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A Membrane Filtering Method for the Purification of Giant Unilamellar Vesicles

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ABSTRACT

The use of giant unilamellar vesicles (GUVs) for investigating the properties of biomembranes is advantageous compared to the use of small-sized vesicles such as large unilamellar vesicles (LUVs). Experimental methods using GUVs, such as the single GUV method, would benefit if there was a methodology for obtaining a large population of similar-sized GUVs composed of oil-free membranes. We here describe a new membrane filtering method for purifying GUVs prepared by the natural swelling method and demonstrate that, following purification of GUVs composed of dioleoylphosphatidylglycerol (DOPG)/dioleoylphosphatidylcholine (DOPC) membranes suspended in a buffer, similar-sized GUVs with diameters of 10-30 μm are obtained. Moreover, this method enabled GUVs to be separated from water-soluble fluorescent probes and LUVs. These results suggest that the membrane filtering method can be applied to GUVs prepared by other methods to purify larger-sized GUVs from smaller GUVs, LUVs, and various water-soluble substances such as proteins and fluorescent probes. This method can also be used for concentration of dilute GUV suspensions.

Key Words:

Giant unilamellar vesicle (GUV), oil-free membrane, purified GUVs, swelling method, concentrated GUVs, separated GUVs.

1. Introduction

Giant liposomes or giant unilamellar vesicles (GUV) of lipid membranes with diameters greater than 10 μm have been used to investigate the physical and biological properties of membranes such as elasticity, shape change, and phase separation (Evans and Rawicz, 1990; Farge and Devaux, 1992; Saitoh et al., 1998; Olbrich et al., 2000; Tsumoto et al, 2001; Tanaka et al., 2002; Yamashita et al., 2002; Baumgart et al., 2003; Lopez-Mentero et al., 2005; Khalifat et al., 2008). The shape of a single GUV, and its physical properties such as phase separation in water, can be measured in real time. Moreover, changes in the structure and physical properties of single GUVs, induced by interactions with substances such as peptides and protein, can be observed as a function of time and spatial coordinates. If we make this kind of experiment using many "single GUVs" under the same condition and make the statistical analysis of the results, such as the changes in the physical properties of a single GUV, over many "single GUVs", we can obtain detailed information (such as a kinetic constant) on elementary processes of various phenomena such as membrane fusion and the antimicrobial peptide-induced pore formation in lipid membranes. (the single GUV method) (Tanaka and Yamazaki, 2004; Tanaka et al., 2004; Tamba and Yamazaki, 2005, 2009; Tamba et al. 2007, 2010; Yamazaki, 2008). Therefore, studies of biomembranes using GUVs have many advantages over comparable studies using small-sized vesicles such as large unilamellar vesicles (LUVs) of lipid membranes.

The efficiency of many experiments involving GUVs, and particularly experiments utilizing the single GUV method, would be much improved if purified, similar-sized GUVs could be used. Several methods for the preparation of GUVs have been reported (Walde et al., 2010). The natural swelling method and the electroformation method (Angelova and Dimitrov, 1986) are popular and have the advantage that the membranes of these GUVs do not contain hydrocarbon oils. However, the GUVs produced by the natural swelling method have a large size distribution. On the other hand, several methods such as the water droplet transfer method (Pautot et al. 2003; Yamada et al., 2006) and the lipid-coated ice droplet hydration method (Sugiura et al., 2008) can produce similar-sized

GUVs, which can be useful in experiments that use the GUVs as vessels such as artificial cells and chemical reactors. However, the disadvantage of these methods (Pautot et al. 2003; Yamada et al., 2006; Sugiura et al., 2008) is that the GUV membranes contain significant amounts of hydrocarbon oils, which can greatly affect the physicochemical properties of lipid membranes.

Investigations into the structure and dynamics of lipid membranes, and into the interaction of substances such as proteins with lipid membranes, require the use of similar-sized GUVs which do not contain hydrocarbon oils. Consequently, the disadvantage of the natural swelling method must be overcome. We here describe a new method, the membrane filtering method, for producing a large population of GUVs of oil-free membranes with similar radii. Using this method we purified GUV suspensions prepared in a buffer by the natural swelling method and obtained similar-sized (10-30 μm) GUVs.

