Advanced Cell Biology Labs 12.
Cell cultures

May 2nd, 2011

Background

I. Introduction

In today's lab, we will practice the handling of human cells growing in culture. Much of what we know about the function of specific cells of the immune system (and virtually all eukaryotic cell biology) comes from studying cells/cellular activities in vitro (outside the organism). All the techniques used to maintain and manipulate cells in vitro are referred to as tissue culture or cell culture. Two major goals of growing cells in culture are to:

1. Provide the necessary nutrients and environment for cells to divide regularly, but NOT to change in major characteristics (i.e., inhibit differentiation)
2. Maintain sterility (prevent bacterial and fungal contamination)

Cell lines are established from tissues by dissociating the cells from the tissue and providing them the proper conditions for their growth. Dissociated cells can divide for some period of time, but then cease to divide or senescence. True cell lines require some genetic change to become immortal (divide forever). This can occur spontaneously (cancer cells), or be induced with chemicals or cancer-causing animal viruses. Hematopoietic cell lines are established from blood cells, often from individuals with leukemia or lymphoma. In this case the cells have already undergone the genetic changes that make them grow indefinitely while in the person having leukemia or lymphoma.

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 two ways: either attached to a substrate (such as a plastic flask, 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. Today we will practice with suspension cell culture techniques using a cell line derived from a leukemia patient. The cell line is called **THP-1**. Some cells are viable, but some are dead, and these will be stained with Trypan Blue because it will go inside dead cells (e.g., cells with damaged membrane) only.

II. Using a Counting Chamber

For microbiology, cell culture, and many applications that require use of suspensions of cells it is necessary to determine cell concentration. A device used for determining the number of cells per unit volume of a suspension is called a counting chamber. The most widely used type of chamber is called a *hemacytometer*, since it was originally designed for performing blood cell counts.

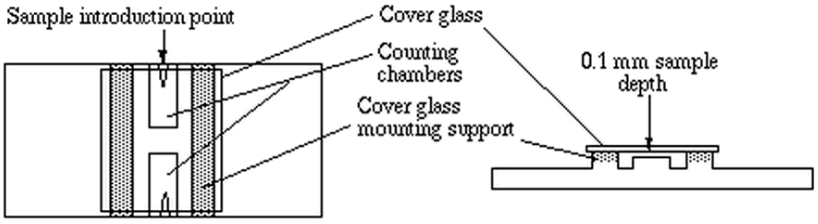

Fig. 1. Hemacytometer.

To prepare the counting chamber the mirror-like polished surface is carefully cleaned with lens paper. The coverslip (coverglass) is also cleaned. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid. The coverslip is placed over the counting surface prior to putting on the cell suspension. The suspension is introduced into one of the V-shaped wells with apipette. The area under the coverslip fills by capillary action. Enough liquid should be introduced so that the mirrored surface is just covered. The charged counting chamber is then placed on the microscope stage and the counting grid is brought into focus at low power.

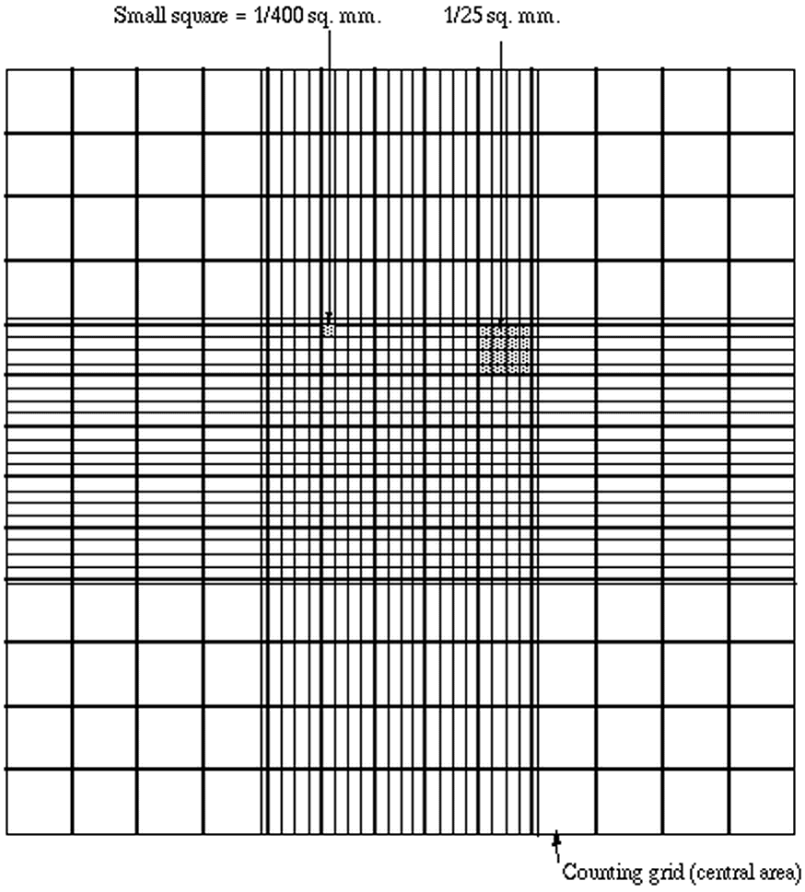

Fig. 2. Counting grid.

