Protocol for Electroformation of Giant Unilamellar Vesicles (GUVs)
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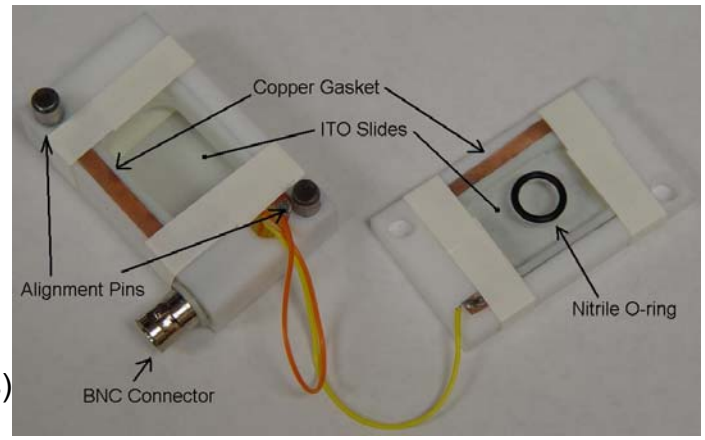
last updated 10/5/07 - See 'Notes' below.



Materials:

- Ethanol, DI water and Kimwipes for cleaning
- 5-10 μl glass syringe (Hamilton Co.)
- 200 μl pipette (with wide mouth tips)
- two Eppendorf tubes (1ml)
- vacuum desiccator
- One of the following
    - nitrile O-ring (9.25 mm ID x 1.78 mm radius)
        - vacuum grease required
        - ~135 μl fill volume
    - Grace Biolabs FW9-2.0 FastWell
        - no grease required
        - ~96 μl fill volume
- appropriate buffer
    - low salt (<10mM)
    - DDI H_2O, sugars (sucrose and glucose) and other non-ionic compounds are OK up to ~500mM.
- desired lipid or lipid mixture; concentration such that there are ~100 bilayers for a typical lipid film deposition (typically 2mg/ml seems to work well)

Notes on cleanliness:

The strength of bilayers, their elastic properties, as well as their phase segregating properties (with multiple lipid species) are all quite sensitive to contaminants. Hence, everything in the procedure is made of metal (steel / copper), Teflon or glass. Polyethylene pipette tips and Eppendorf tubes can only be used in aqueous conditions. Adhesives, cleaning agents, and other surfactants can lead to anomalous behavior.

Preparation:

- Clean the chamber with ethanol and DI water using Kimwipes alternating the solvents at least twice to make sure any grease or sugars are removed from the slides
- Make sure the ITO slides in the chamber are firmly in place and the copper conductor gasket is in contact at the top and bottom of each slide, check the resistance from BNC to slide, it should be no more than 50 Ohms
- Using the 10 μl glass syringe apply a 2-3 μl (@ 2mg/ml conc.) droplet of the lipid/chloroform solution in the center of the ITO slide on the shorter side of the chamber. The layer should appear greenish under white light reflection (corresponding to ~100 bilayers thick).
    - I find that film deposition is one of the most crucial steps – depositing a uniform green(ish) film is key to high yield electroformation.
- Quickly (within a minute) place the whole chamber in the vacuum desiccator, desiccate for at least 30 mins, preferably 1.5 hrs. This is to remove all of the chloroform from the film.
- Meanwhile, clean the nitrile O-ring or FastWell using a Kimwipe and ethanol. If using an O-ring, use a Que-tip or grease slide to apply a thin layer of vacuum grease to one side of the O-ring.
- Remove the chamber from the desiccator and place the O-ring (greased side down) or FastWell approximately in the center of the short side of the chamber, directly over the greenish film.

- Using the 200 μl pipette, place the appropriate fill volume (see Materials) of buffer solution in the O-ring or FastWell.
- Put the top half of the chamber on using the alignment pins, ensure there are no bubbles (very small bubbles are ok).
- Carefully bring the chamber over to the microscope and position over objective (20X is fine).

Procedure:

- Place the chamber on the microscope stage, lipid coated side down.
- Secure the chamber with the spring clips on the microscope stage.
- Attach the BNC connector to the chamber.
- Set the voltage amplitude between 1V and 4V, never exceed 5V as this will damage the ITO coating. Set the driving frequency to ~10Hz. One can vary the amplitude and frequency to suit the lipid/buffer composition (vesicles have been formed with frequencies as low as 2Hz and as high as 20hz and 1-5V).
    - My general recipe is 120 minutes, 1V, 10Hz; 50 minutes,1V, 2Hz
    - Note: multicomponent membranes need to be formed at higher temperatures (~50C)
- Wait and watch as vesicles begin to form over the course of 2-3 hours. Blistering of the lipid layer should be noticeable within a few minutes.
- When the formation is done, turn off the equipment and remove chamber from microscope. Using a wide-mouth tip, pipette out ~100 μl of the solution from the chamber into the Eppendorf tube already filled with ~1 ml desired buffer. *Slowly* mix this solution in the centrifuge tube. Depending on the yield, a 10:1 dilution of this solution may be in order. The vesicles are now ready! They are good for ~24 hours.

Note on Tension in the membrane:

Regulating the internal and external osmolarity is the key to having tense or floppy vesicles. My experiments usually require floppy vesicles which requires careful control of the difference in osmolarity between the inside and outside of the vesicle. If the vesicles are too floppy, protrusions, tubes, buds and other nasties form. I usually form vesicles with 100mM sucrose inside and 102-108mM glucose outside. Additionally, this will cause the vesicles to sediment upon viewing.

Note on Lipid Storage:

Many lipids can be oxidized, and hence ruined if exposed to improper conditions. All lipids should be stored in Teflon capped, glass vials, under argon at -20C. When you are about to use a lipid mixture, wait until it has reached room temperature before opening – this will prevent water condensation inside the vial from damaging the lipid. Labeled lipids should have minimum exposure to light. The shelf-life of a properly stored lipid is about a year.

Note on the Chamber Construction:

ITO slides can be purchased from Sigma-Aldrich (~200$ per 10) and the rest of the materials are widely available. The body of my formation chambers are made of machined Teflon. Teflon tape is used to hold on copper gasket to the ITO slide. Occasionally, I use lab quality masking tape. A function generator or LabView DAQ card are suitable for applying the AC field.