**THE CELL CULTURE EXPERIMENT**

Even though all of your cells contain the same DNA, you have a wide variety of cell types throughout your body. Liver cells help to maintain metabolic homeostasis, brain cells allow you to think, and blood cells carry oxygen. In order to study the nature of these different cell types, scientists have developed methods that allow us to culture cells outside of the body in flat, plastic bottles. These *in vitro* (in glass; outside the living body and in an artificial environment) models allow experimental manipulation and direct observation of living and developing cells. A major advantage of cell culture systems is that we can study the effects of toxins or drugs without introducing unwanted variables such as tissue distribution or metabolic degradation encountered in multi-component systems. It is a highly artificial system to study cell behavior and leads to questions about the validity of the results in a living organism. Nevertheless, cell cultures can give us a first approximation to how exogenous (things external to the system) materials affect cell physiology.

There are two types of cell cultures. Cells obtained directly from a living animal and maintained *in vitro* are **primary cultures**. Primary cultures can be further categorized into either dissociated cultures or organotypic cultures. **Dissociated cultures** are those in which the connection between cells has been broken either mechanically or chemically. These cultures contain individual cells that can be plated into a culture dish. **Organotypic cultures** contain whole pieces of tissue are placed in a culture vessel. Cells grown as organotypic cultures, also called explant cultures, maintain their relationships to surrounding cells. Organotypic cultures are more appropriately called tissue cultures since the tissue relationships present *in vivo* (in the body) are maintained. Primary cell cultures have a limited life span and can be maintained for only a short period of time.

The other type of cell culture is one in which the cells have been grown from a single cell and is called a **secondary culture**. Because these cell lines have been propagated for many years, they are less differentiated than cells *in vivo*. Therefore, caution must be used in extrapolating information gained from the use of secondary cell cultures back to *in vivo* situations. However, they offer us an opportunity to work with a cell system and ask some pretty basic questions about how cells are affected by certain chemicals. For this lab, you will be using a secondary cell line that has been obtained from a certain type of cancer cell.

**SH-SY5Y Cells & Your Term-Long Experiments**

The secondary cell line we will be using is called SH-SY5Y and is a cell line established in 1970 from a neuroblastoma, or neural brain tumor, of a cancer patient. The cells have two X chromosomes indicating they were isolated from a female patient. Another interesting feature of these cells is that they contain 47 chromosomes (you have 46 chromosomes). The cells have a third copy (you have two copies) of part of chromosome 1 (your largest chromosome). In the proper culture conditions, the cells will double every 48 hours. The cells propagate via mitosis and differentiate by extending neurites (any propagation from the cell body of a neuron). To the right is a photograph of SH-SY5Y cells magnified to 200X. The long thin filaments extending from the cell bodies are the neuritis.

Your task this semester will be to feed and care for your own cell cultures and observe the effects that an exogenous compound has on their morphology. Your test compound is anything that you would like to test. For example, you may have heard about the health issues related to ingesting too much Na⁺ in your diet, so you could devise an experiment to see what effects sodium ion has on this cell line. The trick will be to choose something you can rationalize. It is not a very compelling reason to test a substance “just because”. The one...
exception to the test is that you may not use alcohol. Being a disinfectant, you should not be surprised that it will simply kill the cells and make for a very boring experiment.

PART I - THE EXPERIMENTAL PROPOSAL

Planning for a new experiment is a lot like baking a cake. You have to know what ingredients you’ll need, how long to allow for preparing and baking the cake. There is even some level of justifying why you want to bake a cake in the first place. In order to give you the opportunity, OK, force you to think about the experiment before doing it, I am requiring you to submit an experimental proposal in which you explain your experiment. This is to be a 1-2 page description of

- what substance you are using to treat your cell culture,
- why you have chosen this substance complete with some evidence that it might have an effect,
- the experimental question your are trying to answer, and
- how you will prepare the test substance and controls for the experiment.

