Cancer Biology
Biol 445

Lab 1
Cancer cells/Cell culture

A major advance in biological research came with the ability to grow cells in culture. As you are beginning to understand, normal tissue cells don't grow well outside the host organism. Cells can be dissociated from a tissue and placed in a nutrient-rich medium where they may divide for a short time (perhaps 50 doublings), after which they will enter senescence and eventually die. These cultures, called ***primary cell cultures***, can be used for short term studies.

In the early 1950s, after years of unsuccessful attempts, the first immortal cell culture was established. The cells came from **Henrietta Lacks** who had an unusually aggressive cervical cancer. A biopsy of her tumor was placed in a liquid medium including animal serum which was assumed to contain growth chemicals to promote cell division. Immortal cell cultures are now called ***cell lines*** and technology for establishing them has improved steadily since the **HeLa** cell line was first grown. HeLa cells are arguably one of the most valuable tools developed for basic and applied human biological research. (You will read more soon).

Many cell lines are now grown from animal and human tumors. Cancer cells by definition grow abnormally, usually dividing rapidly and ignoring normal signals to differentiate or die. Cell lines can also be established by introducing **oncogenes** into a cell type of interest, mimicking cancer and making the cell type immortal.

Cell lines usually require specific nutrients formulated in a specific *media*. Other factors called *growth factors* are provided in serum. Fetal calf/bovine serum (FBS) is often used as a supplement to the specific medium providing growth factors, some of which are still undefined. Cell culture media often has an additive to monitor the pH. As cells become more crowded, waste accumulates, lowering the pH. pH changes can be seen as a change in color of the media with these additives.

Cell lines grow in one of 2 ways, either *attached* to a substrate (such as a plastic flask (Like HeLa cells), or in *suspension* (floating around). Hematopoietic cells such as lymphocytes grow in suspension, whereas macrophages tend to attach to a substrate. Fibroblast cells (e.g. from skin) tend to grow as attached cells.

Cancer Biology
Lab 1

Lab 1 introduces us to cell culture (also called tissue culture). We will work with and observe a hematopoietic cell line called MonoMac 6 or **MM6**. MM6 was established from a human with leukemia. (see background sheet). The cells grow in suspension in specific media (RPMI-1640) supplemented with 10-20% FBS.

The goal of lab 1 is several fold:

1. To gain general experience in cell culture, specifically in maintaining cells in a sterile environment.
2. To monitor cell division/growth of MM6 in culture.
3. To induce and observe cells differentiating in culture in response to specific agents.

The differentiation agent we will use is 12-O-tetradecanoylphobol-13-acetate called **TPA** or **PMA**. It is a commonly used agent for promoting differentiation of myeloid cells in culture.

Protocol to follow.

Cancer Biology
Lab 1

Protocol—done in Moore 216

Part 1—Observing cells in culture---gross morphological characteristics/cell count

Materials

- 1 6-well culture plate
- Inverted microscope
- Flask of MM6 cells
- Sterile pipettes, electric pipettor
- Micropipettor, sterile tips
- 1 tube of TPA (1 X 10^{-5} Molar)
- Hemacytometer
- Microcentrofuge tubes
- Trypan blue dye
- New, T-25 culture flask

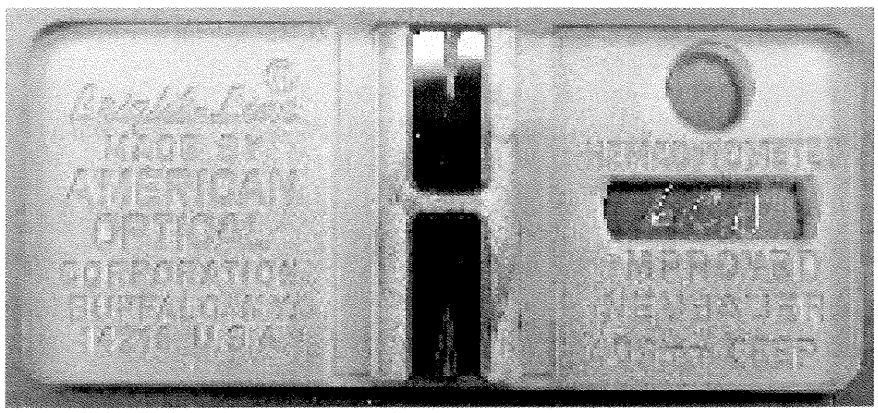
1. Remove the stock cell culture flask of MM6 cells from the CO2 incubator and observe cells using the inverted microscope.
2. Observe and record the appearance of the media and the cells. Shape? Suspension or attached? Single cells or clumpy?
3. Following Dr. Super's instructions:
    a. Wipe down the tissue culture hood, using 70% ethanol
    b. Use a sterile pipette to resuspend the cells (flush the cells through the pipette and off of the flask back surface)
    c. Remove 0.1 ml of cell culture and pipette into a microcentrofuge tube
    d. Combine cells with 0.1 Trypan Blue dye
    e. Load 10 ml of cell-dye mix into each side of a Hemacytometer
    f. Count cells in the 5 large squares of the Hemacytometer grid (see hand out)---use microscopes in 318.
    g. Determine a cell concentration and a total number of cells in the stock flask.
    h. Establish a new flask at 2 X 10^5 cells/ml in 15 ml media.

4. Label 2 wells of the culture plate ***+TPA*** and 2 wells ***–TPA.*** From the new flask of MM6 cells, draw up 4 ml and place in each culture plate.
5. Add 20 µl TPA (10^{-5} molar) to the appropriate culture plate and gently swirl. (What is the final concentration of TPA in your cultures?
6. Place the 6-well plate in the incubator.

7. On Monday, begin daily observations and cell counts.
    a. Sketch and describe as many of the differences as possible in your +TPA and –TPA plates.
    b. Using a 5 ml pipette, gently flush the cell culture. Allow the whole 4 ml volume to rinse the surface of the plate 3-4 times, taking care not to introduce lots of bubbles.
    c. Prepare your cells to count as above. (Steps 3C-3G)
    d. Save data on cell appearance and cell counts for graphing and discussion.

Cancer Biology
Lab 1

Hemacytometer---slide with etched grid at the bottom of a chamber.



Etched grid of hemacytometer