Effective Methods For Culturing Breast Cancer Cell Lines

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Abstract

Cell culturing is one of the most useful and prolific techniques practiced in biological science today. This is because with an effective cell culturing technique one may be able to take as few as a hundred cells and convert them into the millions cells in just a few days. The use of cell lines in drug research and innovation has encouraged many biotechnology businesses to build out their own “cell line manufacturing centers”. To be competitive in this space, the culturing process must deliver high quality cell harvests at a profitable price point.

If these cell culturing methods are ineffective they can cause the death of many cells and lose a huge amount of profit and resources for a company. As the practice of cell culturing has grown, scientists have come up with innovative ways for growing certain types of cells. The purpose of this research project is to examine the effectiveness of the process and protocols used by a major tissue banking company to grow and harvest breast cancer cell lines.

The focus of my analysis was on how well the cells grew and the overall “cell death rate” for each harvested culture. To be considered a “good cell line”, the cell death rate had to be less than two percent (2%). This was an important parameter because it helped me better understand the importance of the cell culturing portion of the business to the profitability of the company.

Introduction

The world of cell line culturing has become a profitable way for biotechnology companies to compete in growing life science industry. The initial culture taken directly from an individual is referred to as the “primary culture” and when diluted and transferred into further containers (a process referred to as “subculture” or “passage”), it becomes a “cell line.” The use of cell lines has lead to many discoveries and innovations. For example, the growth of viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The polio vaccine created by Jonas Salk made a significant impact on the health and lives of millions of people.

One of the more common cell lines produced in laboratory environments are breast cancer cells. Breast cancer is a cancer that starts in the tissues of the breast. There are two main types of breast cancer: (1) ductal carcinoma and (2) lobular carcinoma. Ductal carcinoma starts in the tubes (ducts) that move milk through the breast tissue. Lobular carcinoma starts in the parts of the breast, called lobules, that produce milk.
Breast cancer may be invasive or noninvasive. Invasive means it has spread from the milk duct or lobule to other tissues in the breast. Noninvasive means it has not yet invaded other breast tissue. Noninvasive breast cancer is called "in situ." Over the course of a lifetime, 1 in 8 women will be diagnosed with breast cancer. Some of the risk factors that are linked to this deadly disease include age, family history, and genetics. Early breast cancer usually does not cause symptoms. As the cancer grows, symptoms may include breast lump or lump in the armpit that is hard and has uneven edges.

Cancer cells may be immortal. That is, they can proliferate indefinitely in culture. For example, HeLa cells have been cultured in laboratories around the world. They are all descended from cells removed from a cancer (of the cervix) of Henrietta Lackes. Cancer cells in culture produce telomerase, a ribonucleoprotein. It is found only in the cells of the germline, including embryonic stem cells.

**Hypothesis**

My hypothesis is that the method followed by tissue banking company for growing breast cancer cell lines would produce a death rate of 10 percent or less once cells are harvested.

**Materials**

- Micropipettes
- 1 case of 1000 Micro Liter Pipette Tips
- 1 case of 100 Micro Liter Pipette Tips
- 300 ml of Pre-prepared F-12 culturing medium
- 100 ml of Cell Freezing Solution
- 100 ml of Triple E dissociation medium
- 100 ml of DPS or PPS solution
- 100 ml of Trypan Blue
- 3 Cell Counting Chips
- Digital Microscope
- Breast Cancer Cells
- Automated Pipette
- 10 ml Dispenser ends
- Pneumatic Suction System
- Culturing Flask
- 45 ml Vials
- 1 ml Vials
- 8X8 storage box
- Mr. Frosty deep freeze container
- -80 degree Celsius freezer
**Materials Continued**

- -194 degree Celsius liquid nitrogen storage freezer
- Lab Gloves
- Lab Coat
- Goggles
- Incubator with CO2 controller
- Clean Hood with Air Shield and U.V. Light Emitter
- 70% Alcohol

**Methods**

In order to perform this experiment we used two separate processes to generate our results. The first process was the initial cell seeding process which was the introduction of the breast cancer cells into a cell culturing medium composed of mostly F-12 solution and multiple other solutions, after the cells where properly seeded they where recounted using Trypan Blue and a counting chip.

The second process used was a two part process made up of the re-feeding phase and the freezing phase. In the re-feeding phase the cell culturing medium was removed and the cells where introduced to a disassociation medium to allow them to be properly observed under a microscope, then they where reintroduced to there culturing medium after being briefly washed in medium, once washed they where then counted using Trypan Blue. The freezing process was done right after the feeding process and the last counting of the cells, the cells where removed from their medium and emerge in freezing solution and then pipetted off into holding vials and sent into a deep freezing-80 Celsius refrigerator.

