Optical transport of a single cell-sized liposome

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A closed phospholipid membrane vesicle, or giant liposome, with a diameter of ~10 μm, constitutes a model of a living cell. Since the phospholipid membrane is about 5 nm thick, it is extremely difficult to trap an individual giant liposome with a laser. We report here the intact transport of an individual cell-sized liposome using an infrared laser. It is shown that the optical attractive potential on a liposome with an inner solution of higher refractivity is about one order of magnitude greater than that of a liposome with the same solution both inside and outside. Since this cell-sized liposome is rather stable, the optical manipulation of liposomes could be useful for the realization of a μm-scaled laboratory for use in biochemistry and molecular biology. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1430026]

All living organisms maintain their lives by making a μm-sized biosphere within a closed membrane several nm thick. The main constituent of this biological membrane is a phospholipid bilayer.[1,2] A closed phospholipid bilayer membrane, or liposome,[3] has been actively studied as a model of a living cell membrane. Most previous studies on liposomes have been devoted to rather small vesicles smaller than 0.1 μm in diameter. Experimental procedures to obtain such small vesicles from phospholipids are well established, including sonication and gel filtration. In contrast to the extensive studies on such small phospholipid vesicles, reports on cell-sized liposomes larger than 1 μm are rather limited.[4,5] Surprisingly, there have been very few studies on the preparation of cell-sized liposomes with different chemical compositions inside and outside the membrane. In the present letter, we report a procedure for preparing cell-sized liposomes where the inner chemical composition is markedly different from that of the external environment. We show that such a cell-sized liposome containing a solution of higher refractivity can be efficiently trapped and transported by a focused laser.

Figure 1 shows an overview of the experimental system for optical trapping under microscopy. The infrared laser used for optical trapping was a Nd: yttrium–aluminum–garnet laser (SL902T, Spectron), TEM_{00} beam at a wavelength of 1064 mm. The laser was reflected by a dichroic mirror, and focused by an objective lens (Nikon, Plan Fluor $\times 100$, NA$=1.30$) with a microscope (Nikon, TE-300). The beam power was monitored by a laser power meter (Neoark, PM-345). Liposomes were observed and operated upon through the same objective. The microscope image was captured by a charge coupled device camera connected to a video recorder through an image processor (Argus50, Hamamatsu Photonics). We prepared unilamellar vesicles ranging in diameter from 0.1 to 10 μm (attoliter to nanoliter spheres) by the natural swelling of lipid films of phosphatidyl-glycerol (PG, Sigma).[6–8] Liposomes of PG are negative charged and disperse well in aqueous solution. The refractive indices of the media used in the present study are given in Table I, as measured using a DRM-1021 (Otsuka Electronics) with 633 nm light at 21 °C. Under the same osmotic pressure, D-glucose solution has a higher refractivity than NaCl solution. We prepared liposomes in a solution of either glucose or NaCl, and removed the outside medium by low-speed sedimentation (~10 g). We then added the desired equiosmotic solution to the system. The resulting liposome is stable and does not fuse or break at room temperature for at least one week. The liposome solution was observed and measured in phase-contrast mode.

Figure 2 shows the optical transport of liposomes; in Fig. 2(a) the solutions inside and outside the liposome are the same, pure water, whereas in Fig. 2(b), the inner and outer solutions are 100 mM glucose and 50 mM NaCl, respectively. As expected, it is rather difficult to trap the liposome with the same internal and external solutions. When trapping is tried in a solution with a higher refractivity than pure water, i.e., in salt or glucose solution, trapping is even more difficult. During transport the trapped liposome often escaped from the focus due to the viscosity of the solutions. In addition, the liposome is considerably deformed by the focused laser, as shown in the figure. Thus, local tension is induced at the center of trapping on the membrane. Similar

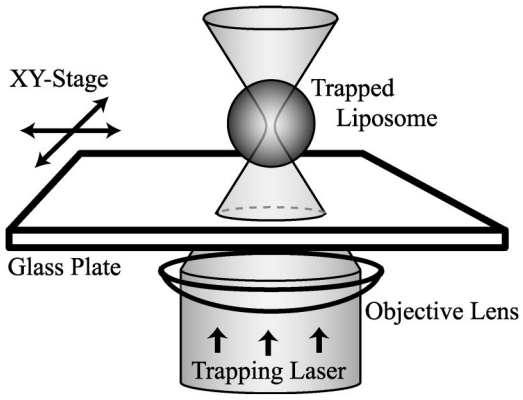

FIG. 1. Schematic representation of the experimental setup. The laser beam for optical trapping was focused through an objective lens with a 1.3 numerical aperture. To transport the liposome, the stage was moved in a horizontal *XY* plane.

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TABLE I. Refractive indices.

