Sonoporation on cells & verification

Technic: Permeabilize the membrane of cells

Goal: To encapsulate rhodamine or gold nanoparticles in cells

Procedure:

1. From BP100 dishes at less than 50% confluence detach cells (see previous protocols) and resuspend them in their old medium in a 15 mL tube.
2. Centrifugate at 300g for 5 minutes then dispose of the surnatant.

Using rhodamine

3. Add 10 µL of rhodamine and culture medium to reach 1.5 mL, then transfer in a microtube.
4. Put the solution in the ultrasound bath for 3 minutes at 37°C.
5. On ice, sonicate the solution with the following protocol: 10% at a 5 seconds ON, 5 seconds OFF cycle for 30 seconds.
6. Add culture medium to the solution to reach 10 mL then pour in a new BP100 dish.
7. Let incubate in the oven for a day.
8. Remove the medium and carefully wash the cells with 2 mL of PBS.
9. Qualitatively look at the fluorescence under a microscope.

Using gold nanoparticles

3. Add 100 µL of AuNps and culture medium to reach 1.5 mL, then transfer in a microtube.
4. Pass the solution in the ultrasound bath for 3 minutes at 37°C.
5. On ice, sonicate the solution with the following protocol: 10% at a 5 seconds ON, 5 seconds OFF cycle for 30 seconds.
6. Add culture medium to the solution to reach 10 mL then pour in a new BP100 dish.
7. Let incubate in the oven for a day.
8. Remove the medium and carefully wash the cells with 2 mL of PBS.
9. With PBS, detach the cells to have them in suspension in a volume of 10 mL. If necessary use trypsin.
10. Carefully sample several 200 µL aliquots in microtubes.
11. Lyse some of them using a defined lysis protocol.
12. Measure the absorbance at 515 nm by spectrophotometer of {sonicated cells+gold+lysis}, {sonicated cells+gold}, {sonicated cells+lysis}, {sonicated cells}.

Reagents and Solutions:

- Filtered sterile PBS at 2 µm
- A BP100 dish of cells (HEK *or* pancreatic) at less than 50% confluence
- R110 at 5 mM *or* AuNps

- Culture medium (89% DMEM or RPMI + 10% SVF + 1% penicillin)
- Trypsin

Equipment:
- Sterile tubes and microtubes
- Pipettes and sterile tips
- 100 mm culture cell dishes (BP100)
- Laboratory aspiration system
- Pipetboy
- Serological pipettes
- Ultrasound bath
- Sonication probe
- Fluorescence microscope
- Spectrophotometer able to read the 515 nm wavelength
- Oven

Estimate time: ~1 hour + 2 hours the next day

Controls and Troubleshooting:
The amount of rhodamine and gold nanoparticles can be changed as well as sonication factors.

Electroporation on cells & verification

Technic: Permeabilize the membrane of cells

Goal: To encapsulate rhodamine or gold nanoparticles in cells

Procedure:

1. From BP100 dishes at less than 50% confluence detach cells (see previous protocols) and resuspend them in their old medium in a 15 mL tube.
2. Centrifugate at 300g for 5 minutes then dispose of the surnatant.

Using rhodamine

3. Add 10 µL of rhodamine and culture medium to reach 1.5 mL, then transfer in a microtube.
4. Perform 10 electroporations of 150 µL each in electroporation cuvettes, then pool the aliquots altogether in a 15 mL tube.
5. Complete with 8.5 mL of culture medium and pour in a new BP100 dish.
6. Let incubate in the oven for a day.
7. Remove the medium and carefully wash the cells with 2 mL of PBS.
8. Qualitatively look at the fluorescence under a microscope.

Using gold nanoparticles

3. Add 100 µL of AuNps and culture medium to reach 1.5 mL, then transfer in a microtube.
4. Perform 10 electroporations of 150 µL each, then pool the aliquots altogether in a 15 mL tube.
5. Complete with 8.5 mL of culture medium and pour in a new BP100 dish.
6. Let incubate in the oven for a day.
7. Remove the medium and carefully wash the cells with 2 mL of PBS.
8. With PBS, detach the cells to have them in suspension in a volume of 10 mL. If necessary use trypsin.
9. Carefully sample several 200 µL aliquots in microtubes.
10. Lyse some of them using a defined lysis protocol.
11. Measure the absorbance at 515 nm by spectrophotometer of {electroporated cells+gold+lysis}, {electroporated cells+gold}, {electroporated cells+lysis}, {electroporated cells}.

Preparation on the electroporation buffer in a sterile environment:

1. Pour 80 mL of sterile water in a beaker on a magnetic stirrer.
2. Add 0.1420 g of disodium phosphate, 0.00952 g of magnesium chloride and 8,58 g of sucrose.
3. Calibrate the pH-meter and dive it in the solution.
4. Add 1 mL of penicillin.
5. Add droplets of HCl or NaOH to reach a pH of 7.4.
6. Complete with sterile water to reach 100 mL.

Electroporation factors:

1. Square waves for HEK293 cells:
    - Number of pulses: 10
    - Voltage: 110 V
    - Pulse length: 25 ms
    - Pulse intervals: 0.1 s

2. Square waves for pancreatic cells:
    - Number of pulses: 8
    - Voltage: 300 V
    - Pulse length: 0.1 ms
    - Pulse intervals: 100 ms

Reagents and Solutions:

- Filtered sterile PBS at 2 µm
- A BP100 dish of cells (HEK *or* pancreatic) at less than 50% confluence
- R110 at 5 mM *or* AuNps
- Culture medium (89% DMEM or RPMI + 10% SVF + 1% penicillin)
- Trypsin
- Disodium phosphate (Na2HPO4) at 10 mM
- Magnesium chloride (MgCl2) at 1 mM
- Sucrose (C12H22O11) at 250 mM
- Penicillin
- HCl and NaOH

Equipment:

- Sterile tubes and microtubes, beaker
- Pipettes and sterile tips
- 100 mm culture cell dishes (BP100)
- Laboratory aspiration system
- Pipetboy
- Serological pipettes
- Fluorescence microscope
- Spectrophotometer able to read the 515 nm wavelength
- pH-meter
- Magnetic stirrer
- Precision balance
- Electroporator
- Electroporation cuvettes

Estimate time: ~2 hour + 2 hours the next day

Controls and Troubleshooting:

The amount of rhodamine and gold nanoparticles can be changed as well as electroporation factors.
All cuvettes had a 2 mm gap for the current to pass through.