BE 167L - Bioengineering Laboratory

Lab 12: Variable Substrate Stiffness and Cell Behavior (Part 2)

Prelab reading

You will evaluate your 3T3 cells on the different stiffnesses of PDMS today. You must passage your T25 flask today in preparation for your independent experiment over the next two weeks (if you plan on using cells). Make sure you have at least 2 new T25 (one per person) flask started today so that you will have enough cells for you experiment to begin next week.

Quantifying cell morphology: Measuring circularity

There are many methods for analyzing cell morphology. One of the measures that could be used for this purpose is circularity which is a dimensionless number that describes the degree of compactness of a shape. A common measure would be the ratio of the area of the shape to the area of a circle having the same perimeter. You could utilize ImageJ software which has the circularity feature to calculate circularity. Note that this is only one possible way of looking at it, and you are definitely encouraged to come up with other ways of looking at the experimental results and analyzing them. ImageJ is a free program available from the NIH. Download the appropriate version for your operating system: http://rsbweb.nih.gov/ij/download.html. After installing, open the program. A tool bar should appear. Drag an image that you captured onto the tool bar to open it.

In the Analyze tab choose Set Measurements. Circularity MAY be one of the options, in which case check `Circularity` and uncheck all other measurements. If it is *not* available, the equation for circularity is $C = 4\pi(\text{area}/\text{perimeter}^2)$. Check area and perimeter while leaving all other measurements unchecked. Click `Ok`. Now select the Freehand selections tool. For the next task you will likely want to Maximize the image to give you the best accuracy. Make a freehand selection of a cell by clicking and dragging the mouse around the perimeter of a cell. When finished select `Measure` under the `Analyze` tab or type `Control-M`. A window should appear containing your measurements (`Circularity` or `Area` and `Perimeter`). Copy measurements for each cell and, if necessary, compute the circularity. Using basic statistics (mean, standard deviation, Student's t-test) compare the cell morphology on the three substrates.

Cell passaging and circularity measurements

Preparation

Reagents

- Your T25 flask from last lab
- Complete medium from previous lab
- Sterile DPBS from previous lab
- Trypsin

Supplies

- Pipettes and tips
- Pipet-aid and serological pipettes
- 1 15 *mL* conical tube
- 1-2 T25 flasks

Equipment

- Phase contrast microscope with camera

- Centrifuge
- Hemocytometer and cell counter

Procedure

1. Passage cells if necessary for your independent projects. If you do not need cells you can aspirate them away.
2. Image your 24 well plate on 20x/40x refer to the section above for specifics. You will want pictures of at least 3 random cells from each sample group (12 total)
3. Clean up after yourself and prepare for independent lab projects and the lab practical next week. You are now done with all of the organized labs!