text{ mm}$), 12 μl ($R^* = 1.42 \text{ mm}$) and 20 μl ($R^* = 1.68 \text{ mm}$), respectively, where $R^* = (3V/4\pi)^{1/3}$. The values of R^* are much smaller than the capillary length of DI water, which is $Ca = (3\gamma_{LV}/\rho g)^{1/2} = 4.72 \text{ mm}$, where $\gamma_{LV} = 72.8 \text{ mN/m}$ and $\rho = 1000 \text{ kg/m}^3$ are the surface tension and density of DI water, and $g = 9.8 \text{ m/s}^2$ is the gravitational

acceleration. On every substrate, for each droplet size, four measurements were carried out at different positions, and values were averaged to give the reported results.

It should be noted that the cleanliness of the substrates is of great importance. Before each measurement, the substrate was rinsed with ethanol and followed by DI water. This is to remove possible contaminants on the substrate surface which could alter the local surface properties and consequently influence the measurement of contact angles. In addition, the dryness of the spreading surfaces is vital to the measurement, and thus the surface is blow-dried by compressed air before each measurement.

3.3 Results and discussion

3.3.1 Wrinkles on substrate surfaces

After being heated to a temperature higher than its T_g , molecular chains of a polymer material become flexible and stretch in various directions, causing the SMP sample to expand to a size larger than its original size macroscopically. After being cooled, the chains shrink to their original coil state and the SMP sample return to its original size. Isotropic wrinkles are formed after this process owing to the mismatch of mechanical properties between the soft SMP sample and the hard coating film.

The surface roughness of the three smooth substrates and the two isotropically wrinkled substrates are listed in Table 3.1. Different coating time was applied to

the substrates, and various thicknesses of the coating films were achieved correspondingly. Theoretically, the relationship between the wrinkle wavelength and the coating thickness is shown in Eq. (3.1) (Volynskii et al. 2000; Genzer and Groenewold 2006; Chung et al. 2007).

$$\lambda = 2\pi h_c \left[\frac{(1-\nu_s^2)E_f}{3(1-\nu_f^2)E_s} \right]^{\frac{1}{3}}, \quad (3.1)$$

where h_c is the coating thickness, ν_s and E_s are the Poisson ratio and the elastic modulus of the SMP sample, and ν_f and E_f are the Poisson ratio and the elastic modulus of the coating film. In this study, different coating thicknesses were applied, from 15 nm to 50 nm. The mechanical properties of the gold film are: $\nu_f = 0.42$, $E_f = 78$ GPa (Buch 1999). The mechanical properties of SMP change with temperature. At 80°C, $E_s = 2.5$ MPa, at 100°C, $E_s = 0.26$ MPa, and at 105°C, $E_s = 0.17$ MPa. At the temperatures higher than its T_g , the SMP is regarded as elastic, and $\nu_s = 0.5$. In real fabrications, due to the preciseness constraint of the equipments and the complexity of the process, the resulted wavelength usually deviates from the theoretical value. However, Eq. (3.1) provides a clear indication on how the wavelength changes with the material properties.

To prepare anisotropically wrinkled substrates, the SMP sample is stretched in one direction at a temperature higher than its T_g , and the stretched temporary shape is frozen when cooling down on the clamps. However, the original shape of the sample before stretching has been memorized by the material as the permanent shape. Once the substrate is heated and freed again, the molecular chains become

flexible and intend to return to their original coil state which is entropy high. However, the gold film could not retract itself, but can be wrinkled by the retraction of SMP where it is coated. Therefore, when cooling down, the stretched substrate retracts opposite the extension direction and goes back to its original shape, resulting in parallel wrinkles perpendicular to the stretching direction. The formation of anisotropic wrinkles is due to the mismatch of mechanical properties between the soft SMP sample and the hard coating film.

Table 3.1 Surface properties of the smooth and isotropically wrinkled substrates.

Substrates	Coating time (s)	R_a (μm)	λ (μm)
Smooth-1 (S1)	200	0.016 ± 0.004	-
Smooth-2 (S2)	400	0.018 ± 0.003	-
Smooth-3 (S3)	600	0.016 ± 0.002	-
Isotropic-1 (I1)	120	0.115 ± 0.003	2.79 ± 0.29
Isotropic-2 (I2)	700	0.299 ± 0.023	17.1 ± 1.79

To prepare anisotropically wrinkled substrates with various wrinkle profiles, parameters were varied as described in Section 3.2.1. Similar to the observation of the isotropically wrinkled substrates, the wavelength of the wrinkles on the anisotropically wrinkled substrates increases with the coating thickness. The measuring results show that the temperature at which the SMP sample is stretched also has influence on the wrinkle wavelength. This can be explained by the change of the elastic modulus of SMP with the stretching temperature. The decrease in the modulus of SMP with the increase in temperature leads to increased wavelength according to Eq. (3.1). In addition, the residual strain after releasing the tensile

stress takes effect in tuning the wrinkle wavelength. Surface properties of the three anisotropically wrinkled substrates used for the droplet spreading experiments are listed in Table 3.2.

Table 3.2 Surface roughness and wrinkle wavelength of the anisotropically wrinkled substrates.

Substrates	R_a (μm)	λ (μm)
Anisotropic-1 (A1)	0.113 ± 0.007	4.09 ± 0.24
Anisotropic-2 (A2)	0.253 ± 0.003	7.04 ± 0.61
Anisotropic-3 (A3)	0.292 ± 0.016	12.5 ± 1.07

Since the polymer material used to prepare solid substrates is a shape memory polymer and the coating film is as thin as several tens of nanometers, the SMP samples can be reused after removing the patterned layer through polishing and retaining their original shapes through heat treatment. Because the pattern profiles could be controlled by quantitatively adjusting the preparation conditions, the resulted substrates are reproducible and reusable, which is favorable in the investigation of spreading phenomenon.

3.3.2 Spreading on smooth substrates

Measured equilibrium contact angles of water droplets with six different sizes on the three smooth substrates are summarized in Figure 3.5(a). The results indicate that droplet size has no effect on the equilibrium contact angles when spreading on the smooth substrates. The results of the equilibrium contact angles are comparable with those reported by Cognard (1984). In addition, it shows that there is no

difference in the equilibrium contact angles on the substrates with different coating thicknesses. This implies that the effect of coating thickness should be negligible when studying the equilibrium contact angles on the isotropically or anisotropically wrinkled substrates.

Figure 3.5 Equilibrium contact angles measured on (a) smooth and (b) isotropically wrinkled substrates. The value for the horizontal line in (b) is 81.2° , obtained by averaging all the contact angles measured on the smooth substrates. The lines connecting data points are used to guide readings only.

The top-view microscopic image in Figure 3.6(a) shows that the three-phase contact line on the smooth substrates is circular while the front-view image in Figure 3.6(b) shows that the cross-sectional profile is part of a circle. These observations indicate that droplets on smooth substrates keep the shape of a spherical cap, and confirm that the gravity is negligible when considering the spreading of liquid droplets with initial droplet radii smaller than the capillary length.

3.3.3 Spreading on isotropically wrinkled substrates

The equilibrium contact angles on the isotropically wrinkled substrates were measured and compared with those on the smooth substrates. Figure 3.5(b) shows that the equilibrium contact angles are similar to those measured on the smooth substrates while there is no obvious effect of surface roughness on the equilibrium contact angles. This observation can be explained by examining the roughness factor of the isotropically wrinkled substrates used in this study.

The Wenzel model (Eq. (2.3)) is often adopted to estimate the contact angles on rough surfaces. For the isotropically wrinkled substrates used in this study, the three-dimensional topography (as shown in Figure 3.1) was obtained by confocal imaging profiler, and the data were used to calculate the true solid surface area and the planar area. The roughness factor, r , was determined to be 1.013 and 1.009 for the substrates I1 and I2, respectively. The small r value is attributed to the low aspect ratio of the wrinkled surfaces. When a water droplet was placed onto an isotropically wrinkled substrate, the radius of the droplet-covered region was in millimeter scale while the depth of the wrinkle grooves was only in submicrometer

scale. Thus, surfaces with shallow wrinkles only have a small roughness factor, r . It is reasonable that such a small r leads to little effect of surface roughness as found from the experimental results shown in Figure 3.5. To obtain appropriate substrates to study droplet spreading macroscopically, both the roughness and the aspect ratio of the patterns should guarantee a large roughness factor. Regarding the droplet shape, Figure 3.6(c-d) show that droplets on the isotropically wrinkled substrates exhibit the shape of a spherical cap as well. The wrinkles in random directions have no influence on the droplet shape.

Figure 3.6 Top-view microscopic images of water droplets on (a) smooth and (c) isotropically wrinkled substrates. Front-view images showing the middle cross sections of the droplets on (b) smooth and (d) isotropically wrinkled substrates. The volume of the droplet is 3 μl , and the substrates are S2 in (a, b) and I1 in (c, d), respectively.

3.3.4 Spreading on anisotropically wrinkled substrates

When a liquid droplet was placed on an anisotropically wrinkled substrate, the equilibrium contact angles were different in the directions parallel and perpendicular to wrinkles. This is due to the anisotropy of the solid-liquid interfacial tension. The equilibrium contact angles measured in the direction parallel to wrinkles (θ_{ey}) were smaller than those measured in the direction perpendicular to wrinkles (θ_{ex}), as shown in Figure 3.7. This is true for all the three anisotropically wrinkled substrates. Unlike on the smooth and isotropically wrinkled substrates, droplet size has effect on the equilibrium contact angles: in both x - and y -directions, the equilibrium contact angles increase with increasing the droplet size. However, this size effect is remarkable only when the droplets are small, as shown in Figure 3.7. When the droplet size is larger than 6 μl , the equilibrium contact angles do not change significantly with the droplet size.

Two measures, contact angle difference ($\Delta\theta_e = \theta_{ex} - \theta_{ey}$) and the droplet elongation (e), are used to quantify the wetting anisotropy. The experimental observations show that the droplets on anisotropically wrinkled substrates exhibit a non-circular contact line. The droplets elongate along wrinkles, and the droplet elongation is quantified as $e = L_{ey}/L_{ex}$, where L_{ey} and L_{ex} are the contact radii in the directions parallel and perpendicular to wrinkles, respectively. $\Delta\theta_e$ and e for the three anisotropically wrinkled substrates at various droplet sizes are compared in Figure 3.8. A larger $\Delta\theta_e$ or e represents a more remarkable anisotropy effect. The elongation e decreases with increasing the droplet size. It reveals that pattern anisotropy has a larger effect on the shape of smaller droplets than on that of

larger droplets. When the droplet volume is as large as 20 μl , the elongation is the least noticeable. Similar results were obtained by Bliznyuk et al. (2009) when chemically stripe-patterned substrates were used in their investigation. Consistent with the trend in equilibrium contact angles, the size effect is only obvious for small droplets.

Figure 3.7 Equilibrium contact angles measured on (a) A1, (b) A2, and (c) A3. (d) Comparison among the three substrates. The solid lines guide the contact angle θ_{ex} and the dashed lines guide the contact angle θ_{ey} .

Figure 3.8 (a) Equilibrium contact angle difference and (b) droplet elongation after spreading on anisotropically wrinkled substrates.

The equilibrium contact angles on the three anisotropically wrinkled substrates are different. Figure 3.7 shows that the two equilibrium contact angles, measured in the direction parallel to wrinkles (θ_{ey}) and in the direction perpendicular to wrinkles (θ_{ex}), increase a bit with increasing the wrinkle wavelength.

The above observations should be attributed to the wetting anisotropy, but not the surface roughness effect. Considering the Wenzel model (Eq. (2.3)), the roughness factor of the three anisotropically wrinkled substrates was calculated to be in the

range of 1.02–1.05, which would result in an apparent contact angle around 81° , and such a small roughness factor would cause little effect on the spreading on anisotropically wrinkled substrates. However, the contact angles measured in the experiments deviate from this prediction, and the spreading behaviors are remarkably different from those on the smooth substrates. Thus, the Wenzel model is not applicable in the present anisotropic spreading phenomenon. If applying the Cassie-Baxter model, apparent contact angle as large as 170° could be expected when the droplets are on the anisotropically wrinkled substrates with air being trapped between wrinkles. However, even for the largest droplet on the roughest substrate, the measured contact angle does not exceed 100° , which implies that the Cassie state is not the dominating mode in this wetting phenomenon. In addition, pinning of the contact line by the wrinkles was noticed from the experimental observations, as shown in Figure 3.9, which implies a local effect of the surface patterns. Thus, the inapplicability of the above-mentioned global models becomes reasonable. An obvious transition could be noticed between the edge along wrinkles and that against wrinkles, as shown in Figure 3.9. However, not all the droplets of various sizes are pinned to the same extent. The pinning becomes less noticeable with increasing the droplet size.

Figure 3.9 Typical microscopic images highlighting the droplet edges pinned by wrinkles on the substrate A2. The volumes of the droplets are (a) 0.5 μl , (b) 1 μl , (c) 3 μl , (d) 6 μl , (e) 12 μl , and (f) 20 μl , respectively.

