EXPERIMENT 1

INTRODUCTION TO CELL CULTURE

LAB REPORT

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Introduction

Cell culture is one of the most important parts of the molecular biology field including drug development, cancer, gene therapy, genetic engineering, etc. Cell culture is the growing of cells obtained from an organism, in another environment (ThermoFisher Scientific,2021). Cell culture aims to proliferate those cells and provide better investigation on the cell structure of the cell. Cell culture is also used for producing antibodies and recombinant proteins (G. Roshan Deen, 2016). Cell culture is important regarding it can decrease the need for conducting experiments on animals (G. Roshan Deen, 2016). Additionally, aseptic conditions are important to take into account regarding the unpreventable contamination risk (Proteintech Group, 2021). Minding the cleanliness of the branch and the equipment to be used is of great importance, which is done using ethanol. Working in a clean and safe environment holds great importance for the reliability of the experiment results. The cell culture experiment hypothesized that HEK293 human embryonic kidney cells can be proliferated when the appropriate conditions are provided. HEK293 cell line is popular in cell culture due to their reliable growth propensity regarding transfection (Beckman Coulter, 2021).

The cell culture experiment aimed to gain knowledge on the growth stages of the cell cultures: log phase, lag phase, and stationary phase, to name them. The purpose of the experiment was to gain expertise and knowledge on subculture methods, where the cells are counted and the living and dead cells are distinguished.

Materials

In order to work in a sterilized workplace, 70% EtOH is used for cleaning the bench. DMEM (10%) is used as the medium for the maintenance of the cells. PBS (phosphate-buffered saline) is used as a salt solution. Trypsin (0.05%) is used to isolate the adherent cells from the surface of the plate. Trypan Blue is used to stain and view the cells under the microscope, to check their vitality, and to count them. Pipettes are used throughout the experiment for measurement and solution traveling and mixing purposes as a generic tool. Dish, falcon, Eppendorf are used for container purposes. Vortex was used to mix. The centrifuge was used to creating a pellet and as a method of separation.

Methods

To start with, the bench to be worked on was made sure to be clean by sterilizing with 70% EtOH, which held great importance for reducing the risk of contamination in the experiment. The HEK-293 cell line human embryonic kidney cell culture was checked under the microscope to make sure that the culture contained cells that were not dead. The medium is removed using a pipette followed by washing the dish using 1ml PBS by moving the PBS around the dish, to collect the cells. Then, 2ml Trypsin of concentration of 0.05% is added to detach the adherent cells from the surface of the plate. The dish was put into the incubator for 2 or 3 minutes at 37°C for providing the enzyme an optimum environment where the enzyme activity was enabled. Later, 2ml of DMEM was added as a medium with a concentration of 10% to prevent cell death and increase cell proliferation by providing the essential substrates for cells to thrive and grow. The medium with the cells is moved to the falcon using the pipette. The falcon has centrifuged at 1500 rpm for 5 minutes. This caused the

cells to form a pellet and supernatant and the supernatant was removed using a pipette. 1ml DMEM was added and using the up and down technique with the pipette, the solution was mixed. The 200 µl of the cells were moved to the Eppendorf. This was followed by adding 20 µl of the cells to another Eppendorf, 80 µl of Trypan Blue of 0.04% concentration were added and mixed using the vortex for staining the cells to aid for better visualization under the microscope. Two samples from the mixed Eppendorf each being 10 µl were moved to the hemocytometer using the pipette. Later, the cells were observed and counted under the microscope, to provide the solution to the experiment.

Results

For the first group which used 20 µl cells and 80 µl trypan blue: 15 cells were counted in the upper left square area, 18 cells were counted in the upper right square, 13 cells were counted for the lower-left square area, and lastly, 15 cells were counted for the lower right square area. The total number of cells is 15+18+13+15=61 cells. The dilution factor is (80+20) /20 = 5.

For the second group which used 25 µl cells and 75 µl trypan blue: 60 cells were counted in the upper left square area, 80 cells were counted in the upper right square, 28 cells were counted for the lower-left square area, and lastly, 52 cells were counted for the lower right square area. The total number of cells is 60+80+28+52=220 cells. The dilution factor is (75+25) /25 = 4.

When 80 µl of Trypan Blue of 0.04% concentration was added to the sample. 305000 living cells were able to be observed under the light microscope. When 75 µl trypan blue was added to the sample. 8800000 living cells were observed. The dead cells were not calculated in this deduction and the number of the living cells were derived by counting the cells in the corner square areas and taking the average of them regarding the dilution factor using the formula:

Cell #/ml= (counted cell #) x (10000) x (Dilution Factor)

Discussion

When the dilution factor is higher, a larger number of cells are yielded in the cell culture experiment. To specify, one group used 25 µl cells and 75 µl trypan blue where the dilution factor was 4, and 220 cells were counted under the light microscope with this dilution factor. The calculated number of cells was 8800000. Another group used 20 µl cells and 80 µl trypan blue where the dilution factor was 5 which is way more than 4. Only 61 cells were counted and 305000 cells were calculated to be living which is about 28 times less than the other groups' results. It seems from this experience that, the dilution factor is inversely correlated with the cell count. The vast difference must have resulted from the different cell concentrations at the beginning of the experiment. Because the concentration of Trypan Blue used to stain the cells was not an affecting factor in the result of the experiment as adding Trypan Blue is sufficient to stain the cells and enable viewing the cells under the light microscope. The dead cells were all blue where the living cells were only blue on the outline.

However, If the amount of Trypan Blue were used, more cells could be stained to be counted, which would have a positive effect on the efficiency of the cell culture experiment. One other aspect is the usage of trypsin which is hazardous for the cell proliferation of kidney cells, which was the cell line that was being worked on. The result was negatively affected by the usage of trypsin because It most probabilities caused cell death to some extent. One other factor might have been the high centrifugation rate which might have caused cell death. Additionally, any error while removing the supernatant could lead to loss of living cells, If more than the necessary amount of supernatant is repelled by the pipette. Less cell death would be observed at the end of the experiment under the light microscope If the cells were treated better during the experiment.

References

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