Selecting a substance. As I mentioned above, you may choose to test any substance you can think of (except alcohol). However, there are some guidelines you might want to keep in mind. First, if you select a test substance that is not pure, such as aspirin, you must know exactly what the components of that substance are. This may require some extra research on your part, but on-line databases, such as PubChem or ChemFinder might provide that information easily.

Although this is not an absolute requirement, I recommend that you choose a substance that is soluble in water. It is possible to test things that are not water soluble, but it makes your job a little more challenging and you will need to consult with the instructors for guidance. It will also be a huge help if you can find something called the CAS number for the substance you want to test. This is sort of like the chemical compound’s social security number and gives you a very powerful search handle for finding information about you substance.

Once you have selected a test substance, you need to find a research article that discusses the cellular effects of the substance. This is necessary because it will help you figure out what concentration you will use in your experiment. There is concern in this experiment about osmotic effects on the cells, so choosing the right concentration range is critical. It is also helpful to know what range you might see some interesting effect rather than just full-out toxicity. In addition to concentration ranges, reading a research article is going to help you figure out what controls you will need to include. Remember that in a good experiment, you change a single variable and compare the results against a second experiment in which the variable was not changed.

Writing the proposal is simply explaining why you are testing the particular substance, providing proof that you have good reason to see an effect, and how you are going to test it. You will also need to explicitly state what your experimental hypothesis is. There is no specific formatting required, just keep it short and to the point. What this amounts to is very much like the Introduction section of your Taste Lab report.
PART II - CARING AND FEEDING OF YOUR CELL CULTURE

Growth conditions and feeding – Being mammalian cells, it shouldn’t be much of a surprise that we need to grow the cultures under conditions that mimic the human body. The most obvious condition is that cells are maintained at body temperature, 37 °C. A slightly less obvious condition that makes sense when you think about it is that the cells need food. Normally, when cells are in the body, they are surrounded by an extracellular fluid that allows for diffusion of nutrients from the capillaries into the cell. Cell cultures also contain an extracellular fluid or medium that contains various nutritional factors necessary to maintain cell growth. The actual formulation of the medium depends on the cell type being grown, but they often contain vitamins, amino acids, and glucose but can also contain undefined components such as horse serum and/or fetal calf serum. The animal sera provide additional nutrient, some of which are unknown or uncharacterized. Despite the various formulations available, we generically refer to the cell culture medium as a “feeding medium” or FEED, for the obvious reason that this is what it does for the cells.

Another important function of the culture medium is to provide the correct pH environment for the cell. Remember that protein function is dramatically altered by pH changes, and if membrane transporters are not working properly, cells do not live very long. Therefore, the FEED contains buffering components such as sodium bicarbonate to maintain the cellular and extracellular pH. Cells are also grown in an atmosphere containing about 5% CO₂. Sodium bicarbonate in the FEED equilibrates with the CO₂ atmosphere maintaining a pH of about 7.4. The pH is visualized monitored using color indicators in the FEED medium. Media with a pH of 7.4 is orange-red, while more acidic conditions are indicated by a more intense yellow color (pH 6.5). The yellow color can be seen in cultures that are very metabolically active, or in flasks containing a large number of cells, either mammalian cells or a bacterial contaminant. The bacteria change the solution pH by metabolizing the nutrients (into organic acids) thereby starving the cell culture. Antibiotics are sometimes added to the FEED to help prevent bacterial contamination.

One last “nutrient” that is often overlooked in working with cell culture is oxygen. Hopefully you remember that cellular respiration is driven by the ability of oxygen to accept electron from the respiratory chain. The trick in supplying enough oxygen to sustain your cell culture is to limit the amount of medium that is covering your cell culture. Oxygen is not very soluble in liquids and thus diffuses poorly through deep pools of media. The cells you are working with are considered “contact-dependent” cultures meaning that they grow attached to the bottom of the flask. As a result, they generally do not float up to the surface gasping for breath and will quickly suffocate it you put too much medium in the plates.