3.4 Summary

Wrinkling has been proven to be an effective method to fabricate isotropically and anisotropically patterned substrates. With this method, the dimensions of wrinkles can be tuned by adjusting preparation parameters, such as the coating thickness and the temperature at which the SMP samples are stretched.

The static state of spreading has been characterized by the equilibrium contact angles and the equilibrium droplet dimensions. The effects of the solid surface patterns and the liquid droplet size on the equilibrium contact angles and droplet dimensions have been investigated. Surface roughness almost has no influence on the equilibrium contact angles when the surface roughness, R_a , is smaller than 0.3 μm and the roughness factor, r , is lower than 1.2 in this study. Droplets exhibit the shape of a spherical cap when spreading on the smooth and isotropically wrinkled substrates. Wetting anisotropy appears when spreading on the anisotropically wrinkled substrates: (i) the equilibrium contact angles measured in the direction parallel to wrinkles are smaller than those measured in the direction perpendicular to wrinkles for the same droplet size; (ii) droplets elongate along wrinkles, showing a non-circular contact line. On the anisotropically wrinkled substrates, droplets could be pinned by wrinkles when spreading in the direction perpendicular to wrinkles. The pinning is less obvious when the droplet is as large as 20 μl . Limited effect of droplet size is observed when spreading on the anisotropically wrinkled substrates. The change of the equilibrium contact angles is significant when the droplet size increases from 1 μl to 6 μl , but insignificant for the medium-sized and large droplets (6 μl to 20 μl). In addition, wrinkle wavelength has influence on the spreading. With the increase in wrinkle wavelength, the equilibrium contact angles become larger, and the droplet elongation increases.

It could be concluded from the study of static spreading of water droplets on wrinkled substrates that spreading behavior could be flexibly tuned by modifying the solid surface properties.

Chapter 4 Spreading of Liquid Droplets on

Anisotropically Wrinkled Substrates: Dynamic Spreading

4.1 Introduction

Most of the studies of the spreading of liquid droplets focus on the static state and only equilibrium contact angles are reported. The wetting and spreading phenomenon can be influenced throughout the whole process by selecting different liquids or choosing various solid surface properties (Kumar and Deshpande 2006). In this chapter, the dynamic spreading of liquid droplets is studied experimentally and numerically on smooth and anisotropically wrinkled substrates. Five liquids with different surface tensions and viscosities are used. A thermodynamic model is adopted to analyze the dynamic spreading process. Comparison between the simulation results and the experimental results of the contact radius gives the mobility of the contact line, which characterizes the spreading speed. The values of the mobility are compared between the direction parallel to wrinkles and the direction perpendicular to wrinkles. The wetting anisotropy is quantified. The mobility is compared among different liquids, and the dependence of the mobility on the liquid viscosity is studied. With a nonlinear relationship between the spreading velocity and the driving force, the curve of the dynamic contact radius is fitted by the simulation curve. A system constant is determined from curve fitting, and its dependence on the liquid viscosity and surface tension is studied.

4.2 Experimental setup and procedures

4.2.1 Materials

The dynamic spreading of liquid droplets was studied with five liquids of different properties in the liquid-vapor surface tension (γ_{LV}) or the viscosity (μ), whose properties are tabulated in Table 4.1. DI water was purified by Milli-Q water system. Sodium dodecyl sulfate (SDS) and glycerol were purchased from Sigma-Aldrich (USA). DI water with surfactant at two concentrations (critical micelle concentration (CMC) and 10%CMC) were obtained by dissolving SDS powder in DI water and referred to as “SDS (CMC)” and “SDS (10%CMC)”, respectively. The water-glycerol mixture was prepared at the volume ratio of 1 to 1 and referred to as “water-glycerol mixture”. In Table 4.1, DI water, SDS (10% CMC) and SDS (CMC) have different surface tensions but almost identical viscosities, while DI water, water-glycerol mixture and glycerol have different viscosities, but their surface tensions are similar.

The volume of the droplets was in the range between 5 μl and 9 μl . No droplet was of extremely small (1 or 2 μl) or large (more than 20 μl) size. The effect of droplet size is negligible when the volume is in the range of 5–9 μl , as discussed in Chapter 3. The initial droplet radii are much smaller than their capillary lengths, as listed in Table 4.1 .

Table 4.1 Properties of the liquids used for spreading experiments

Properties	Density	Viscosity	Surface tension	Capillary length
	ρ (kg/m ³)	μ (mN•s/m ²)	γ_{LV} (mN/m)	Ca (mm)
SDS (CMC)	999.75 [†]	0.975 [†]	33.0 [‡]	3.18
SDS (10%CMC)	998 [†]	0.959 [†]	53.8 [‡]	4.06
DI water	1000 ^{\$}	1.005 ^{\$}	72.8 [‡]	4.72
Water-glycerol	1127 ^{\$}	6.05 ^{\$}	66.9 [#]	4.26
Glycerol	1262 ^{\$}	1499 ^{\$}	63.5 [#]	3.93

[†]The values are estimated from figures reported by Poskanzer and Goodrich (1975).

[‡]The values are cited from Park et al. (1994).

^{\$}The values are cited from Sheely's work (Sheely 1932).

[#]The values are cited from Yang and Leong (2002).

4.2.2 Fabrication of the anisotropically wrinkled substrates

The anisotropically patterned substrates used in this work were fabricated through wrinkling of soft matter coated with hard thin film as introduced in Section 3.2.1 (page 40). They are the substrates A1, A2, and A3 whose surface features, R_a and λ , are listed in Table 3.2 (page 49). A smooth substrate with the same material composition was also used in this work for comparison purpose. It is the substrate S2 whose surface features are listed in Table 3.1 (page 47). The details of the fabrication process can be found in Section 3.2.1 (page 40).

4.2.3 Dynamic spreading of liquid droplets

The experimental setup is schematically shown in Figure 4.1. The dynamic spreading process of liquid droplets on anisotropically wrinkled substrates was recorded by an FTA200 system. Briefly, a liquid droplet was slowly pumped out from a plastic syringe through a blunt-ended stainless steel needle (Precision Stainless Steel Tips #27, Nordson EFD, USA). The droplet grew in size until it detached from the needle and fell onto the substrate. The syringe was clamped at a height that the droplet would not touch the substrate before it detached from the needle and the traveling distance of the droplet was small enough to minimize the potential influence of the impact velocity. The shape evolution of the droplet was recorded at frame rates of 25 frames per second (fps) at the initial stage of spreading and 8 fps at the late stage. The total recording time was about 100 s which was sufficiently long for the droplet to reach its equilibrium state. The spreading process was recorded from the directions parallel and perpendicular to wrinkles, separately, and the measurement was repeated for four times in either direction on each substrate. The recorded images were processed using a customized MATLAB program. Droplet dimensions, including the contact radii (L_x and L_y) and the droplet height (h), and the contact angles (θ_x and θ_y) were obtained.

The dynamic spreading was carried out at the temperature of 24°C and a humidity of 50%. The cleanliness of the substrates is crucial for spreading experiments. Contaminants on the substrate surfaces will alter the local surface properties and consequently influence the measurement of contact angles. Thus, before each measurement, the substrate was rinsed with ethanol and DI water in sequence.

Then the surface was blow-dried by compressed air since the dryness of the substrate surface was also very important for spreading experiments.

Figure 4.1 Schematic diagram of the spreading setup for liquid droplet spreading experiments. The inset schematically shows the droplet shape on anisotropically wrinkled substrates from the top view.

4.3 Experimental results

4.3.1 Equilibrium contact angles and wettability

The experimental observations show that the droplet would finally reach an equilibrium state after changing its contact angles and droplet dimensions with time. The equilibrium contact angles of the five liquids on both smooth and anisotropically wrinkled substrates are summarized in Table 4.2.

Table 4.2 Measured equilibrium contact angles (in degree): θ_{ex} and θ_{ey} on the anisotropically wrinkled substrates and θ_{e} on the smooth substrate.

		SDS(CMC)	SDS(10%C MC)	DI water	Water- glycerol	Glycerol
A1 [†]	θ_{ex}	47.4±0.8	71.4±0.3	80.0±2.4	67.3±0.4	64.3±0.8
	θ_{ey}	40.1±1.6	66.5±1.0	73.2±2.0	61.9±4.0	62.5±0.5
A2	θ_{ex}	51.7±0.9	75.9±1.2	89.4±1.6	68.7±0.9	70.0±1.0
	θ_{ey}	49.9±2.0	75.0±1.4	87.5±1.2	69.3±1.1	69.0±0.3
A3	θ_{ex}	55.2±3.1	75.4±1.2	91.1±2.4	75.9±0.8	77.6±1.9
	θ_{ey}	43.5±2.5	65.5±2.1	85.9±0.6	65.1±1.1	69.2±0.6
S2	θ_{e}	45.2±0.4	71.9±0.4	82.1±0.5	66.7±0.9	66.0±0.8

[†]The substrates A1, A2, A3, and S2 refer to the corresponding substrates listed in Table 3.1 and Table 3.2.

Recalling the definition of wetting states, when $0^\circ < \theta_Y < 180^\circ$, the liquid partially wets the solid surface. The equilibrium contact angles of the five liquids on the smooth substrate are within the range of $40^\circ < \theta_Y < 85^\circ$, showing that all the liquids could partially wet the smooth substrate with a gold film on the surface. In addition, the smooth substrate shows a hydrophilic wetting property to the liquids. The equilibrium contact angles on the anisotropically wrinkled substrates are larger than 40° and smaller than 100° , and no extreme wetting state is observed. Although the equilibrium contact angles on the anisotropically wrinkled substrates deviate

from the values on the smooth substrate due to the wrinkles, the hydrophilicity is maintained except for DI water on A2 and A3. The equilibrium contact angles of DI water in the direction perpendicular to wrinkles are around 90° on A2 and A3.

The comparison of the equilibrium contact angles among DI water, SDS (10%CMC), and SDS (CMC) on the smooth substrate shows that adding the surfactant SDS into DI water could effectively decrease the equilibrium contact angles, which shows the improvement of the wettability of the solid substrate. This improvement is owing to the fact that surfactant molecules on the droplet surface could lower the surface tension of the liquid. This is also true for the equilibrium contact angles on the anisotropically wrinkled substrates, measured in the directions parallel and perpendicular to wrinkles, as shown in Figure 4.2.

Figure 4.2 Equilibrium contact angles of SDS (CMC), SDS (10%CMC), and DI water on the smooth substrate (θ_e) and on the anisotropically wrinkled substrate A1 (θ_{ex} and θ_{ey}).

High surface tension of a liquid leads to a large equilibrium contact angle.

Comparing the equilibrium contact angles among DI water, the water-glycerol mixture, and glycerol, it shows that on the same substrate, the equilibrium contact angles of the latter two liquids are smaller than that of DI water, which should be attributed to the lower surface tension of glycerol and the water-glycerol mixture. The equilibrium contact angles of the water-glycerol mixture and glycerol are similar on the smooth substrate and on the anisotropically wrinkled substrates because the surface tensions of the water-glycerol mixture and glycerol are similar.

4.3.2 Droplet elongation and wetting anisotropy

When a liquid droplet spreads on an anisotropically wrinkled substrate, the droplet evolution has similar trend in different directions. However, the spreading speed should vary in different directions as evidenced by the non-circular contact line. As observed from the experimental results, the contact radius in the direction parallel to wrinkles is larger compared with that in the direction perpendicular to wrinkles, which is attributed to the higher spreading speed in the parallel direction. One measure of the wetting anisotropy, the droplet elongation ($e = L_{ey}/L_{ex}$), is used to characterize the deviation of the contact line shape from a circle. Because the experimental measurements in the two orthogonal directions are carried out with different droplets in this study, the e value was calculated from the dimensionless equilibrium contact radii, L_{ex}/R_x^* and L_{ey}/R_y^* , where $R_x^* = (3V_x/4\pi)^{1/3}$, $R_y^* = (3V_y/4\pi)^{1/3}$, and V_x and V_y are droplet volumes. The droplet elongation for the five liquids on the three anisotropically wrinkled substrates are reported in Table 4.3.

The SDS (CMC) droplets are more elongated by the anisotropic wrinkles on the substrate A3 as compared with DI water droplets. This difference can be explained by the diffusion of surfactant molecules during spreading process. Surfactant molecules diffuse along the slope of wrinkles when spreading in the direction perpendicular to wrinkles, and the macroscopic diffusion rate is low, leading to a much slower spreading in this direction than in the direction parallel to wrinkles (Porter 1994). Thus, the droplet elongation of SDS (CMC) is higher than that of DI water.

Table 4.3 Droplet elongation, e , on the anisotropically wrinkled substrates.

