Budding Dynamics of the Lipid Membrane

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Liposomes are widely used in industrial engineering systems including cosmetics and pharmaceutical and biotechnological applications. A fundamental understanding of the dynamics of lipid bud formation is required to efficiently produce various high-technological liposomes with well-controlled sizes and shapes. In this study, a novel patterning method of controlling the uniformity of the sizes and shapes of liposome buds nucleated from a flat lipid film casted on a flat substrate is reported. The dried-out lipid components had been swelling during aqueous hydration, and they pinched away in approximately one hour. The sizes and spacing of the liposome buds were monitored during hydration. It was observed that the average size of the buds slowly increased, but their spacing did not significantly change. Moreover, the bud size distribution was a very narrow Gaussian, which implies the formation of buds with uniform sizes. The analytical calculation of the equilibrium state was theoretically developed and compared with the experiments. It is envisioned that this study will provide insights on a sustained-release drug vehicle.

Keywords: Liposome, Spin-Coating, Budding.

1. INTRODUCTION

As mimics of the dynamic cellular membranes in a living organism, lipid molecules are primarily self-assembled into spherical bilayers owing to the hydrophobic effect.¹ Even with a low critical micelle concentration (e.g., 0.46 nM in the case of 16:0 phosphocholine), such hydrophobic interaction is sufficient to form a stable bilayer structure, which has been studied in various fields, such as mathematics, physics, chemistry, biology, pharmaceutics, and medicine.³

Owing to the intrinsic biocompatibility and simple manipulability of a vesicle, a liposome have been used as common drug carriers in the pharmaceutical industry.⁴ Several liposome species have been utilized as novel stimuli drug delivery vehicles because they respond to various stimuli, such as the composition, surface charges,⁵ and sizes of liposomes,⁶ and the pH⁷ and temperature⁸ variation in the environment.⁹ In particular, the uniform sizes of liposomes is one of the dominant factors contributing to systemic administration through an intravascular drug delivery pathway.¹⁰,¹¹ It is well known that the liposomal size plays a significant role in in-vivo biodistribution.¹²,¹³ Liposomal size control is currently generally achieved using either of two popular techniques: sonication¹⁴ or an extrusion.¹⁵ Sonication changes the sizes of liposomes through the strong agitation of sound waves.¹⁶ Hence, nucleated liposomes are smaller than those prepared simply by shaking. Two decades ago, Papahadjopoulos et al. developed an extrusion method where liposomes are permeated through a polycarbonate membrane with a regular pore size.¹⁷ Even though this method is very reliable in controlling the liposomal size, it is generally considered inconvenient and time-consuming in the entire process and few studies have been undertaken to allow it to be effectively controlled as designed.

Estes and Mayer et al. have presented a spin-casting method that is capable of producing a homogeneous lipid film on a two-dimensional substrate.¹⁸ They optimized the lipid concentration and the spinning speed to be able to control the thickness and homogeneity of such lipid film, after which they obtained the optimal thickness of the homogeneous lipid film to generate giant liposomes via electroformation. The nucleated giant vesicles made through this method are two to five times larger than those made through the conventional droplet-derived-film technique. Another method involves the use of chemical additives. Kanta Tsumoto and Hideki Matsuo et al. reported that the sugar moieties involved in lipid films may enhance the repulsion between the lipid lamellae,
and they confirmed through the study that this may help obtain more and larger giant liposomes with a narrow size distribution. Alternatively, there have been some efforts to systematically observe and understand the lipid budding process for size control. The budding process of lipid vesicles has been observed with varying lipid compositions to come up with fluid- and gel-like domains. The bud growth results from the fluid-like domains of the lipid vesicles above the phase transition temperature of the fluid-like domain, and a morphological change was observed over time. The modeling study of the budding process has also been investigated.

In this study, the spatial distribution and time evolution of lipid vesicles budding from a flat film were investigated. The combined experimental and theoretical results on the formation of lipid buds are presented. In the experiments, lipids were deposited on a flat surface and were monitored during hydration at a fixed temperature. A theoretical model was developed by considering various energies related to the elastic deformation of lipids. In Section 2, the experimental methods that were used in this study to characterize the dynamics of the lipid budding process are explained. The details of the developed theoretical model are presented in Section 3. In Section 4, the results of the use of the theoretical model are compared with the experimental results. Discussion is featured, and the future research directions are presented, in Section 5.

2. EXPERIMENTAL DETAILS

An experimental method of studying the budding process from the lipid film is presented herein. The strategy for preparing the lipid film for the budding-off test is outlined. A square glass substrate (12 × 12 mm) is first cleaned with a piranha solution (H₂SO₄:H₂O₂=7:3). A dry lipid component, DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids), is completely reconstituted into a mixing co-solvent of chloroform and acetonitrile at a 95:5 ratio. (It is reported that the most homogeneous lipid film formed via spin-coating is successfully achieved under the mixing ratio of 95% chloroform and 5% acetonitrile.) The stock concentration is in 2.5 mg/ml. The 50 µl lipid precursor solution is slowly poured at the center of the glass substrate until the glass substrate is entirely covered with the solution. The glass substrate is then moved to the chamber of a spin-caster and is immediately rotated at the speed of 600 rpm for 60 sec after which the substrate is coated with a homogenous lipid film (DOPC film) that has been dried under high vacuum for over 18 h to remove all traces of mixing organic co-solvents therefrom.

To observe the budding effects in uniform sizes, the dry DOPC film is hydrated by adding 100 µl deionized (DI) water prewarmed at room temperature. Images of the in-situ budding process are taken, using a phase contrast microscope (TE2000-U, Nikon), every min until 60 min after hydration. The images of lipid budding in a dry film and 20, 30, and 40 min after hydration captured and shown in Figure 2(A) are further analyzed using the Image J software (NIH) to determine the sizes (diameters) of the individual liposomes and the distances between them. In the microscope images, the liposomes show bright white dots against the background. The image intensity of the liposomes was measured using the plot profile of the Image J software. The boundaries of the liposome and of the background were estimated, and the number of pixels of the liposomes were converted to micrometer using the scale bars of the images.

3. THEORETICAL MODEL

The formation of micron-sized vesicles is a complicated non-equilibrium process involving elastic deformation and energetic dissipation of the lipid membrane and fluid bulk. When a solvent keeps in touch with a thin layer of membrane attached to a flat substrate, this membrane gets swollen and becomes unstable in space and time. Many small buds are then nucleated from the flat surface and are eventually detached from the remaining membrane. If this process occurs fast, dissipations will become predominant on both the membrane and the bulk. If the process is slow, a system satisfies the quasi-equilibrium state at any instance of time. The experiment that was conducted in this work was performed on time scales of an hour. The budding process was so slow that the equilibrium state is an acceptable assumption for the theoretical model developed herein.

The total energy due to the area increase during the formation of a vesicle mainly consists of viscous dissipations, elastic deformation and stretching. For the slow budding process, the dissipation is neglected because the dissipated energy in both the membrane and the bulk fluid is inversely proportional to the time scale. Then, the total energy is composed of three parts: the bending, edge, and stretching energies.

\[
E = \frac{1}{2} \kappa S \left( \frac{2}{R} - \frac{2}{R_c} \right)^2 + \lambda_2 \pi L + \sigma \left( S - \pi L^2 \right)
\]

where \( R \) is the radius of curvature, \( R_c \) is the intrinsic radius of curvature, \( S \) is the surface area of a bud, and \( L \) is the neck radius as shown in Figure 1. There are several physical parameters, the typical value of the bending modulus \( \kappa \) is about \( 4 \times 10^{-10} \) Nm, that of the line tension \( \lambda \) is about \( 5 \times 10^{-12} \) N, and that of the stretching modulus \( \sigma \) is about \( 1 \times 10^{-7} \) N/m.

