

Using Inquiry-Based Activities to Transform Undergraduate Science Education: A Model Lab for Understanding Cell Growth and Viability

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Abstract

The American Association for the Advancement of Science (AAAS), with support from the National Science Foundation (NSF) published “Vision and Change in Undergraduate Biology Education: A Call to Action” (AAAS, 2011) for distribution nationwide. Vision and Change has a call to action to prepare a new generation of biologists, and also a new generation of citizens who will have to negotiate the implications of new discoveries. In essence, biology instructors and administrators nationwide need to implement real and meaningful change or “transformations” in our biology classrooms and laboratories.

Undergraduate students designed and carried-out an independent and collaborative experiment over a four week period using state-of-the art cell culture techniques and scientific thinking strategies. This lab exposed biology majors to real-world hypothesis generation based on a primary literature review and course lecture content, experimental design, data interpretation, and lab report writing. A case study approach was used to complete a formative evaluation of: (a) how student comfort levels would change after performing guided inquiry-based activities, (b) the beneficial aspects of guided inquiry-based activities, (c) students’ perceived realism of the experiences, and (d) students’ interest level of completing additional inquiry-based research. Key findings indicate benefits and effects on communication, content knowledge, new techniques, technology experience, critical thinking, time management, organization, data collection, interpreting data, scientific writing, and experimental design.

Background and Significance

In 2009, the American Association for the Advancement of Science (AAAS), with support from the National Science Foundation (NSF) published “Vision and Change in Undergraduate Biology Education: A Call to Action” (AAAS, 2011) for distribution nationwide. This document was the culmination of three years of work by the NSF and AAAS, in conjunction with hundreds of biology educators, administrators, and students nationwide to gather information about how to bring biology education into the 21st century. As outlined in Vision and Change, “a revolution is underway in biology” – a revolution caused by technological breakthroughs that allow questions to be asked in ways they have never been asked before. Its “call to action” was the following: To prepare a new generation of biologists, and also a new generation of citizens who will have to negotiate the implications of new discoveries, biology instructors and administrators nationwide need to implement real and meaningful change in our biology classrooms and laboratories. These individuals need to be transformative in the areas that define biology curriculum, promote undergraduate research and innovative pedagogy in the classroom/laboratory, adequately assess new curricula and student learning, continually enhance professional development for biology faculty, and provide institutional-level change that fosters new pedagogies.

Research-oriented instruction emphasizes the central purpose of science—inquiry. Till this day, engaging students in inquiry-based learning is the essence of ongoing science education reforms (American Association for the Advancement of Science [AAAS], 1993; National Research Council [NRC], 1996, 2000). It has been solidified through research that explicit inquiry-based teaching helps students learn science content, master how to do science, and understand the Nature Of Science (NOS) (e.g., Abd-El-Khalick, & Lederman, 2000; Dori, 2006; Khishfe, & Abd-El-Khalick, 2002; Krajcik, Czerniak, & Berger, 1998; Olson & Loucks-Horsley, 2000). A scale of openness to inquiry-based learning initially devised by Schwab (1962) and later formalized by Herron (1971) serves as a basis for defining the types of inquiry recognized today by The National Research Council (NRC, 2000). According to the NRC, inquiry-based activities can be categorized based on the degree of student involvement in the inquiry process, the

degree of teacher intervention in instructing the student, and the student's scientific background regarding the inquiry subject and any relevant working methods. This definition lends itself to three levels of inquiry: the lowest level, *teacher-directed structured inquiry*, defined by strict instructions given to the student by the teacher (working by a research recipe toward a desired outcome); the intermediate level, *guided inquiry*, where an inquiry question is provided to the student who must decide on his or her own what methods to apply in searching for a solution (thus, they encounter and must interpret unforeseen data to make self-conceived conclusions); and, the highest level, *student directed open inquiry*, where all stages of inquiry remain open.

To address how research and innovative pedagogy can be integrated into the classroom, at The Pennsylvania State University - Lehigh Valley, a campus of approximately 1,000 students, a real and meaningful change in the biology laboratory strategy of instruction was implemented. Instead of undergraduate students being provided with a standard two-hour cookbook lab based on a hand-out defined in the category of structured-inquiry, with instructors as chaperones the students performed a guided-inquiry research lab experience in cell biology (BIOL 230W), which allowed students to design and carry-out an independent and collaborative experiment over a four week period using state-of-the art cell culture techniques and scientific thinking strategies. This lab exposed biology majors to real-world hypothesis generation based on a primary literature review and course lecture content, experimental design, data interpretation, and lab report writing. The cell cultures were Vero cells, which were epithelial cells derived from the kidney of a normal adult African green monkey in 1962 by Yasumura and Kawakita at Chiba University in Japan (1963). A wealth of primary literature exists on these cells for students to access and to delve into previous research, and state of the art cell culture techniques are known that can be easily replicated in order to grow these cells easily *in vitro*.

