Summary of Methods to Prepare Lipid Vesicles

(Ranging from 30 nm to 50 μm)

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Part I Diameter: 30 nm - 50 nm (SUV-Small Unilamellar Vesicle)

The shelf-time of these small lipid vesicles is very short due to their high surface tension. They are usually used immediately after preparation. Two methods can be used to produce them.

Method 1: Extrusion

The same procedures as in Part II but choose a PC membrane with smaller pore size (30 nm - 50 nm).

Method 2: Sonication

1. **Make stock solution:** dissolve DMPC lipids in chloroform initially to make 8 mg/ml concentrated lipid solution (stock solution).
2. **Sample solution:** use ~0.23 g of stock solution, which should give ~1.2 mg of DMPC lipid, and dilute the concentrated stock solution in more chloroform.
3. **Evaporation:** Blow-dry the dilute lipid sample using nitrogen for 2 hours to evacuate the solvent of chloroform.
4. **Hydration:** add ~1.2 ml PBS buffer to hydrate the dry lipid sample as soon as the chloroform is all evaporated.
5. **Sonication:** Insert the titanium-tip sonicator inside the lipid suspension and do sonication. Vesicle size and distribution depend on sonication power, frequency and time.

Part II Diameter: 80 nm - 800 nm (LUV-Large Unilamellar Vesicle)

The typical method to make LUV is “extrusion”, whose detailed procedures are summarized as following. Note that vesicles produced by this method are usually more polydisperse at larger size.

1. **Make stock solution:** dissolve DMPC lipids in chloroform initially to make 8 mg/ml concentrated lipid solution (stock solution).
2. **Sample solution:** use ~0.45 g of stock solution, which should give ~2.4 mg of DMPC lipid, and dilute the concentrated stock solution in more chloroform.
3. **Evaporation:** Blow-dry the dilute lipid sample using nitrogen for 2 hours to evacuate the solvent of chloroform.

4. **Hydration:** add ~2.4 ml PBS buffer to hydrate the dry lipid sample as soon as the chloroform is all evaporated.
5. **Incubation:** Place the sample container in a sand bath at **~40 deg. C** for 2 hours. During the 2 hours of incubation, mix the sample once every 10 minutes using a vortexer.
6. **Freeze/thaw:** Immerse the sample in liquid nitrogen followed by boiling water for 5 cycles totally.
7. **Extrusion:** assemble the membrane inside the extruder:
    (1) Wet the Teflon piece with buffer;
    (2) Place **2** pieces of membrane support in the center of the Teflon piece;
    (3) Add a drop of water before putting the PC membrane;
    (4) Put the PC membrane with proper pore size on the **taller** piece of the holder;
    (5) Add a drop of buffer on top of the membrane;
    (6) Place 2 pieces of Teflon holder together;
    (7) Turn up the heater underneath the extruder to above T_m, and place the syringe on the heater to warm up;
    (8) Fill the lipid solution into the syringe, and extrude **11** times;
    (9) Eject the extruded sample from the acceptor syringe. The solution should look clearer than that before extrusion.
8. **Cleaning:** The Teflon piece, o-ring and syringe should be rinsed with copious 2-propanol and DI water after use. Otherwise the residues will contaminate your next sample.

Notes:

- In order to control the pH of the buffer solution, NaH_2PO_4 and Na_2HPO_4 are used as phosphate salt to make pH = 6, $[PO_4^{3-}]$ = 10 mM PBS solution. The molar ratio of the two salts are ~ 7.5 : 1.
- Don't use the same membrane for more than 3 ml of solution.

Part III Diameter: 1 µm - 50 µm (GUV-Giant Unilamellar Vesicle)

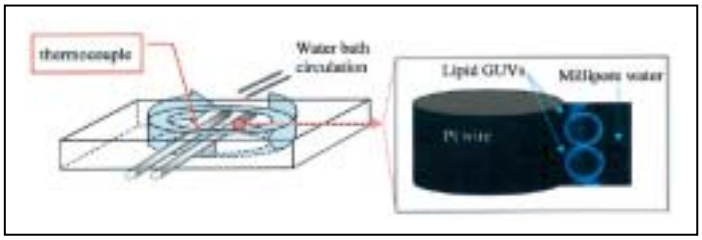
Bearing in mind that even though there are various methods to make GUVs, the key principle is similar, encouraging dried lipid films to swell and form giant vesicles. Here are five typical methods of making GUV.

Method 1: LUV Fusion

As it's well known, LUVs are not stable in suspension because of vesicle fusion with each other. This method is taking the advantage of inter-vesicle fusion to form giant vesicles. This method is very simple, but it is inefficient to produce vesicle with size larger than 10 µm.