	SDS (CMC)	SDS (10%CMC)	DI water	Water- glycerol	Glycerol
A1	1.048	1.046	1.028	1.005	1.048
A2	0.989	1.064	1.040	1.105	0.916
A3	1.133	1.067	1.065	1.159	1.115

As for glycerol and the water-glycerol mixture, the droplet spreading in the direction perpendicular to wrinkles is likely to be pinned by the wrinkles due to their high viscosities. Consequently, the droplet elongation for glycerol and the water-glycerol mixture is much higher than that of DI water on A3.

Figure 4.3 Variation of equilibrium contact angle and droplet elongation with wrinkle wavelength. (a) The equilibrium contact angles (θ_{ex} and θ_{ey}) increase with increasing the wavelength. θ_{ex} : solid lines; θ_{ey} : dashed lines. (b) The droplet elongation increases with increasing the wavelength.

The droplet elongation on A2 and A3 for most liquids used in this study is larger than that on A1, as shown in Figure 4.3. This is because wider wrinkles could facilitate the spreading along wrinkles while hinder the spreading across the wrinkles. The liquid droplet needs to overcome the obstacle of wrinkle edges when it spreads in the direction perpendicular to wrinkles. Spreading over the wrinkles

slows down the whole spreading process in the x direction. When the wrinkle wavelength is larger, it takes more time for the droplet to cross a single wrinkle, and the droplet is more likely to stop its spreading between two wrinkles, leading to a much smaller contact radius macroscopically in this direction, thus a higher value of droplet elongation.

The contact angle difference between the directions parallel and perpendicular to wrinkles is another measure of wetting anisotropy. As shown in Table 4.2 and Figure 4.3, due to the higher γ_{SLx} in the direction perpendicular to wrinkles, the equilibrium contact angles in this direction are larger than those in the direction parallel to wrinkles. Besides, the difference is bigger on A3 than on A1 due to the magnitude of wrinkle wavelength.

4.3.3 Dynamic spreading

The dynamic spreading of a liquid droplet on a solid substrate is characterized by the change of contact angles and the evolution of droplet dimensions. Figure 4.4 and Figure 4.5 show that for all the five liquids on both smooth and anisotropically wrinkled substrates, the contact angles experience a sharp decrease in the initial period, and further decrease slowly until they gain their equilibrium values. During the dynamic spreading of liquid droplets, the driving force at a point along the contact line comes from the contact angle difference between the instant value and the equilibrium value. In the initial period, the instant contact angle ($\theta(t)$) is large, and the difference between $\cos \theta(t)$ and $\cos \theta_e$ is large, which leads to a large driving force, thus a high speed of the contact line. The movement of the contact

line results in the evolution of the droplet shape. When the contact line moves fast, the droplet shape will evolve fast, the contact angle at the three phase contact line will change sharply. When the instant contact angle gets smaller with time, the difference between $\cos \theta(t)$ and $\cos \theta_e$ becomes smaller, and the driving force is smaller, leading to a lower speed of the contact line. Consequently, the change of the instant contact angle becomes slow.

Figure 4.4 Dynamic evolution of contact angles measured on the smooth substrate. At the same capturing speed, the first contact angle that could be measured experimentally from glycerol is much larger because of its a lot higher viscosity than the other liquids.

Figure 4.5 Dynamic evolution of contact angles measured on the anisotropically wrinkled substrate A1. (a) θ_x ; (b) θ_y .

As observed from Figure 4.4 and Figure 4.5, the change of the contact angles of SDS (CMC) is more moderate than that of the contact angle of DI water due to the smaller surface tension of SDS (CMC). On anisotropically wrinkled substrates, the contact angles of DI water in both directions parallel and perpendicular to wrinkles decrease rapidly in the short initial period. After that, they further reduce gradually during about 20 s until they attain their equilibrium values. In contrast, the evolution of the contact angles of SDS (CMC) is not as steep as that of DI water.

They slowly decrease while reducing their change rate with time. The spreading process lasts about 80 s, which is much longer than that of DI water.

Figure 4.6 Dynamic evolution of droplet shapes on the smooth substrate, represented by the contact radius L (mm) and typical images.

Figure 4.7 Shape evolution of liquid droplets on the substrate A3. The instant that the droplet touches the substrate is set as $t=0$ s. (a), (c), (e), (g) and (i): spreading in the x direction. (b), (d), (f), (h) and (j): spreading in the y direction.

The change of the contact angles of glycerol is not as steep as that of the contact angles of DI water. In addition, the change of the contact angles of glycerol could be experimentally captured from much higher values (larger than 90°) compared with the other liquids, as shown in Figure 4.4 and Figure 4.5. These observations are due to the high viscosity of glycerol. After touching the solid substrates, glycerol droplets start to spread at a lower speed than DI water.

To monitor the shape evolution of the droplets on the smooth and anisotropically wrinkled substrates, the contact radii are measured on the images captured by the system schematically shown in Figure 4.1. For the droplets on the anisotropically wrinkled substrates, the contact radii are measured from the x and y directions separately. Typical curves and images showing the evolution of droplet shapes are shown in Figure 4.6 and Figure 4.7. Similar with the observations of Kusumaatmaja et al. (2008), the droplets elongated along wrinkles when the contact line was advancing, leading to a non-spherical shape.

4.4 Non-equilibrium thermodynamics formulation and numerical simulation of liquid droplet spreading on solid substrates

4.4.1 Non-equilibrium thermodynamics formulation

In this study, the analysis of the spreading of liquid droplets on anisotropically wrinkled substrates is within the scope of continuum physics. It is known that for a condensed matter of sub-millimeter scaled size, the surface associated energy dominates the spreading process, and the gravity effect is negligible (Xu et al. 2008).

Considering the spreading of a liquid droplet on an anisotropically wrinkled substrate, the liquid droplet, the solid substrate and the vapor surrounding the droplet constitute the liquid/vapor/solid system, as shown in Figure 4.8. The solid substrate does not react with the liquid or dissolve it. Due to the wrinkles, the interfacial tension between the solid and the liquid varies along the contact line, which will result in different equilibrium contact angles at different contact points along the contact line.

Figure 4.8 Illustration of the droplet dimensions and the interfacial tensions in the liquid/vapor/solid system.

The liquid droplet will spread in all directions and the spreading speed may be different in each direction. The spreading in directions parallel and perpendicular to wrinkles is used to characterize the spreading process. As shown in Figure 4.8, the driving force of the contact line at the contact point with the contact radius perpendicular to wrinkles at the instant t is

$$f_{Lx}(t) = \gamma_{SV} - \gamma_{SLx} - \gamma_{LV} \cos \theta_x(t), \quad (4.1)$$

while the driving force of the contact line at the contact point with the contact radius along wrinkles at the instant t is

$$f_{Ly}(t) = \gamma_{SV} - \gamma_{SLy} - \gamma_{LV} \cos \theta_y(t), \quad (4.2)$$

where γ_{SV} and γ_{LV} are the solid/vapor and liquid/vapor interfacial tensions, γ_{SLx} and γ_{SLy} are the solid/liquid interfacial tensions at the contact points with contact radii perpendicular and parallel to wrinkles, respectively, and θ_x and θ_y are the contact angles that the liquid droplet forms with the solid substrate at the contact points with the contact radii perpendicular and parallel to wrinkles at the instant t . According to the directions of the interfacial tensions and the droplet spreading direction, the driving forces, f_{Lx} and f_{Ly} , are positive when driving the liquid droplet to spread over the solid surface.

Let v_L be the velocity of the contact line. Under the thermodynamics scope, the velocity is taken to be a function of the driving force (Gao et al. 2000), such as

$$v_L = M_L f_L, \quad (4.3)$$

where M_L is called the mobility of the contact line and used as the phenomenological parameter of the system. Equation (4.3) is the kinetic law for

the contact line. It is linear and local, i.e., the velocity at a point only depends on the force at this point.

Regarding the spreading on the anisotropically wrinkled substrates, the driving forces are different in the directions parallel and perpendicular to wrinkles. Thus, the velocities in the two directions should be different correspondingly. They can be written as

$$v_{Lx}(t) = M_{Lx} f_{Lx}(t) = M_{Lx} (\gamma_{SV} - \gamma_{SLx} - \gamma_{LV} \cos \theta_x(t)), \quad (4.4)$$

and

$$v_{Ly}(t) = M_{Ly} f_{Ly}(t) = M_{Ly} (\gamma_{SV} - \gamma_{SLy} - \gamma_{LV} \cos \theta_y(t)), \quad (4.5)$$

which govern the motion of the contact line during the droplet evolution.

Although solid/liquid interfacial tensions are different in the directions parallel and perpendicular to wrinkles, their values should be constant during the spreading process. This is because the wrinkles form periodically in microscopic size and the space between any two neighboring wrinkles are averagely the same while the spreading of liquid droplets are studied macroscopically in the viewpoint of continuum physics. With the above-mentioned driving forces, the droplet shape develops with the dynamic contact angles decreasing with time. Consequently, the driving forces in both orthogonal directions decrease. When the contact angles equal to the equilibrium contact angles in the two directions, the driving forces decrease to zero and the velocities become zero as well. As a result, the droplet stops its spreading and reaches the equilibrium state.

By setting f_{Lx} and f_{Ly} to be zero in equations (4.1) and (4.2), the equilibrium conditions for the contact line could be obtained as

$$\gamma_{SV} - \gamma_{SLx} - \gamma_{LV} \cos \theta_{ex} = 0, \quad (4.6)$$

and

$$\gamma_{SV} - \gamma_{SLy} - \gamma_{LV} \cos \theta_{ey} = 0. \quad (4.7)$$

Equations (4.6) and (4.7) describe the force balance at the contact points with the contact radii perpendicular and parallel to wrinkles, respectively.

4.4.2 Numerical simulation

To demonstrate the feasibility of the model, numerical simulations were carried out. Because the spreading is recorded in the directions parallel and perpendicular to wrinkles separately from two individual droplets due to the lack of three-dimensional imaging system, the evolution of the droplet radii is considered from the two orthogonal directions and the spreading speed is characterized in the two directions, separately. The three dimensional illustration of the droplet and the views from the front and right sides of the droplet are shown in Figure 4.8.

Let δL be the motion of the contact line. From the model proposed in Section 4.4.1, the increase in the contact radii at the contact points with the contact radii perpendicular and parallel to wrinkles are given by

$$\delta L_x = M_{Lx} (\gamma_{SV} - \gamma_{SLx} - \gamma_{LV} \cos \theta_x) \delta t, \quad (4.8)$$

and

$$\delta L_y = M_{Ly} (\gamma_{SV} - \gamma_{SLy} - \gamma_{LV} \cos \theta_y) \delta t. \quad (4.9)$$

Combining with equations (4.6) and (4.7), the above equations can be re-written as

$$\delta L_x = M_{Lx} \gamma_{LV} (\cos \theta_{ex} - \cos \theta_x) \delta t, \quad (4.10)$$

and

$$\delta L_y = M_{Ly} \gamma_{LV} (\cos \theta_{ey} - \cos \theta_y) \delta t. \quad (4.11)$$

The numerical simulations of equations (4.10) and (4.11) were carried out through iteration by pre-assuming the values of the contact line mobility. Taking the simulation for the contact radius in the x direction as an example, if the contact radius of the droplet at the i th state, t_i , is known and an increment of the time is set as Δt_{i+1} , the contact radius change is given by

$$\Delta(L_x)_{i+1} = M_{Lx} \gamma_{LV} (\cos \theta_{ex} - \cos(\theta_x)_i) \Delta t_{i+1}, \quad (4.12)$$

where $(\theta_x)_i$ is measured experimentally and Δt_{i+1} corresponds to the time interval between two successive images. Then the contact radius at the $(i+1)$ th state can be calculated as

$$(L_x)_{i+1} = (L_x)_i + \Delta(L_x)_{i+1}. \quad (4.13)$$

Because the amount of experimental results is only about 1000, interpolation is used to enlarge the amount of simulation results to enhance the resolution of numerical simulation.

Due to the disturbance of impact, a few points at the initial stage were discarded. Only the late stage of the spreading with a relatively low spreading speed was considered in the simulation. The initial contact radius used in the simulation is the first experimental result after discarding the initial points. For a given equilibrium contact angle at the contact point whose normal is in x direction, θ_{ex} , and the

known surface tension of the liquid, the iteration gives the simulated contact radius in the x direction. The mobility of the contact line is found out by comparing the simulation results with the experimental results. When the simulation results fit the experimental results at the late stage, the corresponding value of M_{Lx} is the mobility of the contact line in the x direction.

The evolution of the contact radius in the y direction could be simulated in the same procedure, and the mobility of the contact line can be found out in a similar way. For the droplet spreading on the smooth substrate, the model was applied with γ_{LV} and θ the same at each point along the contact line, and similar simulations could be carried out.

4.4.3 Mobility of the contact line

To characterize the spreading speed of a droplet on a solid substrate, the mobility of the contact line was obtained by fitting the simulation curve with the experimental curve of the contact radius. Only the late stage of spreading was fitted, because the dynamic effect was remarkable and the relationship between the spreading velocity and the driving force deviated from equations (4.8) and (4.9) at the initial stage of spreading. In addition, the modeling in this study was simplified to include only the motion of the contact line. The motion of the liquid-vapor interface may also contribute to the spreading at the initial stage.