To maintain your cell cultures, you will need to change the FEED every 2 to 3 days. Work out the feeding schedule for your cells; if you change the media on Monday, you need to change it again on Thursday. Changing the media on Tuesday requires another change on Friday. Keep an eye on the color of the FEED. Orange is best; if the FEED is yellow, there may be too many cells in the culture or the culture is contaminated. If your culture media is yellow and cloudy, then your cells are definitely contaminated; do not refeed them! Contact an instructor!

Aseptic techniques – Remember how your parents always made a big deal of washing hands, covering your nose when you sneezed, and not spreading germs around the house? They were simply preparing you for this lab (yes, parents are omnipotent after all!) Cell cultures can be contaminated very easily despite the presence of antibiotics in the FEED medium. The slightest touch of the tip of a pipette to an unsterile beaker can destroy your cultures and your project (see, mom is right; germs are EVERYWHERE). To help you avoid contaminating your cultures, there are some standard protocols for working with the cell cultures. I am not expecting you will memorize this list, just look over it and get a sense of how to work aseptically. If you keep the general principle in mind, you’ll pick up the specific habits as you work.

1. All cell culture work is done under a laminar flow hood.
2. Wash your hands with soap and water before working with your cells. Rinse your hands with 70% ethanol (EtOH). EtOH will dry your hands out; use hand cream after you are finished with your cultures.
3. Have everything you need within reach. Do not get up and leave your work area, then come back to it and resume working. Once you start, finish.

4. Loosen the lids/caps of all flasks, bottles, test tubes, etc. but do not remove lids until you are ready. You may have to remove a lid with one hand, loosening the lid will make this much easier. Immediately replace a lid when you are through. If you need to put a cap down on the hood surface, place the inner surface down on top of a paper towel soaked with 70% EtOH.

5. NEVER, NEVER POUR ANYTHING! Pipet all liquids. Never let the pipette touch an unsterile surface. Even touching the tip of the pipette to an unsterile surface means contamination. Never lay a pipette down. If you must put down a pipette and intend on using it again, place it sterile tip down into a sterile test tube in a rack. Also, don’t ever mouth pipette; the last thing you want is a mouth full of cancer cells.

6. If a pipette has touched cells or the sides of a culture vessel, never place it back into a stock solution such as your FEED; this could contaminate your stock solution. You can use the same pipette to feed all your cultures, but be sure not to touch the tip of the pipette to the inside of the vessel; allow the FEED to run down the surface opposite to your cells. Do this gently; the flow of feed directly on top of your cells could dislodge them.

7. Discard old FEED into a sterile beaker. Splashing from an unsterile beaker back onto the pipette that you will use to remove FEED from the next culture will contaminate that culture. After you are done feeding your cells, (replacing feeding medium) add a small amount of bleach to the beaker filled with old FEED and discard contents down the sink.

8. When you are finished working under the hood, remove all your materials, dispose of them properly and wipe down the workspace with EtOH.

Assaying your cell cultures

There are many ways to assess the effects of a chemical on a cell culture, some more complicated than others. We will be using two very simple methods. First is the tried and true visual observation method. To observe your cells, you will be using an inverted microscope that uses special phase contrast optics to enhance the contrast between the background and a living cell. Unfortunately, living cells simply look like bags of water and appear only slightly darker than the background even with the special optics. Therefore, you will also need to treat a portion of your cell culture with a stain to enhance the contrast. Only a portion of the culture is stained because the staining kills the cells.

The other assay method you will use is to count the number of cells using an automated cell counter (the Beckman Coulter Counter). The Coulter Counter, named after Wallace Coulter, detects changes in electrical conductance as fluid containing cells is drawn through an aperture. Cells are non-conducting particles and alter the measured conductance. The Coulter Counter converts this information into cells/mL and total cells. The counter also determines cell viability using the exclusion dye, Trypan Blue. Cells that allow the dye in are dead while cells that exclude the dye (osmoregulation) are living. Your lab instructor will demonstrate how to count your cells using the Coulter Counter.