1. **Prepare LUV:** See Part II for details.
2. **Prepare GUV:** Keep the LUV suspension at room temperature for 1~2 days. Giant vesicles with diameter less than 10 μm formed massively in the suspension.

Method 2: Electroformation – Pt Wire *(Gratton, et al.)*



This method is widely used to produce GUVs with various components and inclusions. Advantages: (a) vesicle size is well controlled by tuning the electric field; (b) detaching vesicles from the Pt wires is possible; (c) One can transfer the GUVs to other medium from the open chamber; (d) All GUVs are unilamellar. Disadvantages: (a) Time-consuming -- each sample preparation needs many hours to clean the chamber; (b) Inefficient -- just a few GUVs can be produced each time due to the small amount of lipids used for each sample; (c) It's tricky to add lipid stock solution onto the Pt wires.

1. **Clean the Chamber:** Sonicate the chamber in soap, ethanol and DI water for 1 hour respectively. Then dry it using nitrogen.
2. **Spread Lipids:** Add 3 μl lipid stock solutions (0.2 mg/ml) to each wire evenly. Keep the whole setup under nitrogen for 2 hours to evacuate the organic solvents.
3. **Glue the Cover Glass:** Use epoxy adhesive to seal the chamber bottom window with cover glass.
4. **Add Water/Buffer:** 2 ml water is enough and make sure there is no leakage of the chamber.
5. **Lipid Hydration:** Turn on the electric field (10 Hz, 3 V) for 90 minutes. GUVs formed along the Pt wires.

Method 3: Electroformation – ITO Glass *(Schwille, et al)*

This method is a derivative of method 2; using ITO glass to replace Pt wire. Compared with method 2, it has the advantages of (a) much faster – no need to clean chamber or other devices before sample preparation; (b) produce many GUVs each time; (c) GUV patterning on ITO-coated glass surface is possible. Disadvantages: (a) Since the two ITO glasses are glued together, it's hard to collect GUVs and transfer them somewhere else.

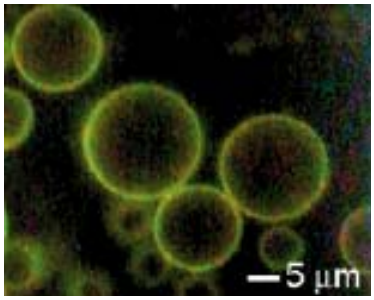
1. **Clean ITO-Coated Glass:** Rinse the glass with 2-propanol and acetone.
2. **Spread Lipids:** Add lipid stock solutions (0.2 mg/ml) onto one of the ITO glass surface. Keep it under nitrogen for 2 hours to evacuate the organic solvents.
3. **Add Water/Buffer:** Add buffer solution to the dried lipid films.
4. **Glue the Two ITO Glasses:** Use epoxy adhesive to glue the two glasses together.

5. **Lipid Hydration:** Turn on the electric field (10 Hz, 3 V) for 90 minutes. GUVs formed on the ITO glass.

Method 4: Dehydration-Rehydration *(Orwar, et al.)*

Advantages: (a) This method is very simple; (b) It takes several minutes to form GUVs; (c) Most of the GUVs are unilamellar. Disadvantages: (a) GUV size is relatively small, ~10 μm; (b) It's hard to remove vesicles from the substrate surface;

1. **Prepare LUV:** See Part II for details.
2. **Dehydrate LUV:** Add a droplet of the LUV suspension (5 μl) onto a hydrophilic substrate such as quartz and glass. Place the substrate into a vacuum oven at room temperature of 10 minutes to dehydrate lipid vesicles.
3. **Rehydrate LUV:** After the LUVs are dry, add 5 μl buffer solutions to dydrate the lipid membrane. GUVs appear in couple of minutes.



Method 5: Sucrose Hydration *(Kinosita, et al.)*

Advantages: (a) Efficient – each sample preparation produces many GUVs; (b) It's very easy to generate osmotic pressure to the GUVs by controlling internal and external solute concentration. Disadvantages: (a) It uses too much sucrose, 100-500 mM, which may affect other measurements; (b) GUVs are not always unilamellar.

1. **Make stock solution:** dissolve DMPC lipids in chloroform initially to make 8 mg/ml concentrated lipid solution (stock solution).
2. **Sample solution:** use ~0.45 g of stock solution, which should give ~2.4 mg of DMPC lipid, and dilute the concentrated stock solution in more chloroform.
3. **Evaporation:** Blow-dry the dilute lipid sample using nitrogen for 2 hours to evacuate the solvent of chloroform.
4. **Hydration:** add ~2.4 ml sucrose solution (100-500 mM) to hydrate the dry lipid sample as soon as the chloroform is all evaporated.
5. **Incubation:** Keep the sample container in a vacuum oven at **~37 deg. C** for overnight. GUVs will form and float in the suspension.