For the spreading of liquid droplets on the smooth substrate, the simulation curves and experimental curves are shown in Figure 4.9 and Figure 4.10. The values of

the mobility of the contact line for the five liquids are labeled in the figures. The comparison of the mobility of the contact line among DI water, the water-glycerol mixture, and glycerol, as shown in Figure 4.11, indicates that the mobility of the contact line decreases with increasing the liquid viscosity.

The mobility of the contact line is a system constant, including the effects of solid surface roughness and liquid viscosity. Xu et al. (2008) characterized the mobility of the contact line when studying the spreading of silicone oil droplets on steel plates of different surface roughness. Their results showed that the mobility decreased with the increase in surface roughness. In this study, the effect of the liquid viscosity on the mobility of the contact line is analyzed, which completes the discussion on the influencing factors involved in the description of dynamic spreading via the mobility of the contact line. As shown in Figure 4.11, the mobility of DI water droplets is larger than that of glycerol and water-glycerol droplets.

Figure 4.9 Dynamic evolution of the contact radius L (mm) of droplets of different viscosities spreading on the smooth substrate. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.10 Dynamic evolution of the contact radius L (mm) of droplets of different surface tensions spreading on the smooth substrate. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.11 Dependence of the mobility on the liquid viscosity.

For the spreading of liquid droplets on the anisotropically wrinkled substrates, the dynamic evolution of the dimensionless contact radii with time is shown in Figure 4.12 and Figure 4.13. The simulation curves fit well with the late stage of the experimental curves. By matching the simulation results with the experimental results of the dimensionless contact radii, the mobility of the contact line in the directions parallel and perpendicular to wrinkles were obtained, as labeled in Figure 4.12 and Figure 4.13. Comparison of the mobility between the two orthogonal directions shows that the mobility in the direction parallel to wrinkles is larger than that in the direction perpendicular to wrinkles, leading to a higher spreading speed and consequently a larger contact radius along wrinkles. In addition, the dependence of the mobility on the liquid viscosity is also observed from the spreading on the anisotropically wrinkled substrates. The mobility of the contact line for DI water is larger than that of glycerol either in the parallel direction or in the perpendicular direction.

Figure 4.12 Dynamic evolution of the dimensionless contact radii (L_x/R_x^* and L_y/R_y^*) of the liquids of different viscosities spreading on the anisotropically wrinkled substrate A3. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.13 Dynamic evolution of the dimensionless contact radii (L_x/R_x^* and L_y/R_y^*) of the liquids of different surface tensions spreading on the anisotropically wrinkled substrate A3. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

4.5 Nonlinear curve fitting of dynamic contact radius

In general, the relationship between the velocity of the contact line v_L and the driving force f_L can be nonlinear,

$$v_L = v_c \left(\frac{f_L}{f_0} \right)^p. \quad (4.14)$$

where v_c is a critical velocity which is a system constant and p is an exponent. Let $f_0 = \gamma_{LV}$, the driving force is normalized, and Eq. (4.14) becomes

$$v_L = v_c (\cos \theta_e - \cos \theta)^p. \quad (4.15)$$

In the special case with $p=1$, $v_c = M_L \gamma_{LV}$, where M_L is the mobility of the contact line.

Following similar formulation procedures as in Section 4.4, the motion of the contact line in the directions perpendicular and parallel to wrinkles during δt can be written as

$$\delta L_x = v_{cx} (\cos \theta_{ex} - \cos \theta_x)^p \delta t, \quad (4.16)$$

and

$$\delta L_y = v_{cy} (\cos \theta_{ey} - \cos \theta_y)^p \delta t. \quad (4.17)$$

The numerical simulation of equations (4.16) and (4.17) were conducted by iteration following similar procedures as in Section 4.4.2. Trials show that when $p=3$, the simulation curves can nicely fit the experimental curves, as shown in Figure 4.14 and Figure 4.15 for the spreading on the smooth substrate and in Figure 4.16 and Figure 4.17 for the spreading on the anisotropically wrinkled substrates, respectively.

Figure 4.14 Nonlinear curve fitting of the contact radius L (mm) of droplets of different viscosities spreading on the smooth substrate. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.15 Nonlinear curve fitting of the contact radius L (mm) of droplets of different surface tensions spreading on the smooth substrate. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.16 Nonlinear curve fitting of the dimensionless contact radii (L_x/R_x^* and L_y/R_y^*) of droplets of different viscosities spreading on the anisotropically wrinkled substrate A3. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

Figure 4.17 Nonlinear curve fitting of the dimensionless contact radii (L_x/R_x^* and L_y/R_y^*) of droplets of different surface tensions spreading on the anisotropically wrinkled substrate A3. The solid lines represent the experimental results while the scattered symbols represent the simulation results.

When liquid droplets spread on the smooth substrate, comparing $v_{c,p=3}$ among DI water, the water-glycerol mixture and glycerol, Figure 4.18(a) shows that the value of $v_{c,p=3}$ decreases with increasing the liquid viscosity, which is in consistence with the trend of mobility of the contact line as discussed in Section 4.4.3. The dependence of $v_{c,p=3}$ on the liquid viscosity is also observed when spreading on the anisotropically wrinkled substrates, as shown in Figure 4.19(a).

Figure 4.18 Dependence of $v_{c,p=3}$ on (a) liquid viscosity and (b) surface tension when a droplet spreads on the smooth substrate.

Figure 4.19 Dependence of $v_{c,p=3}$ on (a) liquid viscosity and (b) surface tension when a droplet spreads on the anisotropically wrinkled substrate A3.

As shown in Figure 4.18(b) and Figure 4.19(b), the value of $v_{c,p=3}$ increases with the increase in the surface tension of the liquid. Thus, on a certain substrate, DI water has the highest value of $v_{c,p=3}$, which is consistent with the experimental observations. When liquid droplets spread on the anisotropically wrinkled substrates, the critical velocity, $v_{c,p=3}$, is larger in the direction parallel to wrinkles

than that in the direction perpendicular to wrinkles, as shown in Figure 4.19. The wetting anisotropy is quantified.

4.6 Summary

The dynamic spreading of liquid droplets on the smooth and anisotropically wrinkled substrates has been studied experimentally and numerically. Five liquids of different properties in surface tension or viscosity have been used. The anisotropically wrinkled substrates have been fabricated through the simple and effective wrinkling method. They are the same with the substrates used in Chapter 3. The spreading process has been recorded and analyzed by measuring the contact angles and droplet dimensions. The equilibrium contact angles are smaller for the liquids of lower surface tension than those of higher surface tension. Droplets elongate along wrinkles. Wrinkle wavelength has been found to have effect on the equilibrium contact angles and droplet elongation. For most liquids, the equilibrium contact angles increase with the increase in wrinkle wavelength, and droplets elongate more dramatically on the substrate with larger wrinkle wavelength. The liquids exhibit similar dynamic spreading behaviors, but the spreading of SDS (CMC) and glycerol is more moderate than that of DI water.

A non-equilibrium thermodynamics model has been built and numerical simulations have been carried out to analyze the evolution of droplet shapes. With the linear relationship between the spreading velocity and the driving force, the mobility of the contact line has been obtained by fitting the simulation curves with the experimental curves at their late stage of spreading. The influence of the liquid viscosity on the dynamic spreading speed has been characterized by the mobility of

the contact line. The mobility decreases with increasing the liquid viscosity. The wetting anisotropy has been further characterized in the dynamic spreading. The spreading speed in the direction parallel to wrinkles is larger than that in the direction perpendicular to wrinkles. With the nonlinear relationship between the spreading velocity and the driving force, the whole experimental curve has been fitted by the simulation curve. The critical velocity, $v_{c,p=3}$, decreases with the increase in liquid viscosity, whereas it increases with increasing the liquid surface tension. The critical velocity is larger in the direction parallel to wrinkles than that in the direction perpendicular to wrinkles, showing the wetting anisotropy.

Chapter 5 Spreading of Giant Liposomes on Smooth Silicon Substrates*

5.1 Introduction

Giant liposomes, similar to liquid droplets, can serve as models to study the spreading phenomenon on solid substrates. Studies of the spreading of liquid droplets and giant liposomes together could deepen the understanding of the physics behind the spreading and the related phenomena. However, there are not as many studies of the spreading of liposomes as of liquid droplets. Smaller than droplets, giant liposomes are of the size of tens of micrometers, and they can only be observed under a microscope. Besides, liposomes form and exist in aqueous solution. The study of liposome spreading must be carried out in solution under the microscope, which adds difficulty in operation. In this study, giant liposomes used for spreading experiments are prepared with the electroformation method, the effectiveness of which in producing giant liposomes is compared among different lipid compositions. The dynamic spreading of giant liposomes is studied in a similar way with that used for the spreading of liquid droplets. The liposome spreading takes place on a vertically fixed smooth silicon substrate and is observed in real time. The contact angles and liposome dimensions are measured by image processing. A micropipette is employed to manipulate an individual liposome before it starts to spread on the solid substrate. Due to the small size of the liposomes, the effect of gravitational force is negligible.

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Based on the experimental observations, the theoretical model proposed for the spreading of liquid droplets is adopted to study the spreading of giant liposomes. Numerical simulations are carried out and the results are compared with the experimental results. The mobility of the contact line is obtained from the comparison to characterize the spreading speed.

5.2 Electroformation of giant liposomes

5.2.1 Experimental setup and procedures

Synthetic lipids 1,2-dioleoyl-*sn*-glycero-3-phosphate (DOPA) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) (Avanti, USA), and the mixture of them were used. The molecular structures of these two lipids are shown in Figure 5.1. The stock lipid solutions of DOPA and DOPC were diluted with chloroform to 1 mg/ml. The mixture was prepared by mixing the diluted lipid solutions at the volume ratio of 1 to 9 (DOPA:DOPC). In the whole experimental process, lipid solutions were handled with glass microsyringes (Hamilton, USA). Under nitrogen, the diluted lipid solution or the lipid mixture was deposited onto the conductive surface of a piece of ITO coated glass plate (ITO coating thickness: 1200-1600 Å, resistance: 1-15 Ω, Delta Technologies, USA). Droplet deposition technique was employed to prepare the lipid film because it has several advantages over the spin coating method as discussed by Kuribayashi et al. (2006). It was simple and required no other equipment. The plate was then put into a vacuum oven and maintained for at least 6 hours to completely remove the organic solvent.

Figure 5.1 Molecular structures of the lipids DOPA and DOPC.

Before electroformation, the glass plate with dry lipids on the ITO coated surface was assembled together with another piece of glass plate to form a formation chamber, as shown in Figure 5.2. The two pieces of glass 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, UK) which generated an AC electric field. An AC electric field (0.2 V peak to peak, 10 Hz) was applied to the formation chamber when sucrose (Sigma-Aldrich, USA) aqueous solution (100 mM) was gently introduced into the chamber through an opening in the silicone spacer. The voltage was immediately raised to 2.0 V and maintained for 2 hours after the addition of the sucrose solution. Liposomes were 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; Video recording software: StreamPix4, Norpix, Canada).

The formed liposomes were detached from the glass plate by decreasing the frequency to 0.5 Hz, and transferred into a plastic tube using a micropipette. The liposome suspension was kept in a refrigerator at 4°C for future use.

Figure 5.2 Schematic diagram of the experimental setup for giant liposome electroformation.

5.2.2 Characterization of electroformed giant liposomes

Liposomes begin to form just after adding the sucrose solution into the chamber and applying the AC electric field, as shown in Figure 5.3(a). Sucrose solution goes through lipid bilayers and causes hydration and swelling of the membranes. Bilayers separate and bend to form liposomes. In this process, sucrose solution is internalized and liposomes inflate to bigger sizes. Figure 5.3(b) and (d) show liposomes under the AC electric field 0.5 hours and 2 hours after adding the sucrose solution, respectively. In the experiments, 2 hours is demonstrated to be enough for liposome formation under the electric field (Bagatolli et al. 2000; Estes and Mayer 2005; Kuribayashi et al. 2006). From 0.5 hours to 2 hours after adding the sucrose solution, the average diameter of the liposomes increases from 10 μm to 27 μm as measured from the images.

Figure 5.3 Liposomes prepared from DOPC via electroformation method. (a) 5 minutes, (b) 0.5 hours, (c) 1 hours, and (d) 2 hours after adding the sucrose solution.