2. Materials and Methods

2.1. Materials

1, 2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and 1, 2-dioleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DOPG) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 2-(6-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (NBD-PC) was purchased from Invitrogen Inc. (Carlsbad, CA). Calcein was purchased from Dojindo Laboratory (Kumamoto, Japan). Bovine serum albumin (BSA) and $CoCl_2$ were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan).

2.2. Method for Purifying GUVs

GUVs were prepared in a buffer by the natural swelling of a dry lipid film at 37 °C as follows (Tanaka et al., 2002; Yamazaki, 2008). First, 100-200 μL of 1 mM 50 mol% DOPG/50 mol% DOPC (in short, DOPG/DOPC) in chloroform were placed in a glass vial (5 mL) and dried under a stream of N_2 gas to produce a thin, homogeneous lipid film. The solvent was completely removed

by placing the bottle containing the lipid film in a vacuum desiccator connected to a rotary vacuum pump for more than 12 h. Next, 10-20 μL water was added to the dry lipid film and the mixture was incubated at 45 °C for 10 min (prehydration). The hydrated lipid film was then incubated with 1 mL buffer A (10 mM PIPES, pH 7.0, 150 mM NaCl and 1 mM EGTA) containing 0.1 M sucrose for 2 to 3 h at 37 °C. For some experiments, we prepared these GUVs in a corresponding buffer containing 1 mM calcein. For most experiments, we prepared GUVs in 2-3 glass vials at the same time. After incubation, the GUV suspension was centrifuged at 14,000×g for 30 min at 20 °C to remove multilamellar vesicles (MLVs) and lipid aggregates. Between 1.5-1.8 mL of supernatant was used per GUV purification experiment. In some cases we filtered the supernatant containing GUVs after centrifugation with a Nylon net filter (41 μm diameter pores, Millipore Co., Billerica, MA) to remove completely MLVs and lipid aggregates.

For purification of GUVs we used the membrane filtering method. In this method, a GUV suspension such as the supernatant containing GUVs after the centrifuge described above is filtered through a nucleopore polycarbonate membrane with 12 μm diameter pores (Nomura Micro Science, Atsugi, Japan) using a homemade apparatus (Fig. 1A). In this apparatus, the nucleopore membrane is clamped in a polypropylene filter holder (Swinnex, ϕ = 25 mm, Millipore Co., Billerica, MA), and then the upper and lower side of the filter holder are connected to a 10 mL plastic (polypropylene) syringe (Top Co., Tokyo, Japan, or Terumo Co., Tokyo, Japan) (Syringe A in Fig. 1A) and a tube made of polypropylene (inner diameter 5 mm and length 11 cm) (Tube in Fig. 1A), respectively. The other end of the tube is connected to another 10 mL plastic syringe (Syringe B in Fig. 1A). After the apparatus was filled with buffer A containing 0.1 M glucose, all the air bubbles were carefully removed. Then, after removing the buffer from syringe B (~1 mL buffer should remain), a GUV suspension in buffer A containing 0.1 M sucrose was added to syringe B. After diluting the GUV suspension with buffer A containing 0.1 M glucose in syringe B, buffer A containing 0.1 M glucose was added to syringe B at a constant velocity (see below, and Table 1)

using a peristaltic pump (SJ-1220, Atto Co. Tokyo, Japan), and simultaneously the buffer in syringe A was removed at the same constant velocity using another peristaltic pump. The direction of the flow of buffer at the filter was from the bottom to the top, shown in Fig. 1A, so the smaller vesicles passed through the filter into the upper syringe (syringe A). After filtering for a defined time, the addition of buffer A containing 0.1 M glucose to syringe B was stopped, and then the buffer inside syringe A was removed by the pump at the same flow rate. Finally, the suspension in the tube and the filter holder was collected and used as a purified GUV suspension.

For the experiments to confirm the location of GUVs in the apparatus following purification (section 3.5) and those of the concentration of GUVs (section 3.6), we used a 1-way luer stopcock made of polycarbonate (VXB1055, ISIS Co., Ltd., Osaka, Japan), which was inserted between the filter holder and the tube (Fig. 1B). A GUV suspension was filtered using the apparatus (Fig. 1B) while the stopcock was open, and after the filtering the stopcock was closed. Then the solutions above the stopcock (i.e., in the space under the filter membrane) and in the tube below the stopcock were collected.

