Cell biology and cellular biophysics (TFY 4260)

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# Lab Exercise 4: Cell Cultivation

Activity period: Week 10 - 11
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## 1 Aim

The main purpose of this lab exercise is to understand how cells are cultured in laboratories and how cell-culturing environmental conditions, such as $CO_2$, humidity, temperature, pH and growth medium, affect cell activity and proliferation. The lab activity is focusing on detaching of cells grown as a monolayer in the 25 $cm^2$ tissue culture flask and manual cell counting by using a Bürker chamber. Three different cell lines of human cervix cancer cell (HeLa), osteosarcoma cell (OHS) and human prostate cancer cell (PC3) are cultured and studied. The growth curves that characterizing three phases of a cell growth in culture, are created to determine the doubling times of the observed cell lines.

## 2 Theory

### 2.1 Growing Cell in Culture

Cells can be removed from a living organism and cultured *in vitro* in a favorable artificial environment in laboratories. There are two type of cell lines; finite and continuous cell line. A finite cell line of normal cells is genetically determined by proliferation limit and senescence. Such cells are short-lived, from few days to weeks. When a finite cell line undergoes transformation, it becomes a continuous cell line acquiring the ability to divide indefinitely and uncontrolled cell growth. The cancer cells HeLa, OHS and PC3 are examples of such immortal cell lines.

Culture conditions vary for each cell type. In a culturing vessel, for instance cell tissue flask, it must contain a substrate/medium that supplies necessary nutrients, e.g. amino acids, carbohydrates, vitamins and minerals, growth factors, hormones and gases $O_2$ and $CO_2$. The physicochemical environmental factors, such as pH of 7.2, osmotic pressure and temperature of 37 °C, must be regulated precisely.

Cells grown floating in the culture medium are called a suspension culture, whereas an adherent monolayer culture consists of anchorage-dependent cells cultured by attaching to a (semi-) solid substrate in the bottom of the tissue culture flasks. [1].

### 2.2 Cell Growth, Phases and Growth Curve

Three different phases of cell growth in culture are observed as shown in figure 1 [1]:

1) *Lag Phase:* during the first 1-2 days, cells are maturing, and not yet able divide. There is thus no or little increase in the amount of cells. The cells adapt themselves to their new culture environment (growth medium) and undergo internal cytoskeletal changes that make them able to attach the surface of the cell tissue flask.

2) *Logarithmic (Log) Phase:* cells begin to actively proliferate and the amount of the cells increases exponentially. Each cell line shows different cell proliferation kinetics. The growing rate (or doubling time) is determined by growth conditions such as available nutrients in the medium and space in the flask. The number of cells N is given by:

$$N = N_0 e^{kt} \tag{1}$$

where $N_0$ is the cell population at time t = 0 and $k = \ln 2/T_d$ is the growth constant. The equation 1 can be used to determine the doubling time of a cell culture when N and $N_0$ are known.

3) *Plateau (or Stationary) Phase:* cell proliferation is stabilized that the cell number remains constant due to growth limiting factors, e.g. confluence and depletion of an essential nutrient. The total growth rate is equal to the death rate resulting in horizontal linear slope.