In a lipid-water system, there are various lipid phases, such as micelles and lamellar phases. In the process of liposome electroformation, dry lipid films are firstly formed on the ITO-coated glass plate. When the aqueous solution is introduced, lipids are at the lamellar phase which could exist in different states (Heimburg 2007). Liposomes may form only when the lipid bilayers are in disordered fluid phase. The critical temperature, indicating the transition from ordered gel phase to disordered fluid phase, is the melting temperature. The melting temperatures of the lipids used in this study are -8°C for DOPA and -20°C for DOPC, which are much lower than room temperature (22°C). Therefore, the temperature during the liposome preparation is higher than the phase transition temperature of the lipids, which is one of the important requirements for liposome

formation (Bagatolli et al. 2000). Another important requirement to prepare giant liposomes is to prevent agitation of samples during the liposome formation. The microscope used in this study is set up on an anti-vibration table, which minimizes the disturbance to the electroformation chamber. Therefore, agitation to the samples could be controlled at a low level.

When pipetting out a bit liposome suspension and depositing it onto a piece of glass slide immediately after the electroformation, the liposomes are difficult to be observed in bright field under a microscope. This is because (i) the liposomes flow fast in the buffer solution, and (ii) the lipid membranes are too thin (about 5 nm) to be focused on. To enhance the contrast between the inner solution and the outer solution, the liposome suspension was diluted using glucose (Sigma-Aldrich, USA) aqueous solution (102 mM). The difference in the refractive index between the solutions inside (sucrose solution) and outside (glucose solution) of the lipid membrane enables direct observation of liposomes. The liposomes prepared from DOPC in diluted suspension are shown in Figure 5.4. Although the size distribution of liposomes prepared through electroformation is narrower than that of liposomes spontaneously formed in aqueous solution, liposomes still could not be of an equal size (Bagatolli et al. 2000). As shown in Figure 5.4, some liposomes can be as large as 58 μm in diameter which greatly facilitates both the handling of a single liposome and the observation of liposome membrane behaviors.

Figure 5.4 Diluted DOPC liposomes in glucose aqueous solution. The profiles of some liposomes are not as clear as those of the others, because the liposomes are at different depths in the solution and they could not be focused simultaneously using normal optical microscope.

5.2.3 Comparison between electroformation and spontaneous formation

Without an AC electric field, giant liposomes were formed spontaneously in the same condition, and the process lasted for 4 hours. Lipid films are hydrated and swollen in aqueous solution even without an electric field. However, the spontaneous formation is slower than the electroformation process to obtain giant liposomes. The average size of the liposomes obtained from spontaneous formation is smaller than that of the electroformed liposomes. Besides, more defects are found from the spontaneously formed liposomes, as shown in Figure 5.5(a).

Figure 5.5 DOPC liposomes produced from (a) spontaneous formation (4 hours after adding sucrose solution) and (b) electroformation (2 hours after adding sucrose solution).

The prerequisite for liposome formation from dry lipid in aqueous solution is the bilayer separation and bending. External electric fields could facilitate both: they can decrease the inner-membrane attraction and can induce instability of bending (Angelova and Dimitrov 1986). The enhanced undulations of lipid bilayers could increase the repulsive forces between adjacent membranes, leading to the

separation of bilayers. Thus, electroformation can induce lipid swelling, and the method is more efficient and effective for the production of giant liposomes.

5.2.4 Comparison of liposomes prepared from different lipid compositions

To study the influence of lipid compositions and type of electric charge on the production of giant liposomes, different lipids were employed to produce liposomes using the electroformation method. Besides pure neutral DOPC, negatively charged DOPA and the mixture of DOPA and DOPC (1:9) were used and compared. Figure 5.6 shows that under the same preparation conditions, compared with pure DOPC, almost no liposomes are formed when pure DOPA is used while liposomes can grow to even a bigger size when the mixture of DOPA and DOPC is used. Liposomes as big as 20 μm in diameter are found only 5 minutes after adding the buffer solution.

The observations reveal that the electroformation process would not be impeded when a small amount of negatively charged lipids are added, but the method is not applicable to pure negatively charged lipid, such as pure DOPA. The addition of negatively charged lipids to the neutral lipids can enhance the force normal to membrane due to the electrostatic repulsion between lipid bilayers. However, when the amount of the negatively charged lipids is large or pure negatively charged lipids are used, the electrostatic interactions between the field and the lipid charges probably hinder the formation of liposomes.

Figure 5.6 Electroformation is applied to different lipid compositions. (a) Almost no liposomes are found after 2 hours for DOPA; (b) many liposomes could be observed for DOPA/DOPC only 5 minutes after adding the sucrose solution.

5.3 Adhesion and spreading of giant liposomes on smooth substrates

5.3.1 Materials and spreading device

Giant liposomes were prepared from DOPC with the electroformation method as introduced in Section 5.2. The spreading experiments were carried out on a home-made spreading device. 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 spreading device is schematically shown in Figure 5.7.

Figure 5.7 Schematic diagram of the experimental setup for liposome spreading on solid substrates. The gravitational force is negligible due to the small size of liposomes, which enables the study of spreading on vertically fixed substrates.

5.3.2 Spreading of giant liposomes on vertically fixed smooth substrates

The experiments on liposome spreading were carried out at the temperature of 24°C and a humidity of 50%. The procedures are as follows. Firstly, the liposome suspension was diluted with glucose aqueous solution (102 mM) at the volume ratio of 1 to 10 to enhance the image contrast, and the silicon substrate was pre-coated with polylysine to promote membrane adhesion. It is known that polylysine

with high molecular weight could promote membrane adhesion on various solid surfaces (Mazia et al. 1975; Huang et al. 1983). In this study, the silicon substrate was pre-coated with polylysine aqueous solution (0.01% w/v; M_w = 150 000 – 300 000, Sigma-Aldrich, USA) 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-shaped spreading device. The vertically fixed silicon sheet served as the spreading substrate and the polished side would be the spreading surface. The surface roughness of a well polished silicon wafer with an isotropic surface is about 20 nm which is low enough to consider the surface as smooth (Tay et al. 2004). Thus, the influence of surface roughness on the spreading process is considered as negligible.

Secondly, a drop of diluted liposome suspension (about 30 μ l) was added along the silicon substrate under the microscope. A single liposome was 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 turning the operation handle.

Finally, the aspirated liposome was transferred to approach to the silicon substrate until it touched the silicon surface. The micropipette was retracted immediately and the liposome spread on the silicon surface. Some liposomes could spread until

they reached the equilibrium states. The adhesion and spreading process was observed under the microscope and recorded by a CCD camera.

5.3.3 Experimental results

After touching the silicon substrate, the liposome adheres onto the silicon surface and starts to spread. During the initial period, the liposome spreads very fast, resulting in an increase in contact area with the silicon substrate. After a short period, the spreading slows down until the contact line stops its motion and reaches the equilibrium state. The evolution trend of the liposome height (h_{lipo}) is opposite to that of the contact radius (L_{lipo}). The liposome height dramatically decreases during the initial short period and slowly decreases further to reach its equilibrium value. The contact angle (θ_{lipo}) that the lipid membrane makes with the silicon substrate decreases with time and it also experiences an initial fast change followed by a slow change with time. Typical evolution of the contact radius and liposome height with time is shown in Figure 5.8. When the liposome shape is assumed to be a spherical cap, the liposome volume calculated from L_{lipo} and h_{lipo} is consistent with the liposome volume measured before spreading which is $3.58 \times 10^4 \mu\text{m}^3$, and the volume is constant during the spreading process as evidenced by Figure 5.8, indicating that there is no leakage of liquid or pore opening during the spreading process.

Figure 5.8 Evolution of liposome dimensions (L_{lipo} and h_{lipo}) with time. The volume of the liposome is calculated from L_{lipo} and h_{lipo} , and is almost constant during the shape evolution of the liposome spreading.

It should be noted that the liposome spreading during the initial period is quite fast and that the beginning of the spreading cannot be captured by a normal CCD camera. On the first image taken by the camera, the contact angle was already lower than 130° , as shown in Figure 5.9, while the starting contact angle was expected to be 180° . In order to fully show the initial fast stage of the spreading process, a high speed camera is needed for the experiments.

Figure 5.9 Evolution of the contact angle θ_{lipo} with time. The microscopic images show the evolution of liposome shapes. Half of the liposome profile is highlighted in dashed lines.

5.4 Non-equilibrium thermodynamics formulation of liposome spreading on smooth substrates

In this project, the analysis of the spreading process is within the continuum physics scope for both liquid droplets and giant liposomes. The theoretical model proposed to describe the spreading of liquid droplets was applied to study the spreading behaviors of giant liposomes. Because the spreading of liposomes took place on a smooth substrate, without losing the key features of the problem, the shape of the liposome was approximated by a spherical cap during the spreading process. In order to explicitly illuminate the model specifically for the spreading of liposomes, the energy and the driving force in the spreading process are considered, and the equilibrium condition for the system is presented in this section. In addition, numerical simulations were carried out, and the simulation

results were compared with the experimental measurements to demonstrate the feasibility of the model in describing the dynamic spreading of liposomes.

5.4.1 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 Figure 5.10. 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. Although some experimental and analytical investigations on the pore opening phenomenon indicate that the membrane surface tension, which predominantly controls various shape transformations of liposomes, is variable during the deformation process (Sandre et al. 1999; Karatekin et al. 2003; Rodriguez et al. 2006), in some cases, the surface tension of the lipid membrane could be simplified to be constant. The difference in surface tension between lipid membranes and normal liquids is originated from their compositions as discussed in Chapter 2. After a liposome is formed, the number of lipid molecules constituting it is constant, and no more lipid molecules could come to fill the enlarged intermolecular space during the shape transformation process. Thus, the membrane surface tension changes with the surface area. When the membrane is stretched to a large extent, the membrane surface tension may increase greatly and membrane failure would occur. In this study, from the experimental observations of the spreading process, no visible pore is found on the membrane, and the change

of the membrane surface area is small. Therefore, in this model, the surface tension of the membrane, σ , is regarded as constant.

Figure 5.10 Shape of the adhered liposomes on solid substrates is approximated by a spherical cap.

Within the scope of continuum physics, for a condensed matter of macroscopic size, the volume related energy dominates the dynamic spreading process (Dimitrakopoulos and Higdon 1999), while for a condensed matter of sub-millimeter scaled size, the surface associated energy becomes dominant (Kwon et al. 2009). 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 (Fan 2006). For the liquid/membrane/solid system in this study, 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, in most cases, line tension contributes only when there is pore or domain on lipid membranes, and it 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 (Karatekin et al. 2003; Tian et al. 2007). Therefore, this study will focus on the description and formulation of the membrane itself. Even though the matter inside of the lipid membrane contributes to the liposome shape and the internal pressure, its associated energy is negligible.

Because the solid substrate has a smooth surface and the lipid membrane is considered as homogeneous without any phase separation or domain, the contact line is a circle with a contact radius of L_{lipo} , and the driving force is uniformly distributed along the contact line. The geometric relations among the contact radius L_{lipo} , the liposome height h_{lipo} , the sphere radius R_{lipo} and the contact angle θ_{lipo} are shown in Figure 5.10.

In the viewpoint of thermodynamics, the change of the system's energy is the product of the driving force and its associated displacement. The shape evolution of the spreading liposome with time is shown in Figure 5.11. For the liquid/membrane/solid system, the energy change could be represented by the sum of the interfacial energy changes,

$$\delta G = \sigma \delta A + \sigma_{s1} \delta A_{s1} + \sigma_{s2} \delta A_{s2}, \quad (5.1)$$

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.

Figure 5.11 Evolution of the liposome shape during spreading process. The contact radius increases by δL_{lipo} when time passes by δt .

