BE 167L - Bioengineering Laboratory

Lab 9: 3D Encapsulation and Preparing Varied Stiffness Substrates

Prelab reading

Read the first 5 pages of the posted article “Encapsulation of Cells in Alginate Gels”. You do not have to read the methods unless you are curious. The procedure outlined below is different from that described in the article. There are two parts to your lab today. You may be asked to start with either the 3D encapsulation or the substrate preparation depending on the time. You will encapsulate your 3T3 cells in alginate hydrogel beads for culture today, and evaluate their viability at different encapsulation densities in the next lab period. The second section of this lab is preparation for your last two defined labs next week. You will prepare substrates of PDMS with varying stiffnesses so that you can seed your 3T3 cells onto them and evaluate their behavior in response to different stiffnesses.

Watch the lab primer video.

3D encapsulation

If time permits, your TA will give a demonstration on how to encapsulate cells in alginate beads before you begin work with your cells. Once you have encapsulated your cells, you will allow them to incubate until the next lab, when you will evaluate them using a new technique that identifies individual live and dead cells.

Preparation

Reagents

- Your T25 flask from last lab
- Complete medium from previous lab
- Sterile DPBS from previous lab
- Trypsin
- Sodium alginate
- 100 mM $CaCl_2$ solution

Supplies

- Pipettes and tips
- Pipet-aid and serological pipettes
- microcentrifuge tubes
- 15 mL conical tubes
- 1 6-well cell culture plate
- 1 T25 flask

Equipment

- Phase contrast microscope
- Centrifuge
- Hemocytometer and cell counter

Procedure

1. Check confluency of your T-25 and begin passaging (further detailed in step 3). You will use these cells for today's encapsulation and seeding another T-25 flask.
2. While one member passages, aseptically aliquot 5 mL of sodium alginate solution. (The concentration of sodium alginate is 15 mg/mL.)
3. Basic passaging procedure
    a. Rinse with DPBS (2mL)
    b. Trypsinize (2 mL)
    c. Deactivate trypsin with media (4 mL)
    d. Centrifuge for five minutes at 150 x g/rcf (Check with the TA prior to centrifuging)
    e. Aspirate supernatant and resuspend in 1 mL of media
    f. Count resuspended cells
4. Seed a new T-25. You will use these cells for labs next week, so you can seed at a lower density. ~100000 cells per flask is acceptable.
5. Split your remaining cells into 3 new tubes (1:2, 1:3, 1:6). So if you had 900 µL of cell solution left, you should have 3 tubes, one with 450 µL of cell solution, one with 300 µL of cell solution, and one with 150 µL of cell solution. Make sure you record how many cells are in each tube.
6. Fill the 3 cell solution tubes to the 5 mL mark with DPBS, and centrifuge. You are rinsing the cells one more time prior to encapsulation in this step.
7. Aspirate the DPBS and resuspend in 1 mL of the alginate solution for each tube. You should now have 3 tubes with different cell density that you can calculate from before.
8. Bring a 6 well plate into the hood, and aliquot 2.5 mL of 100 mM calcium chloride solution per well. Make sure this is done under sterile conditions. You can label your 6 well plate for the 3 different densities at this time as well.
9. Use a 1000 µL pipette to dropwise from the appropriate tube to each well. Do this slowly, and be careful to not aggregate beads. Your TA may demonstrate this part of the procedure for you at this time.
10. Incubate at RT for 10 minutes, then rinse 4 times with sterile DI H_2O. These rinsing steps remove the excess calcium ions. Using a 1000 µL pipette to remove the water is easiest, an aspirating pipette may suck up the alginate beads.
11. Fill each well with cells with 5 mL of media. The encapsulated cells require more media to survive.

Preparing varied stiffness substrates

You will make thin layers of PDMS using 3 different ratios of base to cross-linker (10:1, 30:1, and 60:1) in order to get different stiffnesses. These substrates will be used for next week's lab. It is important to keep this layer as thin as possible which will make microscopy possible. It is also important to keep the layers the same thickness between replicates so that their bulk stiffnesses are the same. Thickness of less than 2*mm* is realistically achievable.

Preparation

Reagents

- Sylgard 184 base
- Sylgard 184 curing agent

Supplies

- Plastic transfer pipettes
- weigh boats/50 mL conical tube
- 1 24-well plate

Equipment

- Oven at 60°C
- Vacuum dessicator

Safety

Wear your usual protective equipment. Take care when handling the Sylgard reagents. They are very oily and difficult to remove. You may need to change your gloves frequently to keep from spreading this reagent to your bench space, equipment, and clothes. Keep some paper towels nearby to help contain any spills or accidents.

3.2 Procedure

1. Make about 1-3 mL of your three base and curing agent mixtures. The ratios are 10:1, 30:1, 60:1 (do not waste curing reagent! You can use either a 50 mL tube to mix or a weigh boat.
2. Dispense a small and equal amount of PDMS to individual wells of a 24 well plate. You want at least 3 wells of each condition. You want the amount of PDMS in each well to be equal, and to fully cover the well. Use whatever transfer method works best for you, and to fully cover the well you can rotate the well plate to fully cover the bottom. You will want to aim for ~200 µL of solution per well.
3. De-gas in the vacuum dessicator for 30 minutes and then place your plate in the oven for at least 3 hours. It is ok if the plates are left in the incubator until the next lab.