We investigated the size distribution of the GUVs using fluorescence, phase contrast microscopy. Purified GUV solution (300 μl; 0.1 M sucrose in buffer A as the internal solution and 0.1 M glucose in buffer A as the external solution) was transferred to a handmade microchamber (Tanaka et al., 2002; Yamazaki, 2008). A glass slide was coated with 0.1 % (w/v) BSA in buffer A containing 0.1 M glucose. GUVs in the chamber were observed using an inverted fluorescence phase contrast microscope (IX-71, Olympus, Tokyo, Japan) at a defined time (such as 30 min) after the suspension was transferred to the chamber. Phase contrast and fluorescence images of GUVs were recorded using a high-sensitivity fluorescence EM-CCD camera (C9100-02, Hamamatsu Photonics K.K., Hamamatsu, Japan) and stored on a hard disk.

2.3. Removal of calcein and LUVs during the purification of GUVs using the membrane filtering method

LUVs of DOPC/NBD-PC (molar ratio: 90/10) were prepared by the extrusion method (MacDonald et al., 1991) using 200 nm-pore membranes (Tamba and Yamazaki, 2005). The appropriate amounts of lipids in chloroform were mixed and dried under a stream of N_2 gas, then the solvent was completely removed by placing the sample in a vacuum desiccator connected to a rotary vacuum pump for more than 12 h. To prepare MLVs, 1 mL of buffer A containing 0.1 M glucose was added to the dry lipid film, and the suspension was vortexed several times for approximately 30 s at room temperature. Next, the suspension of MLVs was subjected to 4-5 cycles of freezing in liquid N_2 for 1 min, followed by warming to room temperature for 20 min (i.e., freeze-thawing). The resulting solution was extruded through a 200 nm-pore membrane using a LF-1 LiposoFast apparatus (Avestin, Ottawa, Canada) until the solution became transparent.

The concentration of calcein and of NBD-PC in LUVs was determined for various GUV suspensions by absorbance measurements using a Shimadzu UV-1200 spectrophotometer (Shimadzu Co., Kyoto, Japan). The absorbance wavelength used for calcein and NBD-PC was 493 and 465 nm, respectively. The concentration of total phospholipid in LUVs in the GUV suspensions was 0.1-0.2 mM, as determined by the absorbance of NBD-PC at 465 nm (using a molar extinction coefficient of 21,000; Invitrogen Inc.).

3. Results and Discussion

3.1. Effects of filtering the GUV suspension on the size distribution of the GUVs

DOPG/DOPC-GUVs in buffer A were purified using the apparatus shown in Fig.1A for 30 min at a flow rate of 2 mL/min. First, DOPG/DOPC-GUVs were prepared in buffer A containing 0.1 M sucrose in a glass vial, then were transferred to an Eppendorf tube and centrifuged to remove MLVs and lipid membrane aggregates. The GUVs in the supernatant were observed using phase contrast microscopy (Fig. 2A). A histogram of the distribution of the diameter of the GUVs (Fig. 2B), produced by analyzing the images of the GUVs, indicated that the fraction of GUVs with diameters less than 10 μm was very large. We confirmed the reproducibility of this result (n =2, n is

the number of independent experiments) and also this is almost the same data in Fig. 3B. In contrast, observation of the GUVs prior to transfer and centrifugation showed a smaller fraction of GUVs with diameters less than 10 μm. It appears that transfer of the GUV suspension from the glass vial to an Eppendorf tube by pipetman, and centrifugation, resulted in the production of many smaller GUVs, since these operations exert large forces such as shear stress on the GUVs. In contrast, analysis of the images of the GUV suspension after filtering for 30 min showed far fewer vesicles with diameters less than 10 μm, and a large population of GUVs with diameters of 10-30 μm (Figure 2C, 2D). The fraction (%) of vesicles with diameters less than 10 μm among all the vesicles was 13 ± 1 % (n =2). No vesicles with diameters less than 2 μm were observed. This suspension of purified GUVs was filtered again using a new polycarbonate filter for 30 min at 2 mL/min, resulting in a sharpened distribution of GUVs around 10-30 μm (Figure 2E, 2F). The fraction (%) of vesicles with diameters less than 10 μm among all the vesicles was 4 ± 1 % (n =2). These data indicate that the GUV suspension was purified to provide similar-sized GUVs with diameters of 10-30 μm. Hereafter we call this method for the purification of GUVs the membrane filtering method.