As shown in Figure 1, cap area \( S \) is expressed as

\[
S = 2 \pi R^2 \left( 1 - \cos \theta \right) = 4 \pi R^2 \sin^2 (\theta/2) = 4 \pi R^2 B_\alpha,
\]

and the difference between the cap area and the projected area is

\[
S - \pi L^2 = \Delta S = S \delta = 4 \pi B_\alpha R^2 B_\alpha.
\]

where the radius of the projected area is

\[
L = \sqrt{\frac{2}{\pi} (1 - B_d)} = 2 R \sqrt{B_d (1 - B_d)}.
\]
where $B_d$ is the budding parameter describing the membrane geometry (i.e., $B_d$ is equal to zero for a flat surface and approaches unity for a $\theta = \pi$ closed spherical bud). Finally, total energy is calculated using the following equation:

$$E = 8\pi\kappa B_d(1 - R/R_c)^2 + \lambda 4\pi R^2 \sqrt{B_d(1 - B_d)} + \sigma 4\pi R^2 B_d^2$$

When the bud is about to be detached from the substrate, $B_d$ becomes unity, and the line tension is not important. At this moment, the energy has a local minimum value at a given $R$. This minimum value can be obtained by taking a derivative on the total energy with respect to $R$. The energy minimum is achieved when the bud radius becomes

$$R = \frac{R_c}{\sqrt{\sigma R_c^2 + 1}}$$

where $\sigma = \sigma / 2\kappa$ is about $1.25 \times 10^{11}$ m$^{-2}$. The bud radius ($R$) has a maximum value when $\frac{\partial R}{\partial R_c} = 0$, that is, when $\sigma R_c^2 = 1$. When put back to Eq. (1), $R$ will have a maximum value of about 1.4 $\mu$m, which is close to the observed value (the average diameter of the ~2 $\mu$m bud). The uncertainty comes from the estimated values of $\kappa$ and $\sigma$.

At this point, the above analysis is generalized to predict the changes in the bud size over time. In this case, it is not necessary for budding parameter $B_d$ to be in the middle of the budding process. By modifying the above analysis, the energy is minimized at

$$R = \frac{R_c - R_c^2 \lambda \sqrt{(1 - B_d)/B_d}}{\sigma R_c^2 B_d + 1}$$

where $\lambda = \lambda / 4\kappa$. Then, the bud size will have a maximum value when the intrinsic radius becomes

$$R_c = \frac{-\lambda \sqrt{(1 - B_d)/B_d} \pm \sqrt{\lambda^2(1 - B_d)/B_d} + \sigma B_d}{\sigma B_d}$$

The time scale of the budding process is presumably governed by the molecule diffusion to the membrane matrix. It is assumed that the surface area of the membrane increases in time as $\sqrt{S/\pi} \sim \sqrt{D t}$, where $D$ is the diffusion coefficient. This implies that budding parameter $B_d$ linearly increases in time as $B_d \sim t / T$, where $T = 4\pi R^2 / D$. By substituting $B_d$ with the normalized time in Eqs. (2) and (3), the time evolution of bud radiiuses can be obtained. (The further results are shown in the next session.)
**Fig. 2.** (A) Images of the lipid film before and after hydration, obtained using a brightfield/phase contrast microscope. The lipid film had a clear, flat surface before hydration and began to wrinkle and bud after hydration (scale bar: 10 μm). (B) Average diameter and spacing of the buds. The bud size slowly increased while the spacing between the buds was almost constant over time. (C) Size distribution of the buds depending on the time (min). (D) Spacing distribution between the buds depending on the time (min). The size and spacing distribution of the buds followed the Gaussian distribution.

**Fig. 3.** Equilibrium size of a bud versus a normalized time. The blue line is from theoretical model and the red circles are from the experiments.

which is not yet well understood. This may provide size-controllable and systematically patterned liposomes with the higher loading efficiency of drugs.

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**References and Notes**

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