Figure 5.11 shows 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 liquid decreases. The increased area equals the decreased one. If the contact radius is L_{lipo} and the increment of the contact radius is defined as δL_{lipo} , the change in the interfacial area between the membrane and the solid substrate could be written as

$$\delta A_{s2} = 2\pi L_{lipo} \delta L_{lipo}. \quad (5.2)$$

Similarly, the change in the interfacial area between the solid substrate and the surrounding liquid is

$$\delta A_{s1} = -2\pi L_{lipo} \delta L_{lipo}. \quad (5.3)$$

As for the spherical cap, the change in the interfacial area between the membrane and the surrounding liquid is

$$\delta A = \delta \left(\pi \left(L_{lipo}^2 + h_{lipo}^2 \right) \right) = 2\pi L_{lipo} \delta L_{lipo} + 2\pi h_{lipo} \delta h_{lipo}. \quad (5.4)$$

The relationship between δL_{lipo} and δh_{lipo} could be found from the volume conservation of the spreading liposome. Since no visible pore or substance exchange is observed in the experiments, the volume of the spreading liposome, V_{lipo} , is constant with $\delta V_{\text{lipo}} = 0$. In this condition, we have

$$\delta V_{\text{lipo}} = \delta \left(\frac{\pi}{6} h_{\text{lipo}} (3L_{\text{lipo}}^2 + h_{\text{lipo}}^2) \right) = 0, \quad (5.5)$$

which leads to

$$\delta h_{\text{lipo}} = -\frac{2L_{\text{lipo}} h_{\text{lipo}}}{L_{\text{lipo}}^2 + h_{\text{lipo}}^2} \delta L_{\text{lipo}}. \quad (5.6)$$

Therefore, by substituting Eq. (5.6) into Eq. (5.4), the area change of the membrane cap as a function of δL_{lipo} can be obtained as

$$\delta A = \delta \left(\pi (L_{\text{lipo}}^2 + h_{\text{lipo}}^2) \right) = 2\pi L_{\text{lipo}} \delta L_{\text{lipo}} \frac{L_{\text{lipo}}^2 - h_{\text{lipo}}^2}{L_{\text{lipo}}^2 + h_{\text{lipo}}^2}. \quad (5.7)$$

Considering the spherical cap, the geometric relationship between the contact angle θ_{lipo} and the liposome dimensions, L_{lipo} and h_{lipo} , is

$$\cos \theta_{\text{lipo}} = \frac{L_{\text{lipo}}^2 - h_{\text{lipo}}^2}{L_{\text{lipo}}^2 + h_{\text{lipo}}^2}. \quad (5.8)$$

Substituting Eq. (5.8) into Eq. (5.7), and considering together with equations (5.2) and (5.3), the energy change (shown in Eq. (5.1)) becomes

$$\delta G = 2\pi L_{\text{lipo}} \delta L_{\text{lipo}} (\sigma \cos \theta_{\text{lipo}} + \sigma_{s2} - \sigma_{s1}). \quad (5.9)$$

The system energy is lowered by the thermodynamics driving force. Here, the driving force in the 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

$$\delta G = -f_{\text{lipo}} \times 2\pi L_{\text{lipo}} \delta L_{\text{lipo}}, \quad (5.10)$$

where f_{lipo} is the “thermodynamic driving force” and the energy should decrease under the rule of non-equilibrium thermodynamics. By comparing between Eq. (5.9) and Eq. (5.10), the driving force can be expressed as

$$f_{\text{lipo}} = \sigma_{s1} - \sigma_{s2} - \sigma \cos \theta_{\text{lipo}}, \quad (5.11)$$

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

5.4.2 Motion equation and equilibrium condition

Let δL_{lipo} be the motion of the contact line. Under the scope of thermodynamics, the velocity of the contact line is a function of the driving force, and the motion can be represented by the driving force. Here, the linear relationship is adopted and the motion is written as

$$\delta L_{\text{lipo}} = M_{\text{lipo}} f_{\text{lipo}} \delta t, \quad (5.12)$$

where M_{lipo} is called the mobility of the contact line and is used as the phenomenological parameter of the liquid/membrane/solid system. Equation (5.12) is the kinetic law for the contact line. The combination of equations (5.11) and (5.12) leads to the expression for the motion of the contact line,

$$\delta L_{\text{lipo}} = M_{\text{lipo}} (\sigma_{s1} - \sigma_{s2} - \sigma \cos \theta_{\text{lipo}}) \delta t. \quad (5.13)$$

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 being at its equilibrium value, θ_{elipo} . The driving force vanishes to zero, and the contact line stops moving. By setting f_{lipo} to be zero in Eq. (5.11), the equilibrium condition for the contact line could be obtained as

$$\sigma_{s1} - \sigma_{s2} - \sigma \cos \theta_{\text{elipo}} = 0, \quad (5.14)$$

which is the famous Young equation.

5.4.3 Numerical simulation

From the non-equilibrium thermodynamics formulation of the liposome spreading process, it is known that the liposome dynamic spreading can be characterized by the mobility of the contact line. Based on the theoretical model, numerical simulations were carried out.

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

$$\delta L_{\text{lipo}} = \sigma M_{\text{lipo}} (\cos \theta_{\text{elipo}} - \cos \theta_{\text{lipo}}) \delta t. \quad (5.15)$$

By normalizing δL_{lipo} with the contact radius at the equilibrium state, L_{elipo} , Eq. (5.15) becomes

$$\frac{\delta L_{\text{lipo}}}{L_{\text{elipo}}} = \frac{\sigma M_{\text{lipo}}}{L_{\text{elipo}}} (\cos \theta_{\text{elipo}} - \cos \theta_{\text{lipo}}) \delta t. \quad (5.16)$$

With the dimensionless form of the equation of evolution, Eq.(5.16), the characteristic spreading time could be defined as

$$t_{\text{lipo}} = \frac{L_{\text{elipo}}}{\sigma M_{\text{lipo}}}. \quad (5.17)$$

The combination of equations (5.16) and (5.17) leads to

$$\frac{\delta L_{\text{lipo}}}{L_{\text{elipo}}} = (\cos \theta_{\text{elipo}} - \cos \theta_{\text{lipo}}) \delta \left(\frac{t}{t_{\text{lipo}}} \right), \quad (5.18)$$

by which the evolution of the contact radius could be simulated.

The numerical simulation of Eq. (5.18) was carried out by a simple iteration. If the contact radius of the liposome at the i th state $\left(\left(t/t_{\text{lipo}} \right)_i \right)$ is known and an increment of dimensionless time is set as $\Delta \left(t/t_{\text{lipo}} \right)_{i+1}$, the contact radius change is given by

$$\Delta \left(\frac{L_{\text{lipo}}}{L_{\text{elipo}}} \right)_{i+1} = (\cos \theta_{\text{elipo}} - \cos (\theta_{\text{lipo}})_i) \Delta \left(\frac{t}{t_{\text{lipo}}} \right)_{i+1}. \quad (5.19)$$

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

$$\left(\frac{L_{\text{lipo}}}{L_{\text{elipo}}} \right)_{i+1} = \left(\frac{L_{\text{lipo}}}{L_{\text{elipo}}} \right)_i + \Delta \left(\frac{L_{\text{lipo}}}{L_{\text{elipo}}} \right)_{i+1}. \quad (5.20)$$

According to Eq. (5.6), the increment of the liposome height is

$$\left(\Delta h_{\text{lipo}} \right)_{i+1} = \left(-\frac{2L_{\text{lipo}}h_{\text{lipo}}}{L_{\text{lipo}}^2 + h_{\text{lipo}}^2} \right)_i \left(\Delta L_{\text{lipo}} \right)_{i+1}, \quad (5.21)$$

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

$$\left(h_{\text{lipo}} \right)_{i+1} = \left(h_{\text{lipo}} \right)_i + \left(\Delta h_{\text{lipo}} \right)_{i+1}. \quad (5.22)$$

The contact angle could be calculated from the contact radius L_{lipo} and the liposome height h_{lipo} as

$$\cos (\theta_{\text{lipo}})_{i+1} = \left(\frac{L_{\text{lipo}}^2 - h_{\text{lipo}}^2}{L_{\text{lipo}}^2 + h_{\text{lipo}}^2} \right)_{i+1}. \quad (5.23)$$

The initial condition of the iteration was set as $(L_{\text{lipo}})_0 = 0$, $(h_{\text{lipo}})_0 = 2 \times (3V_{\text{lipo}}/4\pi)^{1/3}$ with V_{lipo} to be the known liposome volume, and $(\theta_{\text{lipo}})_0 = 180^\circ$. The time step for iteration $\Delta(t/t_{\text{lipo}})_{i+1} = 0.001$ 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.

5.5 Matching simulation results with experimental measurements

The experimental results of the shape evolution in Figure 5.8 show that the liposome immediately adheres onto the silicon surface and starts to spread after touching the silicon substrate. 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. However, the experimental results can only provide us with the information of liposome spreading at the late stage as discussed in Section 5.3.3. Although there is such obstacle in experiments, fortunately, the theoretical model can solve this problem without any difficulty and provide us with the information at the early stage.

To include the early stage of the liposome spreading process, in the numerical simulation, the initial contact angle of the liposome is set as $(\theta_{\text{lipo}})_0 = 180^\circ$, which implies a liposome, just touching the silicon substrate, with a full spherical shape.

The final equilibrium configuration is set based on the experimental results of DOPC liposome spreading on a smooth silicon substrate, *i.e.*, $\theta_{\text{elipo}} = 107.5^\circ$ and $L_{\text{elipo}} = 21.8 \text{ } \mu\text{m}$ for a liposome of the initial radius of about $20.5 \text{ } \mu\text{m}$. The simulated results are shown in Figure 5.12 with a complete spreading process. Initially, once the liposome touches the substrate, the dimensionless contact radius is 0 and the dimensionless height is 1.88, exhibiting a spherical shape. Because of the large difference of the instant contact angle from the equilibrium value, the driving force, $\sigma \left(\cos \theta_{\text{elipo}} - \cos \left(\theta_{\text{lipo}} \right)_0 \right)$, 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 in contact radius and the decrease in liposome height, the contact angle of the liposome decreases, as shown in Figure 5.12(b). As the contact angle $\left(\theta_{\text{lipo}} \right)_t$ becomes smaller, the driving force, $\sigma \left(\cos \theta_{\text{elipo}} - \cos \left(\theta_{\text{lipo}} \right)_t \right)$, 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_{elipo} . Without force to drive the liposome to spread on the silicon substrate, the shape of the liposome reaches and keeps the equilibrium profile, and the spreading process completes.

Figure 5.12 (a) Dynamic evolution of the liposome shape (dimensionless contact radius $L_{\text{lipo}}/L_{\text{elipo}}$, dimensionless liposome height $h_{\text{lipo}}/L_{\text{elipo}}$, and dimensionless volume $V_{\text{lipo}}/L_{\text{elipo}}^3$) with time. (b) The change of contact angle with time. The scattered symbols represent the experimental results while the solid lines represent the simulation results.

After the simulation, the simulation results were fitted with experimental results using the least square method to determine the characteristic spreading time t_{lipo} . After normalizing the experimental time by the characteristic spreading time, the simulation results agree well with the experimental results, as shown in Figure 5.12, which demonstrates that the theoretical model is applicable and reliable to

study the dynamic spreading of giant liposomes. The mobility of the contact line could be obtained from Eq. (5.17) as

$$M_{\text{lipo}} = \frac{L_{\text{lipo}}}{\sigma t_{\text{lipo}}}. \quad (5.24)$$

We can find out from Eq. (5.24) that the mobility of the contact line 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 (Jähnig 1996), it could be estimated from some theoretical models, such as the polymer brush model proposed by Rawicz et al. (2000). They modeled a lipid monolayer as an idealized polymer brush, and predicted the surface tension of a monolayer to be $K_A/6$, where K_A is the direct elastic stretch modulus. Based on the polymer brush model, the author borrowed the data of the surface tension of a DOPC bilayer from their work (Rawicz et al. 2000), where $\sigma = 88$ mN/m. Substituting it into Eq. (5.24), together with the equilibrium contact radius $L_{\text{lipo}} = 21.8$ μm and the characteristic spreading time $t_{\text{lipo}} = 1.15$ s, the mobility of the contact line is estimated to be 2.16×10^{-4} $\text{m}^2/(\text{N}\cdot\text{s})$ for a giant liposome made of DOPC lipid spreading on a smooth silicon substrate. This value is lower than the mobility of a silicone oil droplet spreading on stainless-steel plates (Xu et al. 2008) and that of a liquid droplet (e.g. water droplet) spreading on gold-coated smooth substrates as reported in Chapter 4.

As shown from the above results and analysis, the linear relationship between the spreading velocity and the driving force works well in modeling the dynamic

spreading of giant liposomes. This is because the experimentally recorded spreading process is mainly at the middle and late stage when the spreading speed is relatively low. When the spreading speed is low, the linear relationship could well describe the spreading process as discussed in Section 4.4.3.

It should be noted that a simulation procedure different from that for liquid droplet spreading in Chapter 4 is adopted in this study. Though assumption is made about the liposome shape and a characteristic spreading time is introduced, the simulation works well as evidenced by the matching between experimental results and simulation results as shown in Figure 5.12. In addition, the mobility value is very close to that obtained with the simulation method described in Chapter 4 which is $2.10 \times 10^{-4} \text{ m}^2/(\text{N}\cdot\text{s})$.

5.6 Summary

Liposomes could be prepared from DOPC and the mixture of DOPA and DOPC using either the electroformation method or the spontaneous formation method. The electroformation method has been proven to be more effective in producing giant liposomes with fewer defects from DOPC and DOPA/DOPC mixture. The results show that electroformation is more favorable for neutral and slightly negatively charged lipids than pure negatively charged lipids. With these liposomes, the spreading behavior on smooth silicon substrate has been investigated with the home-made spreading device.

According to the continuum physics theory, the spreading of micrometer-sized liposome should be controlled by the surface related energy of the system. In this

study, the dynamic spreading of giant liposomes has been experimentally examined without considering the gravitational force. The theoretical model proposed for the spreading of liquid droplets has been applied to the spreading of giant liposomes by assuming the liposome shape as part of a sphere. From numerical simulations, the evolution of the liposome shape during the whole spreading process has been visualized. By matching the numerical simulation curve with the experimental curve, the applicability and the feasibility of the model have been demonstrated. For the spreading of giant liposomes on the smooth silicon substrate, the value of the mobility of the contact line has been estimated, which is lower than the mobility of the contact line of liquid droplets spreading on stainless-steel plates or gold-coated shape memory polymer substrates.