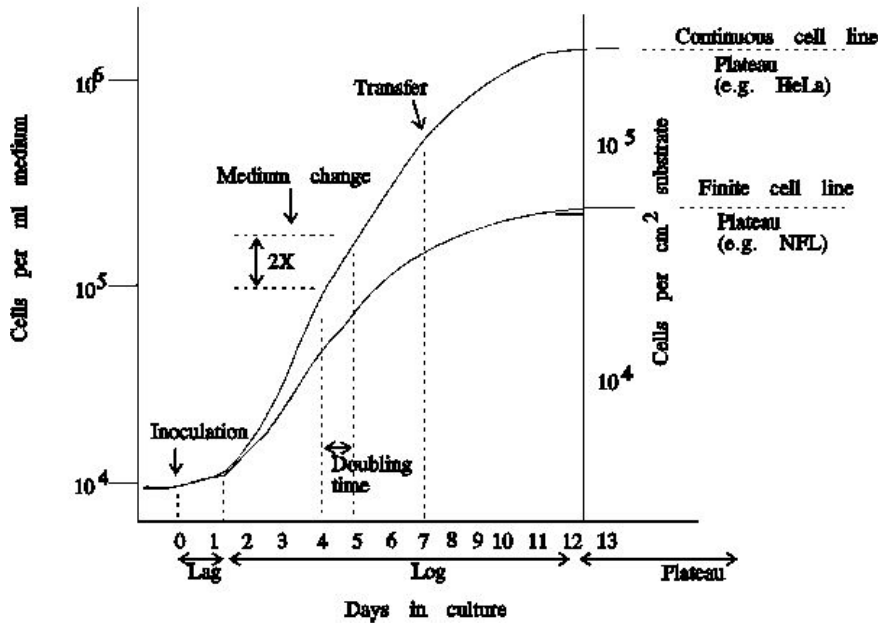


Figure 1. Characteristic growth/kinetic curve of cultured cells showing the cell density versus the time spent in culture. The growth of cells in culture proceeds from the lag phase, log phase to plateau phase [1].

# 3 Materials and Methods

## 3.1 Detaching Cells from 25 $cm^2$ Cell Culture Flasks

Cell detaching from prepared cell lines was performed in a cell culture hood, i.e. biosafety cabinet, to maintain an aseptic work area that is restricted to cell culture work. The working environment is protected from dust and contaminants by constant, unidirectional laminar flow of HEPA-filtered air inside the hood [2]. Hands must be washed thoroughly before putting lab coat and gloves on.

Phosphate buffered saline (PBS), trypsin and growth medium were firstly heated to 37 °C, the optimal growth temperature for most human and mammalian cell lines. The sterile bench of the cell culture hood and vacuum suction were activated. Equipments need were arranged within the hood; 2 x 5ml pipettes, 1 x 10ml pipette, pipettor and glass Pasteur pipette in the front right, and the chemicals PBS and trypsin and tube rack in the rare middle. Pre-prepared cell lines storing in the $CO_2$ incubator were observed in microscope before starting to detach. The incubator provided the appropriate environment for cell growth. All items and solutions, including hands, inside the hood that come in contact with the cells must be sterile and were therefore disinfected by spraying them with 70% ethanol.

The first step was removal of the growth medium from the cell culture flask with a pasteur pipette attached to vacuum suction followed by an addition of 3 ml PBS and tilting it gently. The purpose of PBS was to wash away the remaining medium containing trypsin-inhibiting serum . After removing the PBS with the vacuum suction, 1.5 ml trypsin was then added for 2 - 4 minutes to detach a monolayer of the cell line from the flask by deactivating those proteins on the cell surface that make the cells adhere to the flask. Subsequently, 8.5 ml growth medium was added to stop the enzyme reaction, using the pipette to re-suspend the solution inside the flask for at least 10 times. The pH of the cell culture was controlled by the growth medium.The cells were now prepared for further counting process [1].

## 3.2 Counting Cells

The Bürker counting chamber consists of nine well-defined squares and 3 to 5 of these should be counted. For our assignment we counted 4 of the areas. The results from the successive counts were to be combined and plotted in a graph, and to be used in calculating the doubling times.

For the actual process of counting, the protocol indicated that a preliminary step would have to be to fasten a cover slip into the Burker chamber. In this step it is important the cover slip is attached so that a correct chamber value is acquired. In the next step we were to place a small drop of the cell suspension on each side of the chamber and check if the drop is uniformly distributed under the cover slip. The actual amount of cells is then counted manually with the help of a microscope. Each participant had to count two areas each (since we were three on a group we evaded this kind of formalism). We were to count the number of cells in each area.

The equation for counting the cells is given by:

$$Total\ number\ of\ cells = (N * \mu l\ of\ cell\ solution)/\ (Nr * 0.1\mu l)$$

In the equation N is the number of counted cells, and Nr is the number of areas counted.

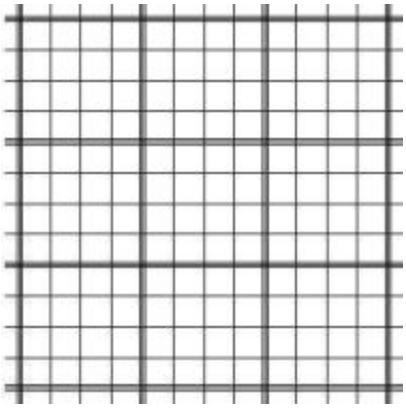

Figure 2 shows how a well-defined area with the dimensions of 3x3 should be.