3.2. Purification of GUVs containing water-soluble fluorescent probes using the membrane filtering method

We next investigated the purification of GUVs containing a water-soluble fluorescent probe, calcein (molecular weight 623, Stokes-Einstein radius 0.74 nm (Yoshida et al., 2000). DOPG/DOPC-GUVs were prepared in buffer A containing 1 mM calcein and 0.1 M sucrose and purified using the membrane filtering method for 1 h at a flow rate of 1 mL/min. After centrifugation (before filtering), the suspension had a strong green color due to the 1 mM calcein. The fluorescence microscope images of the GUV suspension in the presence of 0.6 mM Co^{2+}, a quencher of calcein fluorescence, at 30 min after transfer to the chamber showed that more than 80 % of the GUVs had diameters less than 10 μm (Figure 3A, 3B). We confirmed the

reproducibility of this result (n =2). After filtering for 30 min, the green color of the suspension greatly decreased, and after filtering for 1 h, the suspension became completely transparent, indicating a very low concentration of free calcein. Fluorescence microscope images of the same samples (Figure 3C) in the absence of Co^{2+} show that the fluorescence intensity inside these GUVs was approximately the same as that of GUVs before purification (Fig. 3A), indicating that little or no leakage of calcein occurred during purification. Analysis of the fluorescence images of the purified GUV suspension showed a greatly decreased number of vesicles with diameters less than 10 μm and a large population of GUVs with diameters of 10-30 μm (Fig. 3D). The fraction (%) of vesicles with diameters less than 10 μm among all the vesicles was 14 ± 3 % (n =3). No vesicles with diameters less than 2 μm were observed. We also analyzed the size distribution of the GUVs using phase-contrast microscopy and obtained results consistent with the fluorescence microscopy images shown in Figs. 3A-D.

3.3. Dependence of the efficiency of the purification of GUVs on the flow rate

The dependence of the efficiency of the purification of the GUVs on the flow rate (from 0.5 to 3 mL/min) of the buffer during filtering was investigated. Figures 4(A)-(F) show phase contrast images and histograms of the size distribution of purified DOPG/DOPC-GUVs filtered for 1 h at various flow rates (0.5, 1, and 2 mL/min). All the conditions from 0.5 to 2 mL/min resulted in large populations of GUVs with diameters of 10-30 μm and no vesicles with diameters less than 2 μm. Approximately 12-20% of the vesicles had diameters less than 10 μm (Table 1). Judging from the observation of phase contrast microscopic images of vesicles such as phase contrast of GUV membranes and the undulation motion of the GUV membranes, vesicles other than GUVs (such as oligolamellar vesicles, MLVs, and GUVs containing smaller vesicles in their inside aqueous solution) were present at less than 20% of the total population, irrespective of the flow rate (0.5-2 mL/min). Fractions of these vesicles other than GUVs before and after the filtering were almost

similar, and thereby these vesicles did not increase substantially during the filtering, suggesting that these vesicles were produced in the formation of GUVs and in the processes of GUV suspension before the membrane filtering. Purification at a flow rate of 3 mL/min resulted in very few GUVs and some vesicles other than GUVs, making it difficult to generate a histogram of the size distribution of the purified GUVs. Fraction of the vesicles other than GUVs was large (~40 %, n = 2). Due to the large flow rate, GUVs might pass through the pores in the nucleopore filter, because shapes of GUVs can be deformed relatively easily compared with MLVs and oligolamellar vesicles. Based on these results, flow rates of 1-2 mL/min are optimal for purification of DOPG/DOPC-GUVs under these conditions (buffer, pH, etc.).

3.4. Removal of low molecular weight substances and LUVs from the GUV suspension using the membrane filtering method

The data on the purification of GUVs containing water-soluble fluorescent probes using the membrane filtering method described in Section 3.2 suggest that this method can be used for the removal of water-soluble molecules such as calcein, and also the removal of LUVs of lipid membranes. Visible absorption spectroscopy was used to obtain more quantitative data on the removal of calcein. Before purification, the supernatant of a DOPG/DOPC-GUV suspension was mixed with calcein in buffer A (0.1 mM final concentration of calcein), then the GUVs were purified using the membrane filtering method for 1 h at a flow rate of 1 mL/min. The absorbances (493 nm, for calcein) of the GUV suspension before and after purification were 0.365 ± 0.003 and 0.000 ± 0.003 (n =2), respectively, indicating removal of more than 99% of the free calcein.

These data clearly indicate that the membrane filtering method removes fluorescent probes outside the GUVs. Previously, gel chromatography was used to remove free fluorescent probes from GUV suspensions (Tamba and Yamazaki, 2005; Tamba et al., 2010). However, large-sized GUVs cannot be obtained due to friction between the GUVs and the Sephadex gel during

chromatography. In contrast, using the membrane filtering method, large-sized GUVs with diameters greater than 20 μm are obtained.

We also investigated the removal of LUVs during the purification of GUVs using the membrane filtering method. Fluorescently labeled LUVs (i.e., 90%DOPC/10%NBD-PC) were used to measure the amount of LUVs in suspension. Following centrifugation, DOPG/DOPC-GUV supernatant was mixed with 90%DOPC/10%NBD-PC-LUV in buffer A (0.1-0.2 mM final concentration of lipids in the LUVs) and the vesicles were purified using the membrane filtering method for 1 h at a flow rate of 1 mL/min. The absorbances (465 nm for NBD-PC) of the GUV suspension before and after purification were 0.275 ± 0.003 and 0.000 ± 0.003 (n =2), respectively, indicating removal of more than 99% of the LUVs.

3.5. Effect of solution density on the purification of GUVs

The mechanism behind the purification of GUVs by the membrane filtering method was investigated by studying the effect of a difference in density between the GUVs and the bulk buffer outside the GUV. Although the density of GUVs is much higher than that of the bulk buffer in the above experiments, this difference can be decreased by suspending GUVs prepared in buffer A containing 0.1 M sucrose. We purified DOPG/DOPC-GUVs using buffer A containing 0.1 M sucrose at 1 mL/min for 1 h (Fig. 5A, 5B). The fraction (%) of vesicles with diameters less than 10 μm among all the vesicles was 34 ± 1 % (n =2), which is larger than that purified using the buffer containing 0.1 M glucose (Fig. 2D). This result suggests that under these conditions (i.e., the absence of a difference in density) the GUVs could not be purified efficiently. We initially suggested the following hypothesis to explain the underlying mechanism behind the purification method. In the experimental data shown in Figs. 2-4, these was a significant difference in buffer density inside the GUVs containing 0.1 M sucrose (i.e., higher density) and the buffer containing 0.1 M glucose outside the GUVs (i.e., lower density). Therefore, the GUVs tended to rapidly settle to the bottom of the tube whereas the smaller vesicles remained suspended for a longer time. The

flowing buffer forced molecules and smaller vesicles through the filter (particles with diameters less than 12 μm pass through the filter very easily). To confirm the location of GUVs in the apparatus following purification, GUVs were purified using the apparatus with a stopcock shown in Fig. 1B. After filtering at 1 mL/min for 1 h, the stopcock was closed; no GUVs were present in the tube below the stopcock (Fig. 6B) and all the GUVs were found above the stopcock (i.e., in the space under the filter membrane) (Fig. 6A). We confirmed the reproducibility of this result (n =2). This result suggests that the above hypothesis is wrong, and rather, the mechanism behind the purification of GUVs using the membrane filtering method is simple filtering based on the size of the particle. The higher efficiency of purification of GUVs when there is a large difference in density between the GUVs containing 0.1 M sucrose and the buffer containing 0.1 M glucose may be due to a decrease in the force of the collision of the GUVs with the filter, which decreases the rate at which GUVs pass through the filter pores.

3.6. Concentration of GUVs using the membrane filtering method

The above data suggest that this method can be used for concentration of dilute GUV suspension. After purification of a DOPG/DOPC-GUV suspension using the membrane filtering method for 1 h at a flow rate of 1 mL/min, the GUV suspension was diluted with 4 fold volume of buffer A containing 0.1 M glucose, and then 7.5 mL diluted GUV suspension was filtered again using the apparatus with a stopcock shown in Fig. 1B with a new polycarbonate filter for 10 min at a flow rate of 1 mL/min. Then, the suspension above the stopcock (i.e., in the space under the filter membrane) was collected (~ 1.5 mL), and its phase contrast images show a larger number density of the GUVs with diameter of 10-30 μm (Fig. 7A) compared to that before the filtering (Fig. 7B), indicating concentration of the dilute GUV suspension. If we consider no decrease in the number of GUVs during the concentration of X mL GUV suspension using the apparatus with a stopcock, the GUV suspension can be concentrated to X/1.5 fold of its original GUV concentration, because all the GUVs in the original dilute GUV suspension are concentrated into the space under the filter