One experimental parameter you will need to keep in mind when designing your experiment is that you use the same number of cells in each culture. The density of cells in a culture can affect cell viability and the effects of toxins or stimuli; therefore, it is important that each experimental condition be tested on cell cultures plated at the same density. The number of cells in each well at the beginning and at the end of the experiment must be determined.
PART III - ORAL PRESENTATION OF YOUR EXPERIMENT

Given that you have gone to all the trouble of designing and executing an experiment, it has hopefully been an experience that you want to share with people. In order to share your excitement of your experience, you will be required to give a 15 min oral presentation describing your experimental project during the last week of the block. These are formal presentations (using overhead slides) in which you will clearly 1) justify your experimental design using published research (why you choose to ask the question you did), 2) explain your experimental hypothesis (how you tried to answer the question), 3) interpret the experimental results with respect to the hypothesis (what answer did you arrive at).

The best way to successfully present your work is to follow a set format that is similar to the format of a lab report. It is also important for you to realize that you should not try to present everything you observed in your experiment. Much of the data you gather is supportive and is not necessarily the main message you are trying to communicate. Think about a TV commercial for a car. The ad man picks one thing about that car – high mileage, off-road capabilities, sex appeal – and hammers you with images that convince you it’s true. Ads do not show you the glove compartment or the delayed windshield wipers. They are there but are not the reason you will buy the car. Oral presentations are similar and I am sorry to say that yes, all the hours of work you did can be shrunk down to a short 15 min talk. That is a fact of life you will realize the day you receive a simple sheet of paper for 4 years of hard labor.

Here is my one piece of advice to survive an oral presentation: whatever information you choose to present, you must be able to explain it. This means that if you cite a reference of previous research, you better know what is said in that reference and how it relates to your project. If you present some data, you must be able to explain how you interpret what it means. With that gem of wisdom, here is the format you should follow.

**Introduction:** Present the important background evidence that justifies why you tested the hypothesis you did. “What is the evidence that made you think your experiment might work?” is a simple question to test your ability to justify your experiment. One of the biggest problems with research talks is that the speakers do not provide sufficient background information for their audience to understand their research. Assume the audience understands basic concepts, but is unfamiliar with your specific project. If you go back and review your research proposal, it should help you reflect on the information you need to include as introductory material.

**Experimental Methods:** While everyone is going to be familiar with the general approach to the experiment, you still need to explain the specific details of your experiment. What substance did you use? What solvent did you dissolve it in? What was the final concentration used in the experiment? What were the specific measurements you made? These are the questions that the audience will need to have answered to understand what you did in lab. You might try using a flow chart if you have several steps in your experiment. It is also a good idea to remind the audience what variable your experimental control eliminates from the experiment.

**Results and Discussion:** This is where you bring home your message by highlighting the specific data that proves or disproves your hypothesis. A common trap is to present all of the data you acquired in the experiment. However, lots of data tends to confuse the point you are trying to make. Instead, you should focus only on the data that either 1) shows your hypothesis to be correct, or 2) shows that your hypothesis can’t possibly be correct. Despite the fact you have actually counted the number of cells, you may find that showing only the % change in one culture over another is sufficient to drive your point home. There will be times, however, when you need to discuss the raw data (i.e. number of cells) to account for data you are uncertain about, or data that do not show a clear result. This “speculation” is really about being critical about your experiment. Things do not always turn out to be absolute and there is a need to discuss why that might have happened.

One way to organize a talk that you might want to try is to show the results for a specific measurement in both the control and experimental culture. For example, you might say “We measured the average diameter of the cells in both the control and the test cultures and found that the control cells were slight bigger (by x microns). This suggests that there is an effect of [your substance here] on cell size.” Then move on to the next property you measured and build a case for your overall conclusion. Then at the end, you can summarize each of the results as a collective group and highlight why (or why not) they support your tested hypothesis.
When you do present data in the form of tables or graphs, take the time to explain exactly what is on the graphic. Start your explanation of a table, graph or figure with "This table (graph or figure) represents ……….." Describe to the audience what each axis of the graph or column or row of a table represents. All graphs, tables and figures should have legends. Be sure to analyze the results as you present them. Interpret the results for the audience. Point out the important points and draw the audience's attention to important trends.