Chapter 6 Spreading of Giant Liposomes on Anisotropically Patterned Substrates

6.1 Introduction

From the experimental observations and measurements in Chapter 5, it can be seen that the spreading of giant liposomes can be investigated using a similar method as for the spreading of liquid droplets which is reported in Chapter 4. Surface patterns have been proven to have effect on both static and dynamic spreading of liquid droplets, as studied in chapters 3 and 4, and they may also affect the spreading of giant liposomes (Bernard et al. 2000b). Liposomes were noticed to be elongated when observing the spreading of fluorescently stained giant liposomes on the anisotropically wrinkled substrates. In this study, to further understand the spreading of liposomes, experimental work and numerical simulations are carried out to study the dynamic spreading of giant liposomes on anisotropically patterned substrates.

To quantitatively characterize the shape evolution of liposomes during the dynamic spreading process, the spreading device introduced in Chapter 5 is employed in this study while the smooth silicon substrate is replaced with patterned silicon substrates. Silicon substrates with anisotropic grooves are fabricated and used in this study instead of the anisotropically wrinkled substrates used for liquid spreading, because the anisotropically wrinkled substrates are not suitable for liposome spreading due to the bulk size and the large wrinkle wavelength relative to the liposomes. The patterned silicon substrates have a pattern size an order of magnitude smaller than the liposome size. Therefore, the study is still within the

scope of continuum physics. The theoretical model for the spreading of liquid droplets is applied to the spreading of giant liposomes. Numerical simulations are carried out, and the simulation results are compared with the experimental results. The mobility of the contact line is obtained in the directions parallel and perpendicular to grooves.

6.2 Fabrication and characterization of anisotropically patterned silicon substrates

Anisotropic patterns were realized by fabricating unidirectional parallel grooves on the surface of silicon wafers. A silicon wafer was first rinsed with DI water and spin-dried to remove the residual liquid. There are two main steps of the fabrication process: photolithography and dry etching. The complete fabrication procedure is illustrated in Figure 6.1.

Standard photolithography technique was employed before etching. First, a piece of clean wafer was heated to the temperature of 100°C to remove any moisture that might be present on the surface of the wafer. At the same time, the adhesion promoter, hexamethyldisilazane (HMDS), was applied to promote the adhesion of the photoresist to the silicon wafer. Then a uniform thin layer of positive photoresist (AZ9260) was spin coated onto the silicon wafer. The recipe for the coating parameters, spin speed and coating time, was selected based on the desired etching depth, and the etching depth determined the coating thickness. Second, the silicon wafer with the photoresist layer was prebaked at 110°C on a hotplate for 4 minutes to remove the possible photoresist solvent. Third, a photomask with the desired patterns was aligned over the silicon wafer on a mask aligner and the wafer

was exposed to ultraviolet light (UV light). The 5-inch photomask was made of soda lime and coated with chrome. The exposure type and the exposure time were determined according to the type of the photoresist and the thickness of the photoresist layer on the wafer. Finally, the exposed silicon wafer was developed with the developer AZ400K. It was carried out by immersing the wafer in the developer solution and gently agitating the container until the patterns could be seen clearly. The time for developing was estimated from the type of the photoresist and the coating thickness. After the development, the wafer was mildly rinsed with DI water and spin-dried to remove the residual solution.

Figure 6.1 Illustration of photolithography and etching procedures to fabricate silicon substrates with unidirectional grooves.

Since positive photoresist AZ9260 was used, the exposed areas of the photoresist were soluble in the developer while the unexposed areas remained almost intact on the silicon surface. During dry etching, a thin layer of the silicon wafer, under the exposed areas and without the protection of photoresist, was removed by reactive ion etching (RIE). The etching depth is controlled by the etching time. As compared with wet etching, dry etching can result in better anisotropy of the patterns. After etching, the photoresist on the silicon wafer was thoroughly removed with acetone and rinsed with DI water. Finally, the silicon wafer with patterns on the surface was spin-dried and cut into small pieces (rectangular shape with the dimension of 4 mm $\times$ 15 mm) for the spreading experiment.

Figure 6.2 Schematic diagram of the experimental setup for liposome spreading and the illustration of the anisotropic patterns on the substrates.

Two types of substrates were made with the grooves either parallel or perpendicular to the long edge of the substrate. According to the manner that the substrate is fixed in the spreading device, they are called “horizontal grooves” and “vertical grooves”, respectively, as shown in Figure 6.2. The reason to make two types of substrates is that the spreading could not be observed or captured from the two orthogonal directions simultaneously. The spreading needs to be studied in the

two directions separately. The horizontal grooves are used to study the spreading in the direction parallel to grooves while the vertical grooves are used to study the spreading in the direction perpendicular to grooves.

Figure 6.3 Typical images of anisotropically patterned silicon substrates: (a) two-dimensional surface micrograph, and (b) zoomed three-dimensional topography. Both horizontal and vertical grooves have similar groove shapes and groove dimensions.

The dimensions of the grooves were characterized with a PL μ confocal imaging profiler. The width of both etched and non-etched parts was designed to be 4 μm and actually measured to be 4.5 μm and 3.5 μm , respectively. The depth of the

grooves was measured to be about 1.8 μm . Figure 6.3 shows the two-dimensional and three-dimensional profiles of the grooves.

6.3 Spreading of giant liposomes on anisotropically patterned silicon substrates

According to the experimental results reported in Section 5.2, giant liposomes prepared from the mixture of DOPA and DOPC (1:9) have a larger average size compared with those made of pure DOPC. Larger liposomes are preferred for the study of spreading on patterned substrates. This is because the continuum physics requires the liposome to cover as many grooves as possible, while the smallest groove width that can be achieved is limited by the current fabrication condition. Thus, in this study, giant liposomes prepared from DOPA/DOPC (1:9) with the electroformation method were employed for the spreading process. The spreading experiments were carried out on the previously mentioned home-made spreading device (refer to Section 5.3 (page 105) for details) by replacing the smooth silicon substrate with the patterned silicon substrate, as shown in Figure 6.2. The procedure of the spreading experiments on the patterned silicon substrates is similar to that on the smooth silicon substrates. And the experiments were also carried out at room temperature (24°C) and under the humidity of 50%. The details can be found in Section 5.3.2 (page 106).

As observed from the spreading experiments, giant liposomes preferably spread along grooves, which was evidenced by the smaller equilibrium contact angles and larger dimensionless contact radii measured in the direction parallel to grooves. Like liquid droplets on the anisotropically wrinkled substrates, liposomes also

exhibit wetting anisotropy on the anisotropically patterned silicon substrates. Figure 6.4 shows the typical equilibrium shapes of two liposomes on horizontal grooves and on vertical grooves, respectively. Figure 6.5 shows the dynamic evolution of liposome shapes characterized by the change of contact radii when the liposomes spread on horizontal grooves and on vertical grooves, separately.

Figure 6.4 Typical equilibrium profiles at the two middle cross sections when liposomes spread (a) on the horizontal grooves and (b) on the vertical grooves. Half of the liposome contour is highlighted with dashed lines.

As shown from the experimental observations, after a liposome touches the solid surface, either on the horizontal grooves or on the vertical grooves, it quickly

adheres onto the surface and starts to spread. After a short time of spreading, the liposome reaches its equilibrium state.

Figure 6.5 Experimental results and microscopic images showing the change of contact radii during the spreading process. (a) Spreading on the horizontal grooves; (b) spreading on the vertical grooves. Half of the liposome contour is highlighted with dashed lines in the microscopic images.

Compared with liquid droplets, it takes less time for liposomes to reach their equilibrium states when spreading on solid substrates. The experimental observations show that liposomes made of the mixture of DOPA and DOPC

reached the equilibrium states within 5 s, as shown in Figure 6.5. Therefore, it is challenging to fully record the details of the spreading process. Though the CCD camera was used at the speed of 20 fps, the detailed features of the spreading at the initial stage still could not be fully captured due to the fast evolution and the sheltering of the micropipette before it was completely retracted from the liposome. Since the micropipette has an outer diameter of 100 μm at the tip, it may probably affect the observation of liposomes just after they touch the solid substrate. Thus, the initial stage of the spreading process cannot be experimentally characterized in this experiment.

The equilibrium contact angles were measured to be 113° and 131° (Figure 6.6) in the directions parallel and perpendicular to grooves, respectively. The elongation of the liposome was as high as 1.37, showing that liposomes were remarkably elongated by the grooves.

Figure 6.6 Typical experimental results showing the change of contact angles during the spreading process.

6.4 Non-equilibrium thermodynamics formulation of liposome spreading on patterned silicon substrates

6.4.1 Modeling of the dynamic spreading process

Since the spreading of giant liposomes share similar features with that of liquid droplets, the same formulation method was applied to model the liposome spreading on anisotropically patterned substrates. The modeling procedures are briefly introduced in this section.

The analysis is within the scope of continuum physics. The gravity effect is negligible due to the micrometer scaled size of giant liposomes (Fan 2006). The surface/interfacial tensions govern the spreading process.

Considering a liposome spreading on an anisotropically patterned substrate, the lipid membrane, the solid substrate and the liquid outside the lipid membrane constitute the liquid/membrane/solid system, as shown in Figure 5.10 (page 111). Due to the anisotropic patterns, the interfacial tensions between the membrane and the solid are different between the directions parallel and perpendicular to grooves. As for the substrates used in this study, the equilibrium contact angles measured from the horizontal grooves and the vertical grooves are different.

As shown in Figure 5.10, the driving force of the contact line at the contact point at the instant t is

$$f_{\text{lipo,h/v}}(t) = \sigma_{\text{s1}} - \sigma_{\text{s2,h/v}} - \sigma \cos \theta_{\text{lipo,h/v}}(t), \quad (6.1)$$

where subscripts h and v represent spreading on the horizontal grooves and spreading on the vertical grooves, respectively. The meanings of the symbols used in this section and the following section (Section 6.5) are the same as those in Section 5.4. From the directions of the interfacial tensions and the liposome spreading direction, the driving force, $f_{\text{lipo,h/v}}$, is positive when driving the liposome to spread over the solid surface.

Let $\delta L_{\text{lipo,h/v}}$ be the motion of the contact line in either horizontal or vertical direction. Under the thermodynamics scope, the velocity is a function of the driving force, such as the linear function, which works well for liposome spreading on the smooth substrate. Thus, the motion can be written as

$$\delta L_{\text{lipo,h/v}} = M_{\text{lipo,h/v}} f_{\text{lipo,h/v}} \delta t . \quad (6.2)$$

where $M_{\text{lipo,h/v}}$ is the mobility of the contact line on either horizontal or vertical grooves, and it is used as the phenomenological parameter of the system. Equation (6.2) is the kinetic law for the contact line, and it is linear and local.

Although the membrane/solid interfacial tension is different in the directions parallel and perpendicular to grooves, their values should be constant during the spreading process. Similar explanations apply as for the liquid/vapor/solid system in Section 4.4 (page 77). The membrane surface tension is considered as constant in the model.

With the driving force in Eq. (6.1), $f_{\text{lipo,h/v}}$, the liposome shape changes while the dynamic contact angles decrease with time. Equation (6.1) shows that the driving

forces in both directions parallel and perpendicular to grooves decrease with the decrease in contact angles. The driving forces will become zero when the contact angles achieve the equilibrium values. As a result, the liposome finishes its spreading and reaches the equilibrium state.

By setting $f_{\text{lipo,h/v}}$ to be zero in Eq. (6.1), the equilibrium condition for the contact line could be obtained as

$$\sigma_{s1} - \sigma_{s2,h/v} - \sigma \cos \theta_{\text{elipo,h/v}} = 0. \quad (6.3)$$

The above equation describes the force balance at the contact point either on horizontal or vertical grooves.

6.4.2 Numerical simulation

To demonstrate the applicability of the model in describing the dynamic spreading of giant liposomes, numerical simulations were carried out. The evolution of the liposome shape was considered from the directions parallel and perpendicular to grooves, and the spreading speed was characterized in the two directions separately.