membrane which volume is ~ 1.5 mL. In the above experiment, the theoretical concentration ratio is 7.5/1.5 = 5, which almost agreed with the experimental results of the ratio of the number density of the GUV after the filtering to that before the filtering. We confirmed the reproducibility of the above result of the concentration of the GUVs (n =3).

4. Conclusion

We have developed a membrane filtering method to purify GUV suspensions, prepared in a buffer by the natural swelling method, which produces a large population of GUVs of oil-free membranes with similar diameter (10-30 μm). This method allows the removal of fluorescent probes outside the GUVs, and also the removal of LUVs. The membrane filtering method therefore simultaneously achieves both purposes: the purification of similar-sized GUVs larger than 10 μm, and the removal of free fluorescent probes and LUVs. The membrane filtering method can be applied to GUVs prepared by other methods to purify larger-sized GUVs from smaller GUVs, LUVs, and various water-soluble substances such as proteins and fluorescent probes. The membrane filtering method can be used for concentration of dilute GUV suspension.

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Table 1: Flow rate dependence of the efficiency of the purification of GUVs

Flow rate (mL/min)	The fraction of the vesicles with diameters less than 10 μm (%)	Number of experiments
0.5	20 ± 3	$n = 3$
1.0	12 ± 4	$n = 3$
2.0	13 ± 2	$n = 3$

Figure Captions

Figure1: (A) The apparatus for the membrane filtering method. (B) The apparatus with a stopcock for the membrane filtering method. The details are described in the Materials and Methods section (2.2).

Figure 2: Effects of filtering the GUV suspension on the size distribution of the GUVs. (A) and (B) show a phase contrast image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension after centrifugation (i.e., before purification). We omitted vesicles with diameters less than 2 μm because there were too many to count. (C) and (D) show a phase contrast image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension after the first purification (conditions: flow rate 2 mL/min for 30 min). (E) and (F) show a phase contrast image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension after the second purification (conditions: flow rate 2 mL/min for 30 min). The bars in the images correspond to 50 μm.

Figure 3: Purification of GUVs containing calcein using the membrane filtering method. (A) and (B) show a fluorescence microscopy image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension containing 1 mM calcein before purification. We observed this image in the presence of 0.6 mM Co^{2+}. (C) and (D) show a fluorescence microscopy image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension purified at 1 mL/min for 1 h; this image was observed in the absence of Co^{2+}. The bars in the images correspond to 50 μm.

Figure 4: Dependence of the efficiency of the purification of GUVs on the flow rate. Phase contrast images (A)(C)(E) and the histograms of the size distribution (B)(D)(F) of the purified 50% DOPG/50% DOPC-GUV suspensions for 1 h at various flow rates ((A) (B) 0.5 mL/min, (C)(D) 1 mL/min, and (E)(F) 2 mL/min). The bars in the images correspond to 50 μm.

Figure 5: Effect of the difference in solution density on the purification of GUVs using the membrane filtering method. (A) and (B) show a fluorescence microscopy image and a size distribution histogram, respectively, of the 50% DOPG/50% DOPC-GUV suspension purified in a buffer containing 0.1 M sucrose at 1 mL/min for 1 h. The bars in the images correspond to 50 μm.

Figure 6: Location of GUVs in the apparatus after purification of 50% DOPG/50% DOPC-GUV suspension using the apparatus with a stopcock shown in Fig. 1B. After filtering at 1 mL/min for 1 h, the stopcock was closed. (A) and (B) show phase-contrast images of the GUV suspension above the stopcock (i.e., in the space under the filter membrane) and in the tube below the stopcock, respectively. The bars in the images correspond to 50 μm.

Figure 7: Concentration of GUVs by the membrane filtering method using the apparatus with a stopcock shown in Fig. 1B. (A) and (B) show phase-contrast images of the GUV suspension after and before the filtering of a purified DOPG/DOPC-GUV suspension for 10 min at a flow rate of 1 mL/min., respectively. The bars in the images correspond to 50 μm.

