CHAPTER TWENTY-ONE

Vesicle Extrusion Through Polycarbonate Track-etched Membranes using a Hand-held Mini-extruder

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Abstract

The vesicle extrusion method is used for preparing small (e.g., 100 nm diameter) monodisperse (uniform-sized) vesicles. Polycarbonate track-etched membranes with pores generally ranging from 50 to 200 nm in diameter and a hand-held mini-extruder are

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used for such a procedure. Extrusion through small (100 nm in diameter or smaller) pores also ensures that the vesicle population is predominantly unilamellar.

1. THEORY

The vesicle extrusion method takes advantage of the fact that when vesicles are forced through membrane pores smaller than their diameter, they break down into smaller vesicles closer to the pore size (see Video 1). http://dx.doi.org/10.1016/B978-0-12-420067-8.00021-0. Vesicle extrusion has also been used as a laboratory method to induce protocell division (Hanczyc et al., 2003).

2. EQUIPMENT

Hand-held mini-extruder (http://www.ncnr.nist.gov/userlab/pdf/E134extruder.pdf)
Two gas-tight syringes (often come with an extruder)
pH meter
1.5-ml microcentrifuge tubes
Polycarbonate track-etched membranes, either 13 or 25 mM in diameter (http://www.whatman.com/NucleporeTrackEtchedMembranes.aspx)
10-mm filter supports (http://avantilipids.com/index.php?option=com_content&view=article&id=522&Itemid=293&catnumber=610014)

3. MATERIALS

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (or any phospholipid of choice)
Oleic acid (or any fatty acid of choice)
Lissamine™rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Rh-DHPE) (for fluorescent membrane labeling, if desired)
Bicine (or other buffer of choice, except borate or phosphate buffer, which produces leaky fatty acid vesicles)
8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS, or other water-soluble fluorescent dye of choice)
Sodium hydroxide (NaOH)
Chloroform
Methanol
Deionized water

3.1. Solutions & buffers

Preparation 1-M Na-bicine, pH 8.5

Dissolve 163.2-g bicine in 800-ml deionized water. Adjust pH to 8.5 using NaOH. Add water to 1l

100-mM HPTS

Dissolve 52.4-mg HPTS in 1-ml deionized water

Na-bicine buffer + HPTS

Component	Final concentration	Stock	Amount
Na-bicine, pH 8.5	200 mM	1 M	1 ml
HPTS	2 mM	100 mM	0.1 ml

Add water to 5 ml

Step 1 Na-bicine buffer

Component	Final concentration	Stock	Amount
Na-bicine, pH 8.5	200 mM	1 M	1 ml

Add water to 5 ml

4. PROTOCOL

4.1. Preparation

Prepare vesicles by suspending lipid(s) in a buffer solution (see Preparation of fatty acid or phospholipid vesicles by thin-film rehydration).

4.2. Duration

Preparation	About 1 day
Protocol	About 50 Min

4.3. Tip

If using photosensitive lipids, avoid exposure to light by wrapping aluminum foil around the flask containing the vesicles. Avoid exposure to oxygen by flushing the container with argon or nitrogen gas.

See Fig. 21.1 for the flowchart of the complete protocol.

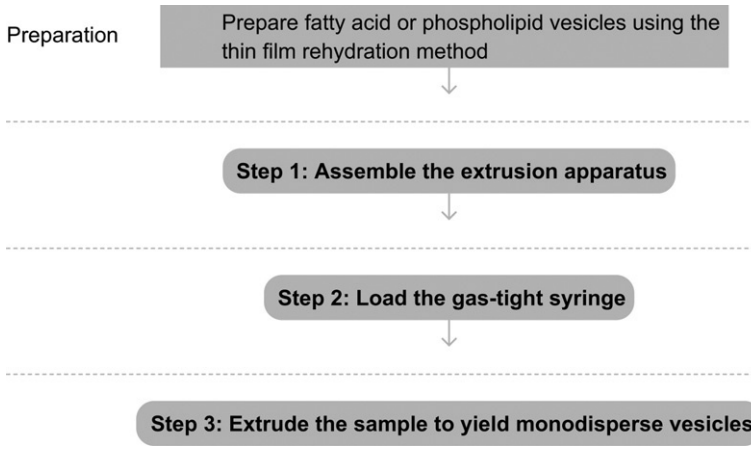

Figure 21.1 Flowchart of the complete protocol, including preparation.

Figure 21.2 Hand-held mini-extruder with two gas-tight syringes.

5. STEP 1 ASSEMBLE THE EXTRUSION APPARATUS

5.1. Overview

Assemble the extrusion apparatus with the polycarbonate track-etched membranes and filter supports (Fig. 21.2 and Video 2). http://dx.doi.org/10.1016/B978-0-12-420067-8.00021-0.

5.2. Duration

5 Min

1.1 Prewet the polycarbonate track-etched membranes of choice (e.g., with 100-nm diameter pores) and the filter supports.

