EXERCISE 7

Migration and Colony Formation of Endothelial Cells

We will be working with Bovine Aortic Endothelial cells (BAE), an adherent cell line. When these cells have the ability to divide in 3 dimensions, as they do "in situ," they can form tubules. We provide a 3-dimensional environment by dispersing them at replating in a medium containing molten soft agar, which then gels on the plate. This protocol is commonly referred to as a "soft agar assay." The experiment will take 14 days to complete, but observations should be made every few days.

Each group will receive two 35 mm^2 plates with BAE cells. From these plates, each group will then make 4 plates total.

PREPARATION OF BASE AGAR

1. Melt 1% agar in a microwave and cool to 40ºC in a water bath (**Note:** This solution is already made).

2. Warm 2X RPMI + 20% FCS to 40ºC in water bath (**Note:** This solution is already made). Allow at least 30 minutes for temperature to equilibrate.

3. Mix the aliquots of the two solutions. (*What would be the final concentration of two solutions?*)

4. Add 1.5 mL of mixture to each empty 35 mm^2 Petri dish and set aside for 5 minutes to allow agar to solidify. After they have solidified, label appropriately.

PREPARATION OF TOP AGAROSE

1. Melt 0.7% Agarose in a microwave and cool to 40ºC in a water bath (**Note:** This solution is already made). Also warm 2X RPMI + 20% FCS to the same temperature.

2. Trypsinize the BAE cells to release them (For these cells, it would require a ~10-minute incubation at 37ºC).

3. Add 0.1 mL of cell suspension to sterile 15-mL centrifuge tubes.

4. Add 3 mL of 2X RPMI + 10% or 20% FCS and 3 mL 0.7% Agarose to a tube of cells. Mix gently by swirling, and add 1.5 mL to each of the four plates with the base agar solidified. Only do one tube at a time so that the Agarose does not set prematurely.

5. Incubate plates at 37ºC in humidified incubator for 10 to 14 days.

6. Stain plates with 0.5 mL of 0.005% Crystal Violet for more than 1 hour at room temperature. The stain is visible without the use of a microscope; however, for lighter-stained cultures, use either a microscope or a dissecting microscope to view the staining better.

** Note the morphology of these cells prior to plating, and compare it to after being mixed with the agar/Agarose. If there is a change in morphology, how could it be explained, given the cell type? What could be tested using this type of assay?

Note: Cells in agar when plated at the appropriate density will form tubes. Cells that grow too densely will not form tubules, but rather sheets that "wave" through the agar overlay that may be difficult to maintain in continuous focus in the microscope.

A good comparison/control is to plate some on culture dishes without agar, which, instead, will yield a simple monolayer. As an additional control, include a transformed line, such as neuroblastoma cells, that yield monolayers and do not aggregate or interact in the soft-agar assay.