BE 167L - Bioengineering Laboratory

Lab 11: Variable Substrate Stiffness and Cell Behavior (Part 1)

Prelab reading

You will seed 3T3 cells onto PDMS substrates of varying stiffness as well as onto the regular tissue culture plastic in your 24 well plate. Stiffness of the culturing surface can affect many aspects of cell behavior. Stem cells may be influenced to differentiate into different lineages based on the stiffness. Smooth muscle cells may produce more F-actin or form more focal adhesions on stiffer substrates. Some cells may increase or decrease their proliferation rate or their rate of migration across the surface based on the stiffness.

Cell seeding

Preparation

Reagents

- Your 24-well plate with PDMS from the last lab
- Your T25 flask from last lab
- Fibronectin (50 µg/mL) in DPBS
- Complete medium from previous lab
- Sterile DPBS from previous lab
- Trypsin

Supplies

- Pipettes and tips
- Pipet-aid and serological pipettes
- 15 mL or 50 mL conical tubes
- T25 flask

Equipment

- Phase contrast microscope
- Centrifuge
- Hemocytometer and cell counter

Procedure

1. Use a microscope to identify which wells you will be using on your 24 well plate. You will want to avoid wells that appear damaged/nonuniform. You may opt to mark these wells with an X to avoid using them.
2. Coat each well you will be using (9 wells with PDMS and 3 without) with 150 uL of fibronectin solution. Please do not take more than needed. Let it sit covered for 15-20 minutes in the BSC.
3. Rinse the surfaces twice with DPBS to wash off the non-adherent proteins.
4. While you are coating your surfaces passage your T-25. You will use the cells from passaging to seed your wells, as well as prepare for your experiments next week.

5. Seed your wells with cells from your T-25. You will want to seed at least 20,000 cells in each well, but no more than 80,000. while still reserving cells total for a new T25 flask.
6. Check your wells under the microscope to make sure your cells are evenly distributed. Do this for both the T-25 and your 24 well plate.
7. Clean up, and prepare for your independent projects. If you haven't already, please check in with your TA regarding your independent lab projects.