1.2 Put one filter support on each side of the two internal membrane supports (white plastic parts), with the prewetted polycarbonate track-etched membrane on top. Assemble the rest of the parts of the extrusion apparatus (Fig. 21.3 & 21.4).

5.3. Tip

Rinse the extruder parts with water before assembly.

5.4. Tip

When prewetting the polycarbonate track-etched membranes and filter supports, rapidly dip the membranes or filter supports into the same buffer used for preparing vesicles.

5.5. Tip

One can choose to double the polycarbonate track-etched membrane to prevent membrane rupture, especially when using high pressure for extrusion.

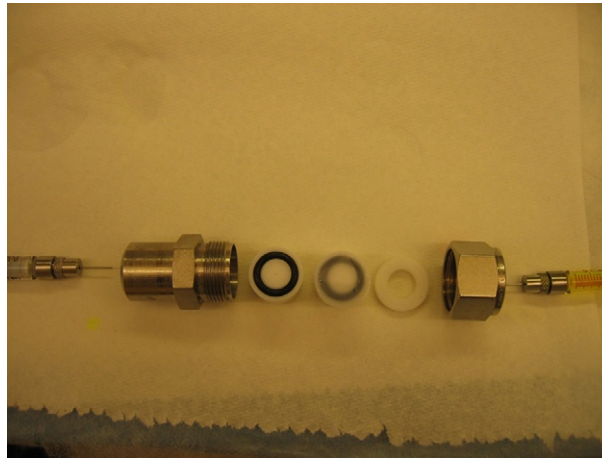

Figure 21.3 Assembly of the extrusion apparatus. On each internal membrane support (white plastic part with a black O-ring) there is a filter support, with a polycarbonate track-etched membrane on top.

Figure 21.4 Assembly of the internal membrane support parts with filter support and polycarbonate track-etched membrane in the middle.

6. STEP 2 LOAD THE GAS-TIGHT SYRINGES

6.1. Overview

Load the gas-tight syringes with vesicle sample.

6.2. Duration

5 Min

2.1 Rinse the syringes with water and then the same buffer used for preparing vesicles.

2.2 Load one of the gas-tight syringes with the vesicle sample in a volume between 0.5 and 1 ml. See Video 3. http://dx.doi.org/10.1016/B978-0-12-420067-8.00021-0.

7. STEP 3 EXTRUSION TO YIELD MONODISPERSE VESICLES

7.1. Overview

Force the vesicles in the two gas-tight syringes back and forth through the hand-held mini-extruder 11 times. See Video 4. http://dx.doi.org/10.1016/B978-0-12-420067-8.00021-0.

7.2. Duration

10 Min

3.1 Lock the gas-tight syringes in place on the hand-held mini-extruder.

3.2 Slowly push one syringe, so that all the solution in it goes through the hand-held mini-extruder to the other side, into the other syringe (Fig. 21.5).

3.3 Push from the other side using the other syringe. Repeat this 11 times through the filter, and collect the final product vesicles. See Video 5. http://dx.doi.org/10.1016/B978-0-12-420067-8.00021-0.

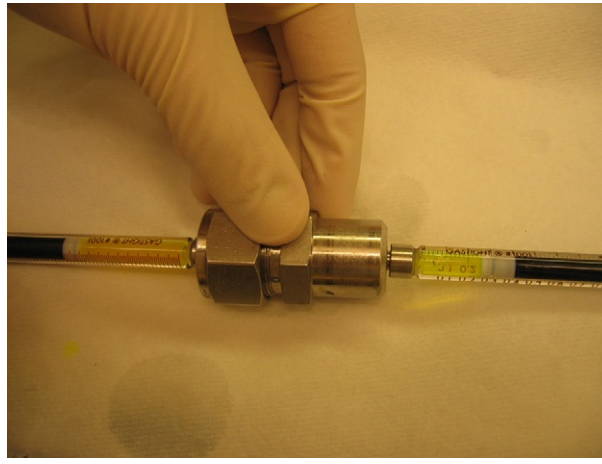

Figure 21.5 Slowly push one syringe (left), so that all the solution in it goes through the hand-held mini-extruder to the other side, into the other syringe (right).

7.3. Tip

For larger pore sizes (>100 nm), slowly push the syringe at a constant rate of 1 ml Min^{-1}. Extrusion at higher flow rates may lead to excessive disruption of vesicles (Hanczyc et al., 2003).

7.4. Tip

Make sure that the final product vesicles are collected from the syringe that was not initially loaded with the vesicle sample.

REFERENCES

Referenced Literature

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Related Literature

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Hope, M. J., Bally, M. B., Webb, G., & Cullis, P. R. (1985). Production of large unilamellar vesicles by a rapid extrusion procedure. Biochimica et Biophysica Acta (BBA) – Biomembranes. *Characterization of size distribution, trapped volume and ability to maintain a membrane potential*, *812*(1), 55–65.

Referenced Protocols in Methods Navigator

Preparation of fatty acid or phospholipid vesicles by thin-film rehydration.