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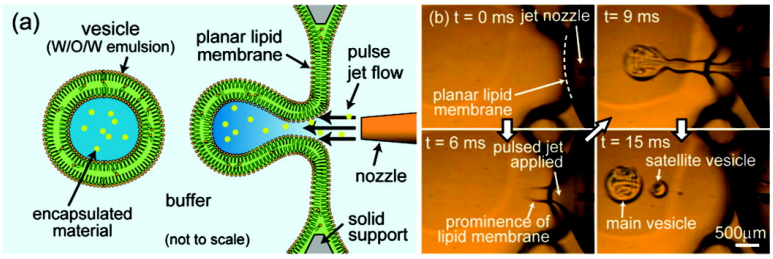

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Formation of Giant Lipid Vesiclelike Compartments from a Planar Lipid Membrane by a Pulsed Jet Flow

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Ⓦ This paper contains enhanced objects available on the Internet at http://pubs.acs.org/journals/jacsat.

Encapsulation of chemicals or biological materials in small vesicles has been recognized as an important technology for medical and biological applications such as drug delivery,[1] bioreactors,[2] and artificial cell systems.[3,4] Size uniformity as well as high entrapment efficiency have become key characteristics for single-vesicle based assays.[3] Liposomes (lipid vesicles) and emulsions are widely used for these purposes.[5] However, despite huge efforts, producing liposomes of a uniform size has proven difficult. Monodisperse emulsions can be achieved using T-junction[6] or flow-focusing[7] in microfluidic systems. To form lipid vesiclelike compartments from emulsions, a chemically engineered process after emulsification has been proposed.[8]

We report a straightforward way of encapsulating biological water-soluble materials inspired by forming soap bubbles from a soap film. In this method vesicles are mechanically blown out of a preformed planar lipid membrane, directly encapsulating ejected materials (Figure 1a). A planar lipid membrane (1×1 mm^2) was formed vertically by contacting two water droplets surrounded by an organic solvent (*n*-decane) that contained phospholipids (L-α-phosphatidylcholine purified from egg, Avanti polar lipids, Inc.).[9] By applying a short pulsed liquid jet flow ejected from a fine jet nozzle brought near the membrane, the membrane deforms, and a vesicle is generated. Using this method allows direct encapsulation of molecules or substances into the vesicles in a short time, eliminating postprocessing such as centrifugation,[3,10] dialysis,[5] and digestion of molecules bound to the outer surface.

A pulse jet flow was created by controlling the opening of the electromagnetic valve of a commercial microdispenser located between a glass capillary nozzle ($\phi = 60$ μm) and an air compressor (see Supporting Information for details). Liquid to be ejected was filled in the tip of the glass nozzle and ejected against the planar lipid membrane. When the valve was opened for 15 ms (dispensing time, t_D) at 60 kPa (pressure at the dispenser, P), vesicles of $\phi = 650$ μm were generated (Figure 1b, also see the movie file). When the jet was applied, the membrane deformed and stretched significantly, and the neck of the stretched column was pinched off in 10 ms. The velocity of the membrane elongation was 0.5 mm s^{-1}. Satellite vesicles were generated occasionally. This process could be repeated to form a number of vesicles (Figure 2a, also see movie).

We tested another dispenser that operated at a shorter t_D in order to produce smaller vesicles and to confirm that this phenomenon does not depend on a specific device. When the jet was applied at $t_D = 1.5$ ms and $P = 400$ kPa, vesicles of $\phi \approx 300$ μm were generated (Figure 2b). By varying t_D, the diameter was controlled in the range between 300 and 400 μm with the coefficient of variation smaller than 10% (Figure 2c, solid symbols). With longer t_D, the planar membrane was greatly stretched, and the size of the satellite vesicles also increased (Figure 2c, open symbols). Note that their diameters were also uniform. The frequency of vesicle generation reached up to 4 Hz.

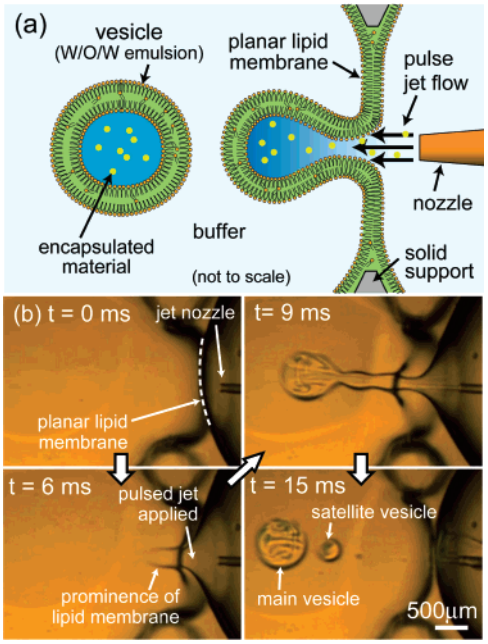

Figure 1. (a) Conceptual diagram of "blowing vesicle" method. Green area represents organic solvent. (b) Sequential images of vesicle formation captured by a high-speed CCD camera. Planar membrane was stretched to form a column and broken up to spherical vesicles within 10 ms.

Ⓦ A movie in MPG format is available.

The most important advantage of this method is that any materials ejected are directly encapsulated, regardless of their size, concentration, or chemical properties. As a demonstration, we encapsulated Jurkat cells and chromosomes from Hela cell cytosolic extract (Figure 3). During the experiment we found that there is a back-flow into the nozzle between consecutive pulses; this back-flow diluted the concentration of the encapsulated materials. Thus, to achieve uniform chemical concentration, the solution around the nozzle has to be the same as that in the nozzle.