Conclusion: This is usually a single slide summarizing the result of your study. It is often the place where you can propose another idea for an experiment based on things you observed in the present study. For example, if you were to find that aspirin caused a tremendous increase in cell viability, you might propose that molecules similar in structure to aspirin be tested for their effect.

Evaluating the presentations

We will be evaluating your presentation for two main characteristics, how well you communicate your project, and how deeply you present/understand the concepts relevant to your project. A maximum of 15 points will be assigned to each characteristic based on the traits listed below.

Communication deals with the manner in which you present your work. If you are communicating effectively, you should exhibit the following traits.

1. The presentation is organized in a way that facilitates the message being delivered.
2. The tested hypothesis and goals of the project are clearly evident.
3. The slides are free of errors and misinformation
4. The audience is “taught” by leading them through individual slides pointing out important information.
5. The presenter is familiar with what they are trying to say and engages the audience with their story.

Content addresses the information you are presenting. You have sufficient command over the content of your presentation if…

1. You have cited and explained specific evidence to justify your hypothesis.
2. You have described the details of your experimental methods
3. You have explained how the controls are adequate for the hypothesis being tested.
4. The presented material is relevant to the conclusion of the study.
5. You have correctly interpreted the experimental evidence in reaching a conclusion.

Tips for a successful presentation

Even after years of lecturing and giving scientific talks, I still get nervous about speaking in front of a group. Most of this probably is based on the fear of exposing my stupidity by saying something dumb, but generally people are civil. It does happen that you occasionally get a heckler, but most of the questions people ask are genuine; you have simply said something they find interesting and want to know more.

There are some things you can do to take the butterflies out of the gut. First and foremost, keep in mind that this class is your training ground. No one is going to believe you are a complete idiot just because you messed up a lab presentation. This is a time for you to get critical feedback so you know what to do better next time. It might also help relieve some of the stress if you remember the three R’s for public speaking:

- Reflect on what you’re presenting
- Rehearse to “hear” yourself, and
- Rewrite your talk to improve clarity.
These are tools you have control over and empower you to be proactive in developing your public speaking skills. It always helps me settle down if there is something I can do to help any situation. Below are a few more guidelines that might clarify what constitutes a “good” presentation.

1. Take the time to practice in front of other people. The best talks are well rehearsed, so that they appear to come naturally and make all of the desired points within the allotted time. A practice audience can give you valuable feedback when you are not clearly explaining something or if you have some funny, distracting habit.

2. Select only a few main points for your presentation. Support them with evidence, and repeat them often.

3. Present information in a brief outline form. Filling a slide with lots of text forces your audience to read and not listen. Limit text to fewer than 5 lines and tables to fewer than 5 rows and 3 columns if at all possible.

4. Make all fonts, symbols, and lines on visual aids bold enough and large enough to be seen from the back of the room. 24 point font is a good size for your typical room and it is better to use a san-serif font like Arial Bold because it is easier to read. Images suitable for a printed-paper are often not suitable for projecting.

5. Present only one idea on a slide. That way you can educate the audience without them getting distracted by other interesting stuff, and it’s less confusing for you.

6. Do not read your talk from cards or sheets of paper. Talk to the audience and use the information on the screen as a guide for what you are going to say.

7. Stand at the screen; it makes pointing at specific information easier plus your audience can see you and the visual information at the same time. This position will, however, force you to be conscious of looking at the audience instead of the slide for your talking cues.

8. Map out your talk for your audience. If you give them a sense of what information they are going to be hearing, you’ll connect with them better.

9. Explain any graphs, figures, or tables that you present. For graphs, explain what each axis represents (even if they are adequately labeled). For both tables, figures, and graphs explain what the data tell us. Point directly to important data.

10. Don’t talk like a space droid. Use you natural speaking voice and engage the audience; this is a performance art!