Fig. 1

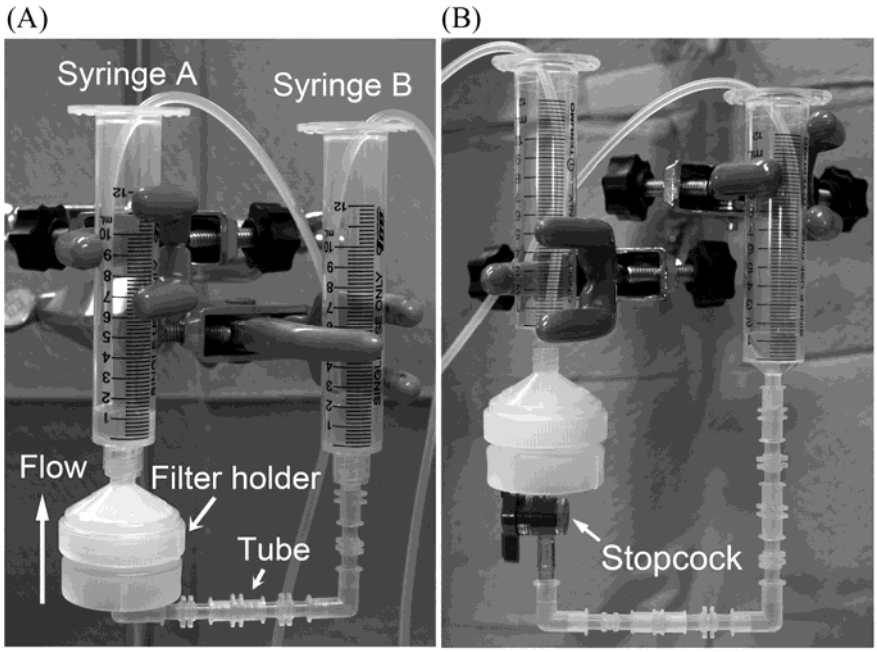
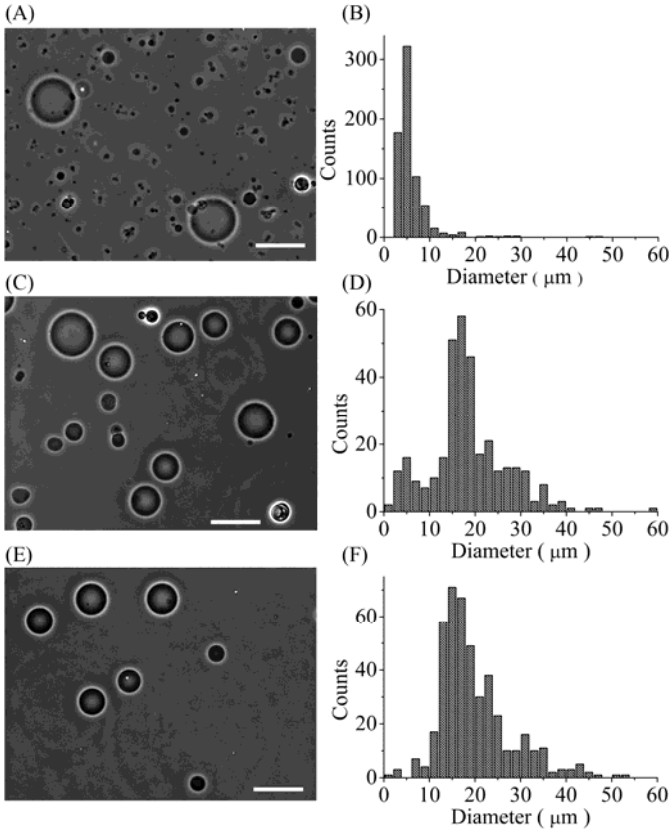


Fig. 2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(B)
300
200
100
0
Counts
0 10 20 30 40 50 60
Diameter (μm)
(C)
(D)
60
40
20
0
Counts
0 10 20 30 40 50 60
Diameter (μm)
(E)
(F)
60
40
20
0
Counts
0 10 20 30 40 50 60
Diameter (μm)