It is essential to be extremely careful with higher power objectives, since the counting chamber is much thicker than a conventional slide. The chamber or an objective lens may be damaged if the user is not not careful. One entire grid on standard hemacytometers with Neubauer rulings can be seen at 40× (4× objective). The main divisions separate the grid into 9 large squares (like a tic-tac-toe grid). Each square has a surface area of one square mm, and the depth of the chamber is 0.1 mm. Thus the entire counting grid lies under a volume of 0.9 mm-cubed.

Suspensions should be dilute enough so that the cells or other particles do not overlap each other on the grid, and should be uniformly distributed. To perform the count, determine the magnification needed to recognize the desired cell type. Now systematically count the cells in selected squares so that the

total count is 100 cells or so (number of cells needed for a statistically significant count). For large cells this may mean counting the four large corner squares and the middle one. For a dense suspension of small cells you may wish to count the cells in the four 1/25 sq. mm corners plus the middle square in the central square. Always decide on a specific counting pattern to avoid bias. For cells that overlap a ruling, count a cell as "in" if it overlaps the top or right ruling, and "out" if it overlaps the bottom or left ruling.

Suppose that you conduct a count as described above, and count 187 particles in the five small squares described. Each square has an area of 1/25 mm-squared (that is, 0.04 mm-squared) and depth of 0.1 mm. The total volume in each square is $(0.04)\times(0.1) = 0.004$ mm-cubed. You have five squares with combined volume of $5\times$ $(0.004) = 0.02$ mm-cubed. Thus you counted 187 particles in a volume of 0.02 mm-cubed, giving you $187/(0.02) = 9350$ particles per mm-cubed. There are 1000 cubic millimeters in one cubic centimeter (same as a milliliter), so your particle count is 9,350,000 per ml.

Cells are often large enough to require counting over a larger surface area. For example, you might count the total number of cells in the four large corner squares plus the middle combined. Each square has surface area of 1 mm-squared and a depth of 0.1 mm, giving it a volume of 0.1 mm-cubed. Suppose that you counted 125 cells (total) in the five squares. You then have 125 cells per 0.5 mm-cubed, which is 250 cells/mm-cubed. Again, multiply by 1000 to determine cell count per ml (250,000).

Sometimes you will need to dilute a cell suspension to get the cell density low enough for counting. In that case you will need to multiply your final count by the dilution factor. For example, suppose that for counting you had to dilute a suspension 10 fold. Suppose you obtained a final count of 250,000 cells/ml as described above. Then the count in the original (undiluted) suspension is $10 \times 250{,}000$ which is 2,500,000 cells/ml.

Assignment

1. Become familiar with THP-1 cell morphology and in vitro growth characteristics
2. Become familiar with hemacytometer technique
3. Determine cell concentration (# cells/ml culture media) and viability (% of non-stained with Trypan Blue cells)
4. ***Provide a resulted cell concentration, viability and a short description of how you calculated it***

Materials

1. Flask of THP-1 cells
2. Trypan Blue
3. Microfuge tube
4. Bleach, ethanol, lab tissue
5. Sterile pipette 2–20 μL
6. Hemacytometer

Procedure

[*Instructor's part is in italics*]

1. Put on gloves
2. Be sure to carefully clean the hemacytometer. Lift off the coverslip and squirt off the coverslip and slide with 10% bleach, into the plastic waste cup. Then squirt off both with 70% ethanol and shine with a lab tissue. When dry, return to the box.
3. *Obtain your flask from the incubator*
4. *Look at it using the inverted microscope*
5. *Loosen the cap on one of your flasks. Gently loosen the cells from the flask bottom. Try to get as many off as possible. Do not remove the cells yet.*

6. *Mix 2 mL of cell suspension with 2 mL of Trypan Blue in a separate tube*
7. *Make 200 μL aliquotes of mixture in microfuge tubes*
8. Using a hemacytometer, charge both sides with cells from microfuge tube (use 10 µL pipetting)
9. Count cells and determine cell concentration (# cells/ml) and viability using both sides
10. Clean the hemacytometer again; be sure to put all waste in a plastic container (**not** in a sink!)