DUAL-NEEDLE MICROFLUIDIC LOCAL DRUG DELIVERY

Materials

1. Pipette puller (e.g. Kopf Model 720 with platinum heater)
2. Omega-Dot borosilicate glass capillary tubing with fiber (Friderick-Haer & Co., OD = 1.2 mm, ID = 0.9 mm)
3. Storage container for pulled microneedles, which may be constructed with a block of foam, a strip of double-sided tape and a 100 mm petri dish.
4. Microforge (Narishigi Model MF-900)
5. Microneedle grinder (Optional, Narishigi Model Eg-4)
6. Bunsen burner
7. Flint glass Pasteur pipettes, pulled to create very long skinny tips for loading solution into microneedles. Hold the pipette horizontally at both ends over the flame of a Bunsen burner. When the pipette starts to turn soft, quickly move it out of the flame and pull immediately.
8. Storage container for pulled Pasteur pipettes. A long, hard plastic kitchen organizer will work.
9. Microscope with a temperature and CO_2 -controlled incubator enclosure over the stage. The box must contain an opening next to the stage for the access of micromanipulator.
10. Agent intended for local release, in base medium (without serum) with 0.1 mg/ml 10-40 kDa fluorescein dextran as tracer.
11. Dual instrument micromanipulator (custom built, see the protocol on Instruments page for details)
12. Precision positive pressure controller (custom built, see the Instruments page for details), connected to house compressed air
13. Negative pressure controller connected to house vacuum, with a needle valve for controlled bleeding and a vacuum gauge. A side-armed Erlenmeyer flask for collecting the fluid is placed between the suction needle and negative pressure controller
14. Beckman TLA-100 tabletop ultracentrifuge and a xx rotor
15. Mineral oil (Sigma-Aldrich M8410)

Procedure

Preparation of needles

1. Pull glass needle on pipette puller, using Omega-Dot glass capillary tubing. Avoid needles with too long a shaft as they plug up easily. Needles may be stored in a storage box inside a desiccator.
2. Load a pulled glass microneedle onto the microforge. Using a 15 x objective lens, position the needle tip near the heating filament.
3. Adjust the x-y-z position of the microneedle so that the tip is close to the heating filament and on the same focal plane.
4. Push the microneedle tip directly into the heating filament so that it breaks cleanly.
5. Estimate the diameter of the broken tip with the eyepiece. Depending on the size of the opening, a diameter of ~5-10 μm is ideal for the release, while a diameter of ~15-50 μm is ideal for suction.
6. Jagged needle tip can be fire polished with a microforge, by turning on the heating filament and holding the needle very close to without touching the filament. Alternatively, the tip may be shaped with a microneedle grinder.
7. Place finished microneedles in a storage container. A number of release and suction microneedles may be prepared at a time and store desiccated.

Setting up the microscope and preparing local drug delivery media

8. Turn on the microscope stage incubator, wait until the temperature stabilizes. Turn on CO_2 and adjust the gas flow rate for maintaining the pH of culture medium.
9. Turn on TLA-100 ultracentrifuge, install the rotor, and wait for the temperature to reach 4°C.
10. Prepare solution for local delivery and place 50 μl in each ultracentrifuge tube. Centrifuge at 30,000 rpm for 30 min.
11. After ultracentrifugation, remove the rotor and take out the centrifuge tubes with a pair of tweezers.
12. Without agitating the tube, carefully remove 40 μl of the solution from the top of the tube into a fresh Eppendorf tube. Keep the pipet tip away from the bottom of the contribute tube.
13. Place the cell culture dish onto the microscope. Carefully add ~2 ml of mineral oil to the medium to cover the entire surface and prevent evaporation.

Loading the microneedles and securing the dual instrument micromanipulator

14. Attach a rubber bulb over the wide end of the pulled Pasteur pipette. Load the solution for delivery media into a pulled Pasteur pipette, by capillary action after dipping in the tip into the solution. Do not apply suction.

15. Using the pulled narrow end of the pulled Pasteur pipette, load the solution for local delivery into a release microneedle.

16. Attach the loaded release microneedle to a needle holder, connect with positive pressure at ~0.3 PSI without pressurizing the solution. Mount the needle holder on the dual instrument micromanipulator. Assuming that micromanipulator is to be controlled with right hand, the needle should be mounted at the position toward the user.

17. Attach a suction microneedle to a needle holder, connect with negative pressure and mount of the dual instrument micromanipulator. Assuming that micromanipulator is to be controlled with right hand, the needle should be mounted at the position away from the user.

18. Move the vertical position control knob of the dual instrument micromanipulator close to the center of the range.

19. Tilt down the release microneedle at around 45^{o}. Position the tip of the suction needle close to the tip of release needle but slightly above and behind.

20. Carefully position the dual instrument micromanipulator on the platform next to the microscope, such that that the microneedles are above the culture chamber and directly over the objective lens. Secure the micromanipulator in this position.

21. Carefully lower the tip of the needle through the layer of mineral oil into the medium. Immediately pressurize the solution.

Performing local drug release with the setup and collecting data

22. Using a 10x objective lens, focus on cells and look for an area of interest.

23. Raise the objective lens such that the cells become out of focus. Carefully lower the dual microneedles while sweeping its horizonal position, look for a moving shadow in the microscope field.

24. Once the shadow of the tips of the needle is identified, carefully bring the tip of the release needle to focus and to the center of the field.

25. Change to 40x. Focus above cells, lower the tip of the release needle until it is in focus. Bring the tip to the center of the field.

26. Fine adjust the position of the suction pipette such that its tip is approximately 20-40 μm behind the release needle at a slightly higher elevation.

27. Apply a few pulses of purge pressure while observing the fluorescence of fluorescent dextran. Make sure that it is flowing out smoothly. It should appear as a fuzzy bolus at the tip of the release needle, the size of which may be controlled by adjusting the negative pressure.

28. Focus down on cells (tips of the needle become out of focus). Carefully bring down the microneedle until the tip of the release needle is right next to the region for treatment and slightly out of focus, being above the focal plane of the cell.

29. Continue to monitor the flow of the solution. Fine tune the positive and negative pressure to obtain the desired rate of delivery and area of treatment, as indicated by the fluorescent dextran in the solution. Apply pulses of purging pressure in case the flow stops.

Notes

1. If the lower arm of the pipette puller does not fully drop down after 15 seconds, manually turn off the pipette puller, check the shape of the heater, and try again with a new capillary tube to avoid internal damage to the equipment.
2. Clean up all visible broken pieces of glass to avoid injury.
3. Finished release and suction microneedles can be stored indefinitely in a desiccator for later use.
4. Pulled Pasteur pipettes can be stored indefinitely.
5. Take care not to introduce air bubbles when loading the release microneedle. Try tapping the needle if this happens. Replace the release needle if air bubbles remain.
6. Do not turn on compressed air valve until needle tips are submerged beneath the mineral oil to minimize clogging.
7. Make sure that the local drug release is reaching the intended target by checking above and below the intended focal plane.

Reference & Citation

O'Connell, C.B., Warner, A.K. and Wang, Y.-L. (2001) Distinct roles of the equatorial and polar cortices in the cleavage of adherent cells. *Curr. Biol.* 11:702-707.