Migration Assay Protocol Week 2

Day 1 (*under the hood*)

1. Under sterile conditions, allow the invasion chamber plate to warm up at room temperature for 10 min.
    a. Plates will be individually packed. Make sure you open them under the hood and label your plate.
2. Rehydrate the membrane layer of the cell culture inserts by adding 100 µL of warm plain media to the inner compartment. Incubate at room temperature for 1 hour.
    a. Make sure the 100 µL is added to the insert only.
    b. Simply leave the covered plate inside the hood for the 1 hour incubation.
3. Prepare a cell suspension containing $1.5x10^5$-$3x10^5$cells/well in serum free media.
    a. In order to make treatment groups prepare 4 mL of cell suspension with a concentration of $0.5\text{-}1.0x10^5$ cells/mL.
        i. This will still give you $1.5\text{-}3x10^5$cells/well but allows for 400 µL extra.
    b. The serum free media should still contain P/S and L-glut as needed depending on your cell's usual media.
    c. Count the cells in your flask according to normal counting methods.
    d. Then make calculations for needed volume and concentration of cells/volume.
4. Divide the 4 mL cell suspension into 4 sterile vials labeled Unt., drug concentration 1, concentration 2, and concentration 3. Each vial should contain 1 mL of cell suspension.
5. Prepare treatment groups by taking out the appropriate volume of media and adding the appropriate volume of drug for each treatment group.
    a. See *Making Drug Treatments* protocol for example calculations.
6. Carefully remove the rehydration medium from the inserts without disturbing the membrane layer. Be sure not to puncture the membrane.
    a. Note: It will not affect the assay performance if a small amount of rehydration medium is left in the compartment.
    b. Vacuum off the media with a Pasteur pipet.
    c. Place a finger on one side of the insert to hold it down while you are vacuuming.
7. Add 500 µL of complete media (with fetal bovine serum) or desired chemoattractant(s) to the lower well of the migration plate.
8. Add 300 µL of the cell suspension solution to the inside of each insert.
    a. Plate 3 inserts from each vial.
    b. Make sure your plate cover is labeled, so you know where each treatment group is located.
9. Cover plate and incubate for 24 hours at 37°C in 5% CO_2 atmosphere.
    a. This is the environment of our normal incubator.
10. When you are done, check with your TA or lab instructor for instructions on cell maintenance.

Day 2 (*not under the hood*)

11. After 24 hours, take the plate out of the incubator.
    a. Make sure to **put on gloves** for the following steps.
    b. The steps below *do not* have to be done under the hood.
12. Carefully lift inserts and vacuum off the media in the lower wells. Place the insert back into the empty well.
13. Put 400 µL of Cell Stain Solution into a clean well for each insert used (should be 12).
14. Completing one insert at a time:
    a. Pick up insert.
    b. Vacuum the media out of the insert.

c. Wet the end of a cotton-tipped swab with 1X PBS and roll it against the side of the tube to get off the excess PBS. Gently swab the interior of the insert to remove non-invasive cells.
    i. Take care not to puncture the polycarbonate membrane.
    ii. Be sure to remove all cells on the inside perimeter of the insert
    iii. The cells that migrated should be on the outside of the membrane because they migrated through it towards the complete media containing the nutrients.
d. Place swabbed insert back in well until all inserts have been swabbed. Then transfer all inserts to the wells containing the Cell Stain Solution at the same time.

15. Once all inserts have been transferred to the Cell Stain Solution, incubate for 10 minutes at room temperature.
16. Gently wash the stained inserts several times in a beaker of diionized water. Carefully vacuum inside the insert to speed up the drying process. Then allow inserts to air dry.
    a. To keep track of which insert is which write labels on the paper towel above where you place each insert.
    b. To air dry place inserts upside down on a folded paper towel.
    c. Note how each set of inserts looks (see below). Take a picture of the inserts beside their labels to later compare to the numbers from the plate reader.
17. Add 400µL of Extraction Solution per well plus 3 wells to use for blanks in a new 24 well plate. Transfer each insert into a well with Extraction Solution in the new 24 well plate.
18. Incubate the plate for 10 minutes on the belly dancer.
19. When complete, remove the inserts from the plate. Keep inserts until plate is read.
    a. Again take a picture of the plate.
20. Remove lid and read the plate in the plate reader using *Hensley NBT assay 560nm 24 well plate*.
21. Save results in an excel file in your folder in the "Cell Biology Fall 2015" folder, and send to your email.
22. Graph results.

Use visual clues to make sure your numbers match what you are seeing.