Combining equations (6.1) and (6.2), the motion of the contact line is obtained as

$$\delta L_{\text{lipo,h/v}} = M_{\text{lipo,h/v}} \left(\sigma_{s1} - \sigma_{s2,h/v} - \sigma \cos \theta_{\text{lipo,h/v}} \right) \delta t. \quad (6.4)$$

Considering together with Eq. (6.3), it can be re-written as

$$\delta L_{\text{lipo,h/v}} = \sigma M_{\text{lipo,h/v}} \left(\cos \theta_{\text{elipo,h/v}} - \cos \theta_{\text{lipo,h/v}} \right) \delta t. \quad (6.5)$$

The numerical simulations of Eq. (6.5) on either horizontal grooves or vertical grooves were carried out by iteration. If the contact radius of the liposome at the i th state ($(t_{\text{lipo,h/v}})_i$) is known and an increment of the time is set as $\Delta(t_{\text{lipo,h/v}})_{i+1}$, the contact radius change is given by

$$\Delta(L_{\text{lipo,h/v}})_{i+1} = \sigma M_{\text{lipo,h/v}} \left(\cos \theta_{\text{elipo,h/v}} - \cos(\theta_{\text{lipo,h/v}})_i \right) \Delta(t_{\text{lipo,h/v}})_{i+1}, \quad (6.6)$$

where $(\theta_{\text{lipo,h/v}})_i$ is measured from experiments and $\Delta(t_{\text{lipo,h/v}})_{i+1}$ corresponds to the time interval between two successive microscopic images. Then the contact radius at the $(i+1)$ th state can be calculated as

$$(L_{\text{lipo,h/v}})_{i+1} = (L_{\text{lipo,h/v}})_i + \Delta(L_{\text{lipo,h/v}})_{i+1}. \quad (6.7)$$

Because the amount of experimental results is less than 50, interpolation is used to enlarge the amount of simulation results to enhance the resolution of numerical simulation.

The initial contact radius is the experimental result measured on the first microscopic image. For a given equilibrium contact angle at a contact point, $\theta_{\text{elipo,h/v}}$, and the estimated surface tension of the lipid membrane, the iteration gives the simulated contact radius. The mobility of the contact line is found out by comparing the simulation results with the experimental results. When the simulation results could fit the experimental results, the corresponding value of $M_{\text{lipo,h/v}}$ is the mobility of the contact line for the liposome spreading on the certain patterned substrate.

6.5 Matching simulation results with experimental measurements

The shape of the liposomes on the anisotropically patterned substrates cannot be described by a simple geometric shape as observed from the experiments, which makes the simulation method in Section 5.4 inappropriate for this problem. Thus, the method used for liquid droplet spreading was employed in the simulations in this study. Though the simulations cannot describe the initial stage, they give the insight information of the mobility of the contact line and help in characterizing the spreading speed.

In this study, the spreading speed is characterized by the mobility of the contact line which is obtained by fitting the simulation results with the experimental results, as shown in Figure 6.7. The values of the mobility of the contact line in the directions parallel and perpendicular to grooves are labeled in the graphs. The mobility of the contact line for the liposome spreading in the direction parallel to grooves is larger than that spreading in the direction perpendicular to grooves. Wetting anisotropy is demonstrated dynamically.

It should be noted that the membrane surface tension must be known to obtain the mobility of the contact line. However, the membrane surface tension is not experimentally accessible, and there is no reference data in the literature for the surface tension of DOPA/DOPC lipid membrane. In this work, the content of DOPA in the lipid mixture is ten percent, which is relatively low, and the experimental results show that the equilibrium contact angle of DOPA/DOPC liposomes on the smooth silicon substrate is in the range of 110–120° which is close to that of DOPC liposomes. Thus, the membrane surface tension of DOPC,

88 mN/m as reported in Section 5.5, is employed to estimate the contact line mobility of DOPA/DOPC liposomes.

Figure 6.7 Dynamic evolution of the contact radii: (a) $L_{\text{lipo,h}}$ and (b) $L_{\text{lipo,v}}$. The scattered symbols represent the experimental results while the solid lines represent the simulation results.

6.6 Summary

The dynamic spreading of giant liposomes on anisotropically patterned substrates has been investigated experimentally and numerically in this study. The spreading process is driven by the surface/interfacial tension forces.

Liposomes spread preferably along grooves while they are trapped by the groove edges in the direction perpendicular to grooves. The difference in the equilibrium contact angles between the two directions has been found out, and the elongation of the liposome has been characterized. Based on the experimental results, the theoretical model under the framework of non-equilibrium thermodynamics has been applied to describe the dynamic spreading process. By matching the numerical simulation results with the experimental results, the validity and the feasibility of the model has been demonstrated. Furthermore, the mobility of the contact line has been determined for the spreading of giant liposomes on anisotropically patterned substrates. The results show that the spreading is faster in the direction parallel to grooves, which is consistent with the results obtained from the spreading of liquid droplets on anisotropically wrinkled substrates.

Generally, the spreading behavior of giant liposomes is similar to that of liquid droplets. However, from the observations in this study, for the spreading of a giant liposome, the change of the contact angles and the liposome dimensions is not as sharp as that of a liquid droplet at the initial stage. In addition, the time to reach its equilibrium state for a liposome is shorter than that for a liquid droplet.

Chapter 7 Conclusions and Future Work

7.1 Conclusions

The objective of this project is to study the spreading of liquid droplets and giant liposomes on solid substrates. The main achievements on the static and dynamic spreading of liquid droplets and giant liposomes on smooth and patterned substrates are summarized in this section.

7.1.1 Spreading of liquid droplets

The static and dynamic spreading of liquid droplets has been studied on the smooth, isotropically wrinkled, and anisotropically wrinkled substrates. Five liquids are used, including DI water, SDS (CMC), SDS (10%CMC), glycerol, and water-glycerol mixture (volume ratio 1:1). Among them, DI water, SDS (10% CMC) and SDS (CMC) have different surface tensions but almost identical viscosities, while DI water, water-glycerol mixture and glycerol have different viscosities, but their surface tensions are similar. The investigation is within the scope of continuum physics. The energy related to surfaces dominates the spreading while the energy related to volumes is negligible. The effect of droplet size on the equilibrium contact angles and droplet shapes has been studied. Wetting anisotropy has been quantified. A theoretical model has been set up under the framework of non-equilibrium thermodynamics. The spreading speed has been characterized by the mobility of the contact line which has been obtained from the comparison between simulation results and experimental results. Nonlinear curve fitting has been conducted to further quantify the spreading speed through the critical velocity. The results show:

- Wrinkling is an effective method to fabricate model substrates for the study of spreading. The resulted substrates are reproducible and reusable.
- On the smooth and isotropically wrinkled substrates, droplets keep the shape of a spherical cap. Gold coating thickness and surface roughness has no apparent influence on the equilibrium contact angles. There is no droplet size effect on both smooth and isotropically wrinkled substrates.
- On the anisotropically wrinkled substrates, droplets have different contact angles along the contact line and elongate along wrinkles. There is limited effect of droplet size: the equilibrium contact angles increase with increasing the droplet size; the droplet elongation gets smaller when the droplet size is larger. This trend is more obvious for smaller droplets than for larger droplets. The size effect is due to the anisotropic patterns. Wrinkle wavelength has effect on the equilibrium contact angles and the degree of wetting anisotropy.
- Different liquids exhibit different spreading behaviors. Less viscous liquids reach equilibrium states in shorter time while more viscous liquids take longer time to reach equilibrium states. After adding surfactant (e.g. SDS (CMC)), the change of the contact angles and the evolution of the droplet dimensions with time become less steep than that of DI water.
- The mobility of the contact line is smaller for more viscous liquids than that for less viscous liquids. The mobility is larger in the direction parallel to wrinkles than that in the perpendicular direction.
- The critical velocity is dependent on the liquid viscosity and the surface tension. It decreases with the liquid viscosity, but increases with the surface tension.

7.1.2 Spreading of giant liposomes

Giant liposomes with average diameters larger than 10 μm have been prepared from DOPC and the mixture of DOPA and DOPC (1:9) under AC electric fields. Smooth and anisotropically patterned substrates have been used to study the spreading of giant liposomes. A spreading device has been fabricated using polymer sheets and silicon substrates with the spreading substrate being vertically fixed. The investigation is within the scope of continuum physics. The surface/interfacial tensions govern the spreading process while the gravitational force is negligible. The theoretical model proposed for droplet spreading has been applied to investigate liposome spreading. The spreading speed has been characterized by the mobility of the contact line which has been obtained from the comparison between simulation and experimental results. The results show:

- Electroformation is a more effective way to produce pure spherical giant liposomes than spontaneous formation method. Adding a small amount (10%) of negatively charged lipid (DOPA) to neutral lipid (DOPC) does not inhibit the production of giant liposomes, but results in liposomes with larger average sizes.
- Liposomes exhibit partial wetting behaviors on the smooth and anisotropically patterned substrates with the equilibrium contact angles larger than 90° .
- On the smooth substrates, the equilibrium contact angles are the same along the contact line, and the shape of liposomes can be approximated to be a spherical cap.
- On the anisotropically patterned substrates, the equilibrium contact angles are different when measured in the directions parallel and perpendicular to

grooves. Liposomes elongate along grooves, exhibiting a non-circular contact line.

- The thermodynamic model with the linear relationship between the contact line velocity and the driving force is valid in describing the liposome spreading process.
- The mobility of the contact line is larger in the direction parallel to grooves than that in the perpendicular direction.

7.2 Contributions of this work

To highlight the significance of this work, the main contributions made through this work are briefly summarized in this section.

The dynamic spreading of liquid droplets and giant liposomes is studied experimentally and numerically. Although the spreading of liquid droplets has been investigated by many researchers, most studies are on the static spreading, and only the equilibrium contact angles are reported. In this work, the dynamic spreading is studied with five liquids of different liquid properties and giant liposomes made from phospholipids. This provides information on the dynamic spreading behaviors, such as the evolution of dimensions and the change of contact angles. The influence of liquid properties and the effect of solid surface patterns are observed and characterized in this work. The phenomenological parameter, mobility of the contact line, is used to characterize the dynamic spreading, and its dependence on the liquid viscosity is found out, which contributes to the study on the influencing factors involved in the description of dynamic spreading via the

mobility of the contact line. The work on the dynamic spreading is of great scientific importance and leads to consideration in possible applications.

The spreading of giant liposomes is studied by considering liposomes as a kind of liquid instead of a solid which has been used in some other studies. Like liquid droplets studied in this work, surface tension forces govern the spreading of liposomes, and the spreading can be characterized by the contact angle the liposome makes with the substrate and the liposome dimensions. The experimental results and numerical results both show that liposomes exhibit the property of a liquid in the spreading process.

To study the spreading of giant liposomes, a self-made spreading device is designed and fabricated, which provides a simple and direct method to observe and record the dynamic spreading of giant liposomes at a low cost. Compared with the spreading of liquid droplets, there are extra difficulties to study the spreading of liposomes. The spreading device for liposomes must facilitate the manipulation of liposomes in aqueous solution and the observation of shape evolution under a microscope. If a liposome spreads on a horizontal substrate under a normal optical microscope with a top view, the contact angles could not be measured, and even the contact line could not be differentiated when the contact angle is larger than 90° . Some indirect methods have been employed to study the adhesion and spreading of liposomes by employing sophisticated equipments, such as reflection interference contrast microscope (RICM), but they add difficulty in operation and cost in maintenance. In this work, a spreading device with a vertically fixed spreading substrate is designed and fabricated. With this device, the dynamic

spreading is directly observed from a microscope, and the liposome dimensions and contact angles are measured directly from the microscopic images. Considering the flexibility of this device, it can be used beyond this study: the substrate can be modified chemically or mechanically according to the requirements of the study. In addition, this device could be used to study the interactions between biological entities and solid substrates, such as cell adhesion and spreading.

7.3 Suggestions for future work

The study of wetting and spreading of droplets and liposomes can be extended. Some recommendations are made based on the achievements of this project.

7.3.1 Spreading of liquid droplets and bi-wettability on a single substrate

The observations made in this project show that the degree of wetting anisotropy could be adjusted by varying pattern dimensions. Appropriate pattern designs and pattern dimensions can lead to a large contact angle difference when measuring from the two orthogonal directions. Surfaces exhibiting hydrophobicity in one direction and hydrophilicity in another direction can be fabricated by creating anisotropic patterns on solid substrates. Extreme wetting properties, superhydrophobicity and superhydrophilicity, may be achieved if further applying chemical modification to the patterned substrates.

To study the dynamic spreading of liquid droplets on the substrates of bi-wettability, it is essential to employ a high speed camera for the experiments. As shown in the results obtained from this project, droplets spread very fast in the initial period. Much higher capturing speed than that used in this project is needed to record the spreading process in the very initial period.

7.3.2 Fusion and poration of giant liposomes

The process of fusion is as important as spreading for some biological events. Fusion is the phenomenon that two initially separated liposomes form one bigger liposome by fusing parts of their membranes into a single lipid bilayer. Under an electric field, the shapes of liposomes will be distorted, and fusion is expected to take place. The electrofusion device can be fabricated via microfluidic techniques. The potential influence of electric fields, lipid compositions, and liposome size can be studied.

In addition to electrofusion, electroporation is another important membrane behavior which is of great biological significance. Previous studies have shown that pores can be generated during electrofusion processes. Thus, electroporation can be investigated when studying the electrofusion phenomenon. The effect of lipid compositions and liposome size on the formation of pores can be studied. The parameters of the electric field can be adjusted to facilitate the electroporation process. Similar with liposome spreading, electrofusion and electroporation are also fast events. It will be beneficial to use a high speed camera to study these phenomena. For the electrofusion and electroporation phenomena, theoretical

models may be set up to describe the process and to understand the physics behind the phenomena. Potential applications can be expected.

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