Fig. 3

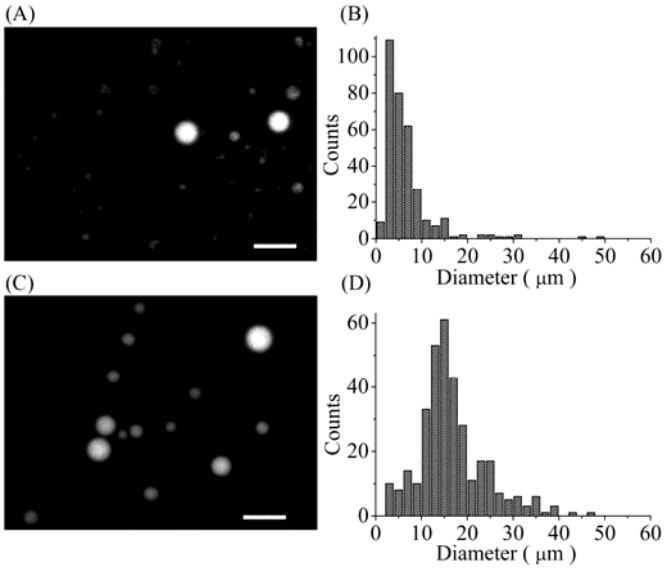

Fig. 4

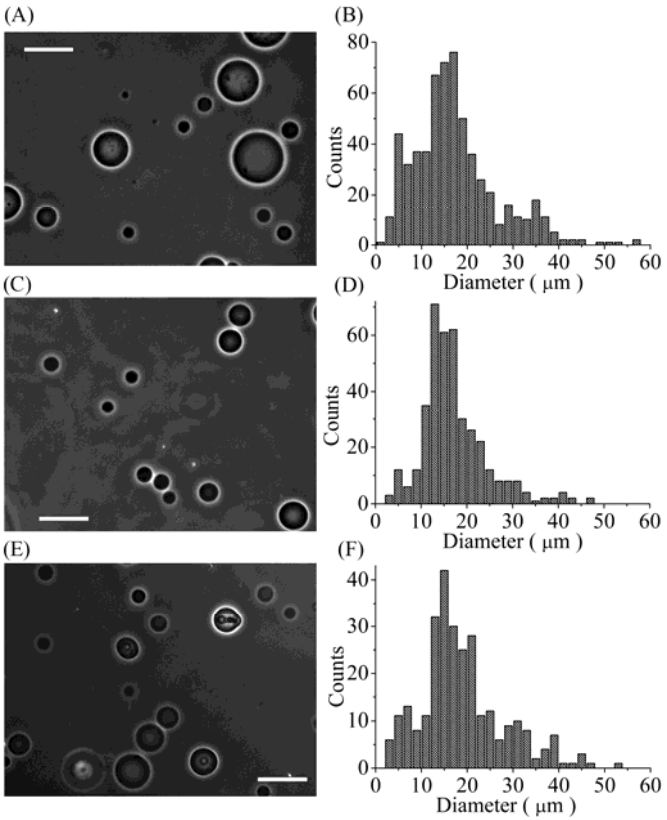

Fig. 5

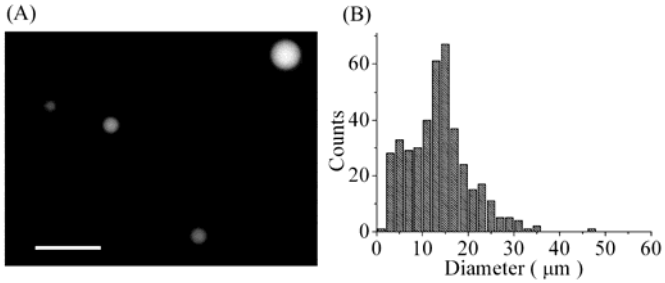

Fig. 6

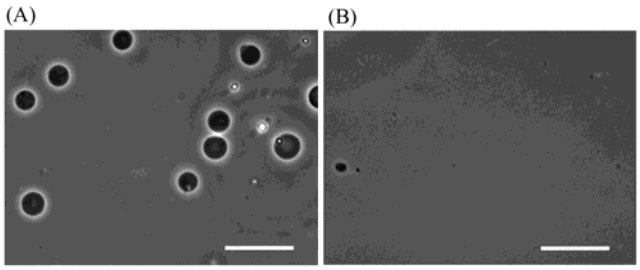

Fig. 7