## 4 Results

The results of cell proliferation for cancer cells of human prostate cancer (PC3), human cervix cancer (Hela) and the osteosarcoma cell line (OHS) are shown in Figure 3. The cells were developed as monolayer under sufficient access to nutrients, but limited by the space in the flask (25 $cm^2$). The number of cells were counted by students using the Bürker counting chamber.

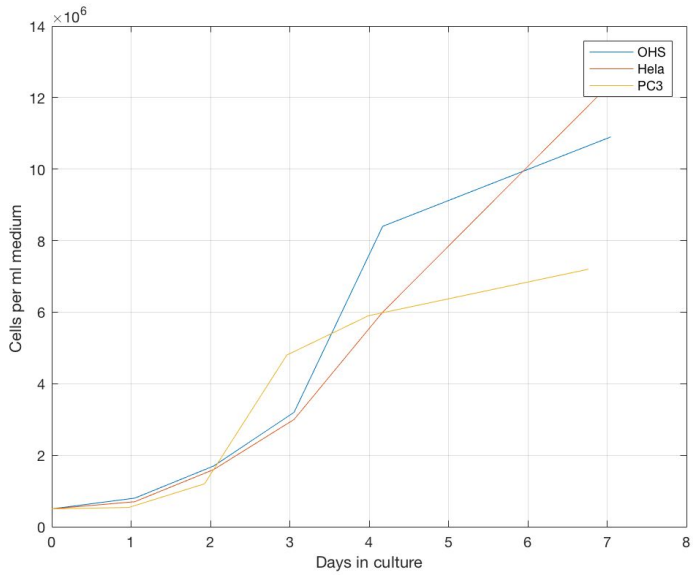


Figure 3. Growth curves of human prostate cancer (PC3), human cervix cancer (Hela) and the osteosarcoma cell line (OHS) cells. The cells grown as a monolayer in a flask with growth area of 25 $cm^2$ and sufficient access to necessary nutrients.

*Logarithmic Phase* of cell growth appear must clearly for OHS between day 3 and day 4 and PC3 from day 2 to day 3. For Hela, the *Log* phase seems to occur from day 3. The doubling time can then be calculated by applying equation (1), but in this case we estimate by using Figure 3. Table 1 shown the calculated doubling time for all three types of cancer cells.

| | OHS | Hela | PC3 |
|---|---|---|---|
| Doubling time [hours] | 16.5 | 24.7 | 8.4 |

## 5 Discussion

In this part, we are to compare the growth curves and doubling times of the cell lines, as done in figure 3. From that figure, we can see that the PC3 clearly has an exponential growth. The same could be said about OHS until day 3, where it starts to deviate. With regards to the cell line we counted as a groupå, Hela, the growth is not exponential. The results start to deviate already from day 2 where its curve is too steep. Then it goes down in day 3 again. And from that day and onwards to days 7, it does not exhibit exponential growth. Our deviating results for Hela could be due to a counting error from day 2 and day 3 (in that period in between one of the groups could possible have a counting error). Also worth pointing out that Hela has a much greater doubling time compared to the two other cell lines.

Sources of error could be, like already mentioned above, manually counting errors. Contamination in both preparation and when evaluated by the different groups could be an another source of error. A last form of error source is the actual plotting of the graph, which could have been erroneous.

## 6 Conclusion

We did achieve our aim considering we were supposed to do several tasks in the laboratory and count the cells. Both of these were performed to sufficient results. With regards to making a sufficient graph and analyzing them, we were dependent on other groups also performing well. Based on the fact that the Hela-cells did not have an exponential growth, we can conclude that this did not happen. But these kind of errors do happen in a laboratory and all in all, we did as good as we could based on the circumstances.

## Reference

[1] Cell biology and cellular biophysics, TFY 4260. (Spring 17). *Lab exercise: Cell cultivation*. NTNU.

[2] *Cell culture basic handbook*. Retrieved 2017, March 9 from https://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf