BE 167L - Bioengineering Laboratory

Lab 10: Evaluating Cell Viability in Hydrogels and Preparing Substrates to Study Cell Behavior

Prelab reading

Read the posted instructions for the Live/Dead viability/cytotoxicity kit from Molecular Probes.

You will evaluate the viability of your encapsulated cells using fluorescent dyes. This type of labeling will enable you to see spatially where dead and live cells are located in your alginate beads. Can you predict where cells will be alive and where they will be dead? Can you predict which concentrations of cells will have more live or dead cells? While you may be able to count the number of dead and live cells in a frame of the microscope to get a % viability, it would take many frames and a lot of time to count all the cells in a single bead, and may not be realistically possible. Challenge question: Think of a way to remove the cells from the calcium alginate beads (hint: how is the gel crosslinked?)

The type of information you get from this assay is different from the previous assays you learned (BCA and MTS). It may be difficult to get an average picture of the entire population in terms of total number of cells or metabolic activity, but it can give you spatial, visual, and morphological information that you wouldn't get otherwise. These are just some of the culture characteristics that you can look for in the microscope if the cells are stained:

- Spread or rounded
- Clear or granular (lots of vacuoles)
- Large or small
- Releasing vesicles from its membrane or not
- Many or few in the dividing stage of the cell cycle
- Clustered in clumps or dispersed evenly throughout

In addition to the proliferation assays that we have covered in this class, there are several other assays which characterize cells in different ways. Can you name an assay which measures an aspect of cell activity?

In the second part of this lab you will prepare your PDMS substrates for cell seeding in the lab next week. You will need to prepare them for cell culture. Your TA may ask you to perform this part of your lab first or second to distribute the use of fluorescent microscopes during your lab period.

Watch the lab primer video.

Live/Dead stain

Your TA will have the reagents warmed to room temperature. In the future, make sure that they are room temperature before you open them, or water will condense in the tube and degrade the reagents.

Preparation

Reagents

- Your 6-well plate with alginate beads from the last lab
- Your T25 flask from last lab

- Complete medium from previous lab
- Sterile DPBS from previous lab
- Trypsin
- Live/Dead kit from Molecular Probes (two reagents: calcein AM and ethidium homodimer)

Supplies

- Pipettes and tips
- Pipet-aid and serological pipettes
- 15 mL conical tubes

Equipment

- Phase contrast microscope
- Centrifuge
- Hemocytometer and cell counter
- Fluorescent microscope

Safety

Wear your safety glasses, lab coat, and latex gloves. Continue to practice good sterile cell culture techniques and proper biohazard waste disposal. Be careful with the Live/Dead reagents. They are dissolved in DMSO, which is an organic solvent and considered hazardous chemical waste. Also remember that calcein AM and ethidium homodimer enter living and dead cells, both of which exist on your skin. Wash promptly with soap and water if you have contact with them. Don't look directly at the fluorescent light beam coming from the microscope.

Procedure

1. Gauge confluency of your T-25 and change media. Your T-25 should not be confluent so all you need to do is change media today.
2. Observe your 6 well plate under the microscope. You may want to take a picture for your own records. Things you should be looking for are contamination, and if your cells have escaped from the beads.
3. Remove medium and rinse twice with DPBS for each well. Use a micropipette and be careful not to lose any beads. Rinse for 5 minutes
4. Aliquot 8 mL of sterile DPBS and add to it 1 µL of calcein AM, and 2 µL of ethidium homodimer. Your TA will have these sterile aliquots for you to add to your tube of DPBS, make sure to mix well using a vortexer.
5. Remove the rinse from your wells and place 2.5 mL of the working solution into each well.
6. Place into the incubator for 30-45 minutes. A longer time may be necessary depending on the thickness of the beads.
7. Visualize under fluorescent microscopy, and take pictures. Look at your samples under the fluorescent microscope, switching between the bright field (with appropriate phase contrast), GFP, and RFP filters to see green and red cells (alive and dead). In your lab report, you will be asked to summarize and describe your alginate beads. Before lab section, think about different ways to characterize your cell populations. Take pictures accordingly, so that you have data representing the different characteristics of the cells. An incomplete list of characteristics includes: bead size, bead geometry, cell viability (percentage in a whole gel), cell viability (as a function of depth into a bead), cell morphology, cell density. Record interesting observations in your lab notebook.

Preparing varied stiffness substrates

Your 24-well plate should be out of the oven now. You will prepare the surfaces for cell seeding today and seed cells on them next week.

Preparation

Reagents

- Complete medium from previous lab
- Sterile DPBS from previous lab
- Your 24-well plate with PDMS substrates from the last lab
- 70% ethanol

Supplies

- Pipettes and tips
- Pipet-aid and serological pipettes

Safety

Wear your usual protective equipment.

Procedure

1. In the hood, add 500 µL of 70% Ethanol to each well with PDMS, incubate with the lid on for 30 minutes. Prolonged contact with ethanol will ensure sterilization of the surface. PDMS is highly hydrophobic and should not take up any of the ethanol.
2. Aspirate the 70% ethanol and rinse with 500 µL of DPBS 4 times. Excess rinses will ensure that any residual ethanol leaves.
3. Store your well plate in the incubator. Do your best to maintain sterility!