We estimated the vesicle shell thickness from the principle of buoyancy (see Supporting Information for detail). When the solution both inside and outside of the vesicle was DI water ($\rho = 1.00$),

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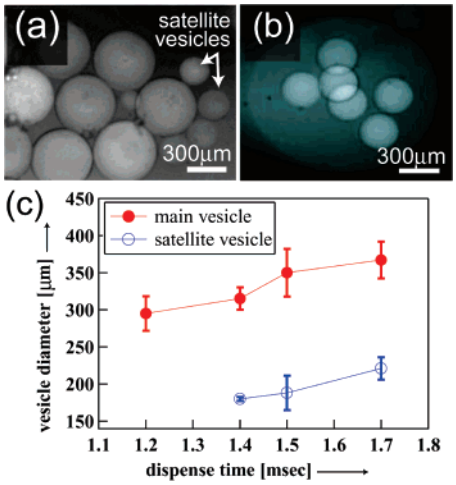

Figure 2. (a,b) Microscopic images of vesicles generated at $t_D = 10$ ms and 1.5 ms, respectively, with different dispensers. Uniform vesicles with 500−600 and 300−400 μm diameter were generated. Fluorescent dye (calcein) was encapsulated for visualization. (c) Vesicle diameter of main and satellite vesicles at varying t_D's.

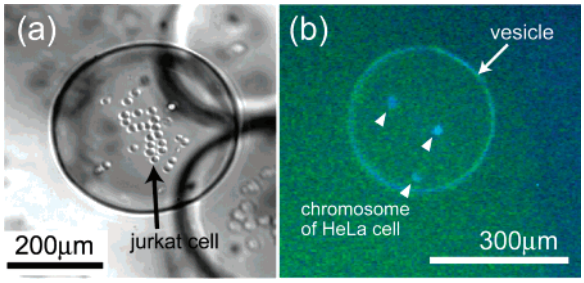

Figure 3. (a) Microscopic image of Jurkat cells and (b) fluorescent image of stained chromosome from HeLa cell, encapsulated in the lipid vesicle.

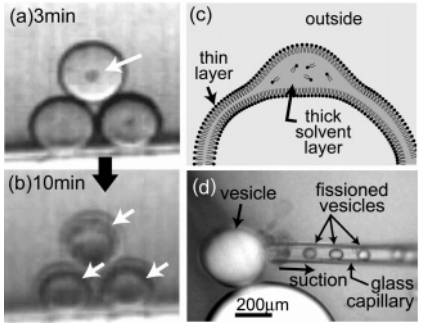

Figure 4. (a,b) Localization of organic solvent to the top of the vesicles (indicated by arrows). (a) 3 min and (b) 10 min after generation. Large circle gradually appeared on top of the vesicles. (c) Model of solvent localization (sectional view of a vesicle). (d) Fission of vesicles. Fine glass capillary (60-μm inner diameter) was brought into contact, and suction was applied. Small vesicles were generated inside the capillary.

Ⓦ Sequential images of vesicle fission captured by a high-speed CCD camera in MPG format is available.

vesicles floated, indicating that there is a layer of solvent (*n*-decane, specific density $\rho = 0.734$) which is lighter than water. Thus, we first estimated the volume of the solvent by observing if vesicles containing solution of larger density sank or floated, and calculated the thickness of the shell. Vesicles of $\phi = 330$ μm containing 300 mM sucrose ($\rho = 1.103$) sank in a 400 mM glucose solution ($\rho = 1.072$), while the same vesicles floated in 300 mM glucose solution ($\rho = 1.054$). With some simple math, the thickness of the vesicle shell turned out to be between 4.6 and 7.5 μm. Hence, vesicles right after being generated were W/O/W emulsions having a thin shell (2% of the vesicle diameter) of organic solvent. We postulate that solvent supporting the outer rim of the planar membrane was engulfed when the planar lipid membrane was stretched.

We also found a phenomenon that indicates thinning of the vesicle shell. For continuous observation, vesicles were sucked into a glass tube and released into another chamber that has an array of pillar cage[11] (see movie via link in the figure caption for Figure 4). Typically after 5 to 10 min, a small ring similar to a bump gradually appeared on top as a result of the accumulation of solvent that was driven by buoyancy (Figure 4a−c). Thus, the shell must have become thinner in the other area. A similar phenomenon was reported in a W/O/W emulsion containing amphiphilic diblock copolymer.[12]

Smaller vesicles need to be produced for applications such as cell-sized bioreactors. However, when the finer nozzle was used, the large pressure drop meant that no strong jet was generated. Thus, the limitation came from the experimental setup, not from the physical mechanism of vesicle generation. Smaller vesicles were produced by sucking a large vesicle (~300 μm) into a fine glass capillary ($\phi \approx 60$ μm, Figure 4e, and movie). In addition, the shell thickness of those fissioned vesicles should be thinner as a result of the larger surface-to-volume ratio.

In conclusion, we demonstrated the direct encapsulation of an aqueous solution into W/O/W vesicles with an extremely thin solvent layer (2% of the vesicle diameter). Vesicles in uniform size ($\phi = 300-600$ μm) were generated repeatedly. While many interesting aspects remain to be characterized, such as the embedded mechanism of membrane deformation and fission and the physicochemical properties of vesicles, we believe this encapsulation method will provide broad applications in chemical and biological researches.

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Supporting Information Available: Materials and methods, and estimation of vesicle shell thickness. This material is available free of charge via the Internet at http://pubs.acs.org.

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