1. Sterilize the clean hood by turning on the air shield and wiping down the inside using 70 percent alcohol, make sure to sterilize hands using 70% alcohol before doing this. Once it has been wiped down turn on ultra violet light for 10 minute to kill of remaining germs. *Note all pre-seeding and pre-counting should be done before this using the standard culturing method for metastatic breast cancer cells*

2. Remove three flasks of cultured breast cancer cells from the incubator that has been set at 37 degrees Celsius and 5% CO2 level and place them in the clean hood along with all other necessary materials such as pipettes and such.

3. Turn on pneumatic suction system and remove all excess culture solution from the culturing flask.
4. Use the automatic pipette and rinse the inside of the flask with either DPS or PPS solution so as to remove any microscopic debris that might have gotten into the culturing medium. This will ensure that the cell culturing medium will properly interact with the cells once re-cultured.

5. Use pneumatic suction to remove excess DPS or PPS and then add in 1 to 8 ml of dissociation reagent which in this case will be Triple E, remember to only use enough of reagent to cover the bottom of the flask. Let flask sit for five minutes with reagent. This will cause the cells to absorb the reagent and become circular in shape thus preventing them from sticking to the sides of the flask and each other.

6. Remove flask from hood and tap each side of the flask while slowly shaking it side to side to loosen up any cells that might still be stuck to the bottom of the flask.

7. Observe the cells under microscope to make sure they are freely floating in solution. Once it is certain that they are quickly deactivate the disassociation medium by flush the flask with F-12 culturing medium at room temperature. If using Tripsum as the dissociation medium use the culturing medium at 4 degrees Celsius to deactivate. Once this is done drain off excess culturing and dissociation medium.

8. To finish re-culturing add in 10 ml of culturing medium to the flask and place the cells back into the incubator at 37 degrees Celsius and 5% CO₂.

9. To proceed on to the freezing process, continue up to step 7 and skip over step 8 and proceed on to this step. Pipette in 10 ml of culturing solution into each of the flask and then pipette out all of the solution including the cells and pipette it into one of the 45 ml vials. 1 vile for each flask

10. Pipette of 10 micro litters of cells and place them into a cells counting chip. Pipette in 10 micro liters of Trypan blue and then place the chip under a microscope to begin counting. Use standard methods for counting and calculating the number of cells in each flask.

11. Once the number of cells has been calculated divide that number by 1 million, this number will be the amount of Cryo-Stat freezing solution in ml you must add to each of the flask to prepare them for deep freezing.

12. Use pneumatic suction and remove excess culturing medium from the vials, then pipette in calculated amount of freezing solution into each vile. Once down pipette off 1 ml of solution and cells and put each ml into a 1 ml vial. This should put exactly 1 million cells in each vial. Place the vials into a Mr. Frosty storage container. Once all the vials have been put into the container place it in the -80 degree deep freezer.

13. After the cells have been freezing for one 24 hour period place them into a 8X8 storage box and put them into the -194 degree liquid nitrogen freezer for permanent storage.
Data/Results

All flasks showed a significant increase in the number of cells from the original number of seed cells. The cell death percentage for the three flasks ranged from 2.6 percent to 8.4 percent. See Figure 1 and Figure 2.

Figure 1

<table>
<thead>
<tr>
<th>Flask</th>
<th>Initial Cell Count</th>
<th>Final Cell Count</th>
<th>Dead Cell Count</th>
<th>Death Rate (%)</th>
<th>Cell Growth Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,980,000</td>
<td>6,439,496</td>
<td>168,504</td>
<td>2.6</td>
<td>116</td>
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<tr>
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<td>6,103,809</td>
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<td>6,664,156</td>
<td>279,843</td>
<td>4.2</td>
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</tr>
</tbody>
</table>
Discussion

Based on the results all three breast cancer cell cultures passed my requirements for being a effective culture. Each flask was able to yield at least 90% living cells from the final culture with the lowest cell yield being only 91.7% and the highest being all the way up at 97.4% living cell yield. The second requirement of which was at least a 100% culture size increase was easily met. These results show that the cell culturing process used here is effective in producing a large quantity of cells in a short period of time but still leaves room for reducing the cell death rates.

To improve on these lost we would have work on the key areas where most cellular death occurs which is in the dissociation phase of the re-culturing process and the freezing process. In the dissociation phase the cells are put into a hypertonic solution where the cells will then begin letting in more particles from its surrounding environment thus swelling and not being able to properly stick to the sides of the flask or other cells. When this happens there is a chance of cell lysis occurring from the cells letting in to many particles from the outside, this even happens in triple E solution, to prevent this better solutions need to be introduced that will either use different mechanisms from osmotic ones to force cells to not stick to each other or use more safely guarded osmotic mechanisms.

The second key area is freezing where if cells run the risk of tearing apart there cell walls from the expansion of freezing water. To prevent this cells are currently put into solutions that will force the most of the water from out of a cell to prevent the cell from overly expanding, to improve this better solutions to more efficiently do this are needed. Once these areas are improved on it should be easy to reach higher living yields of cells in the 98 to 99 percent ranges.

References

4. Cancer Cells in Culture
   http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/CancerCellsInCulture.html