Solution	n
Pure water	1.3333
D-glucose, 100 mM	1.3360
NaCl, 50 mM	1.3339

deformation effects in liposomes induced by a focused laser, such as pearling and fission, have been noticed in past studies.[9–11] On the other hand, when the inner solution has a higher refractive index than the outer solution, the liposome is strongly trapped and safely transported without serious transformation, as in Fig. 2(b). During transport, the trapped position is shifted from the center of the spherical liposome, and this is attributed to competition between viscous dragging and the optical force.

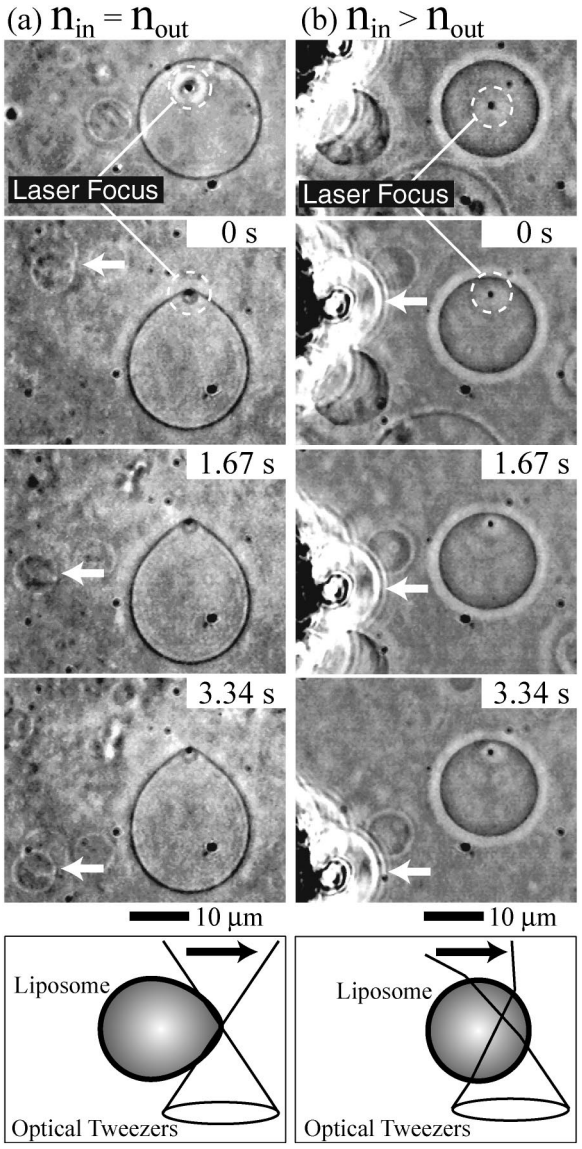

FIG. 2. Transport of giant liposomes by optical tweezers. Scale bar represents 10 μm. The white arrows in the images indicate other liposomes as landmarks in relative motion. The liposomes are trapped and transported by an infrared laser of 500 mW. The dark spots indicate the focus. In the schematic illustrations at the bottom of the figure, the black arrows indicate the directions of transport. (a) The inside and outside solutions are the same [pure water (Milli$Q$)]. (b) The inside solution, 100 mM glucose, has a higher refractivity than the outside solution of 50 mM NaCl.

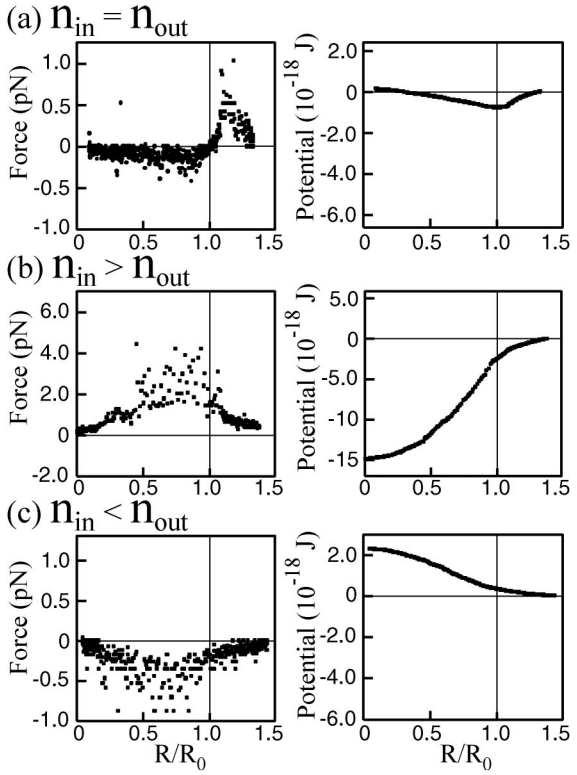

FIG. 3. Optical force and potential as a function of distance from the center of the liposome. R/R_0 is the normalized length, where $R/R_0 = 1$ is the surface of the liposome. (a) Inside and outside, both 100 mM glucose. (b) Inside, 100 mM glucose; outside, 50 mM NaCl solution. (c) Inside, 50 mM NaCl; outside, 100 mM glucose.

To compare the efficiency of trapping in a quantitative manner, we measured the effective optical force by considering viscous drag.[12] The results are summarized in Fig. 3. Using unilamellar liposomes of 5–10 μm ($\gg\lambda$; wavelength of light) in radius, we measured the dynamic responses of the position of the giant liposomes based on an analysis of video images (1/30 s). R/R$_0$ is the normalized radial distance from the center of the liposome, where R/R$_0$ = 1 corresponds to the position of the membrane. The terms n_{in} and n_{out} are the refractive indices of the inner and outer solutions. From the profile of optical force in experimental measurements on the XY plane, we evaluated the effective potential, as shown on the right side of Fig. 3. The effective attractive potential on a liposome with an inner solution of high refractivity [Fig. 3(b)] is about one order of magnitude greater than that of a liposome with the same solution both inside and outside [Fig. 3(a)]. When the refractivity of the inner solution is lower than that of the outer solution, the potential is always positive with respect to infinite distance [Fig. 3(c)]. This again indicates that the solution composition has a greater effect than direct trapping of the thin membrane. Let us briefly discuss the difference in trapping efficiency mentioned above. In the case of trapping with a thin membrane as in Figs. 2(a) and 3(a), we adapt the theory of a Rayleigh regime, since the thickness of the membrane is about 5 nm, which is two orders of magnitude smaller than the wavelength. The optical force acting on point $\mathbf{r}$ of the liposome can be expressed as[13,14]

$$\mathbf{f}(\mathbf{r}) = \alpha(\mathbf{r})\left\{\frac{1}{2}\nabla\mathbf{E}(\mathbf{r})^2 + \frac{1}{c}\frac{\partial}{\partial t}[\mathbf{E}(\mathbf{r})\times\mathbf{B}(\mathbf{r})]\right\}, \quad (1)$$

where **E** and **B** are electric and magnetic fields, c is the speed of light, $\alpha = 3{n_m}^2(m^2-1)/(m^2+2)$ is the polarizability per unit volume under the dipole approximation, n_o and n_m are the refractivities of the object and the surrounding medium, and $m = n_o/n_m$ is the relative index. The total force acting on the liposome is

$$\mathbf{F} = \int \mathbf{f}(\mathbf{r})d\mathbf{r}. \tag{2}$$

Equation (1) means that no optical force acts on the volume of the liposome under the condition $m=1$, i.e., with the same solution inside and outside the liposome. Thus, the laser tends to cause deformation of the liposome rather than transportation. As for stable trapping of the liposome at $n_{\text{in}}/n_{\text{out}} = 1.0021 > 1$, as in Figs. 2(b) and 3(b), the regime of geometrical optics in a dielectric sphere is applicable.[15,16] Irradiated laser light is refracted and reflected at the membrane interface between the inside and outside solutions. The liposome undergoes counteractions from the refraction and reflection of light. In such a ray optics regime, the trapping force is expressed as $F = (n_{\text{out}}P/c)Q$, where $(n_{\text{out}}P/c)$ is light momentum per second, and Q is the trapping efficiency. The efficiency Q in Fig. 3(b) is about 0.01,[17] whereas for the case in Fig. 3(a), $Q \sim 0.001$.

Up to now, optical manipulations of cell-sized liposomes have been carried out almost only for phospholipid membranes containing the same solutions as the bathing solutions. Under such experimental conditions, only the thin layer of the membrane can be effectively trapped by a laser. Thus, the trapping efficiency has been rather low,[18] about 0.4 pN. In addition, serious effects of the focused laser have been noted[10] when the laser power is made strong enough to ''trap the membrane.'' Due to this difficulty in optical trapping, liposomes have historically been manipulated mostly using tapered micropipettes,[19] despite the advantages of laser trapping, handiness, simplicity of the system, and lack of adhesion. In contrast, laser force seems to have been merely used to deform the membrane, thus causing tension and fission,[11] except for the trapping of smaller liposomes 1 or 2 μm in diameter.[20,21]

In the present study, we have demonstrated the efficient optical trapping of cell-sized liposomes. Such cell-sized liposomes can carry various chemicals, including enzymes, DNA and RNA.[22] Giant liposomes are also reasonably stable for up to several weeks, in contrast to smaller liposomes. In addition, the efficiency of trapping can be enhanced by using suitable inner and outer solutions. The application of this technique may be useful for the future construction of a μm-scale laboratory for use in biochemistry and molecular biology.

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