The goals of this lab were specific: (a) to enhance students' experimental techniques in cell-biology, (b) to build on critical thinking and scientific writing skills, (c) to teach students to identify scientific hypotheses and design experiments to test them using a guiding question, (d) to allow students the necessary time and independence to

appreciate and truly comprehend how scientific research is performed, and (e) to provide an experimental strategy that can be replicated in other cell biology labs.

Student perspectives were collated and examined on the guided laboratory research experience. The guided-inquiry-based laboratory was less structured, provided autonomy, and created a more authentic experience than what the population of students had practiced beforehand while undergraduates at Penn State. Grounded theory research in a case study techniques were used to qualitatively assess how these student perspectives can inform university professors concerning the implementation of guided versus open-inquiry laboratory experiences in undergraduate science courses. Faculty also observed student techniques, noted conversations the students were having during their discovery process, and assessed the journal-like scientific paper the students wrote as a final report of the experiment.

Pedagogical Design and Results

Inquiry-based Laboratory Assignment

Vero cells were used as part of a guided-inquiry-based laboratory to enhance and entwine undergraduate research with the teaching of core concepts in cell biology at Penn State Lehigh Valley. Students were informed that for four weeks they would be need to wear the hat of a cell biologist who performs cell culture experiments; and, as such, they at all times, had to critically think about the “life” of the cells they were investigating. Students working in groups of four over a four-week period were required to complete the following elements:

- a. Learn select experimental techniques including the following: basic cell culture techniques; Vero cell culture conditions and passage; and, cell counting (viable and non-viable cells) technique using a hemocytometer.
- b. Devise a hypothesis which explains the changes (life versus death) in freshly passed Vero cells at 10^5 cells/mL that remain in culture for an eight day period without media change when all other culture conditions are kept constant.
- c. Envision a research strategy based on the above hypothesis that included these components: Purpose (which includes background information, hypothesis under

- investigation, and scientific reasoning), Materials, Procedure, Data Interpretation, and References (using a standard “protocol” format). On each day over an eight-day period, microscopic observations of Vero cell density and morphology, and counts of viable and dead cells were noted.
- d. Undertake the experiment as per their research design, collect data, and present research findings in the form of a scientific paper.

Research Design

Grounded theory research techniques were used to uncover the study’s findings of the student perspectives of guided-inquiry-based laboratory experiences in order to generate a theory from the data and through constant comparative methods about what was occurring while the students were completing the experiment (Conrad, 1978). This case study research examined the dynamics and intricacies of student experiences in the guided-inquiry-based laboratory within the Biology 230W course offered at a campus location of Penn State and served as a means of formative evaluation, whose outcomes inform changes to the next iterations of the guided-inquiry-based laboratory. Geertz (1973) prescribed in this method that notes and data must be read and reread to interpret and uncover key themes. This reflective practice and analysis revealed basic assumptions of student perspectives of participating in a guided-inquiry-based laboratory. Using challenging assumptions as baseline questions within the post-course survey, students completed the questions to determine whether or not these challenging assumptions ring true in their laboratory experiences. Key themes from the literature were examined on a deeper level: (a) student comfort levels would change after performing inquiry-based activities, (b) beneficial aspects of inquiry-based activities, (c) perceived realism of the experiences, and (d) interest level of completing additional inquiry-based research. These frameworks were used to understand student perceptions and how they impact the planning and implementation of guided-inquiry-based programs.

This case study approach was selected due to the defined boundaries of the participants and the particular topic being researched (Creswell, 2007, p. 74). The steps taken were to (a) define a problem, (b) review literature for historical context of problem and current research on problem, (c) select participants, (d) schedule guided-inquiry-

based laboratory and observation with participants, (e) conduct surveys and observations, (f) provide feedback on journal-like scientific papers, and (g) analyze and report outcomes. University permission from the Institutional Review Board (IRB) was deemed not required due to administrative nature of the information and its limit of generalization to the case study participants within this pilot study.

Assessment of student perspectives

Twenty students enrolled in Biology 230W and were assigned to the guided-inquiry-based laboratory. Students were observed during their creation of a hypothesis and experiment. After students designed and implemented their open-ended research experiment and submitted a scientific paper for appraisal, their perspectives on and their experience of the laboratory exercise were assessed to inform better teaching practices.

The students rated on a scale of 1 to 5 key components of inquiry-based activities to assess their comfort level of implementing inquiry-based activities (NRC, 2000). The majority of the students found the exercise beneficial in improving their laboratory, data-collection, and analysis skills and described their increased comfort completing experiments. All students responded to the question—“Considering the following key components of inquiry-based activities, how has the cell experiment affected your comfort level and skills in the following areas?”—at a moderate to very significant level of how the laboratory experience affected their comfort level for all key components except taking precise measurements. Figure 1 depicts the number of responses in each of the Likert scale categories for each key component for the post-lab student perspectives of enhanced comfort level and skill level in inquiry-based activities. Additionally, this exercise aided students in performing a systematic scientific literature survey and incorporating publically available scientific literature into laboratory exercises.

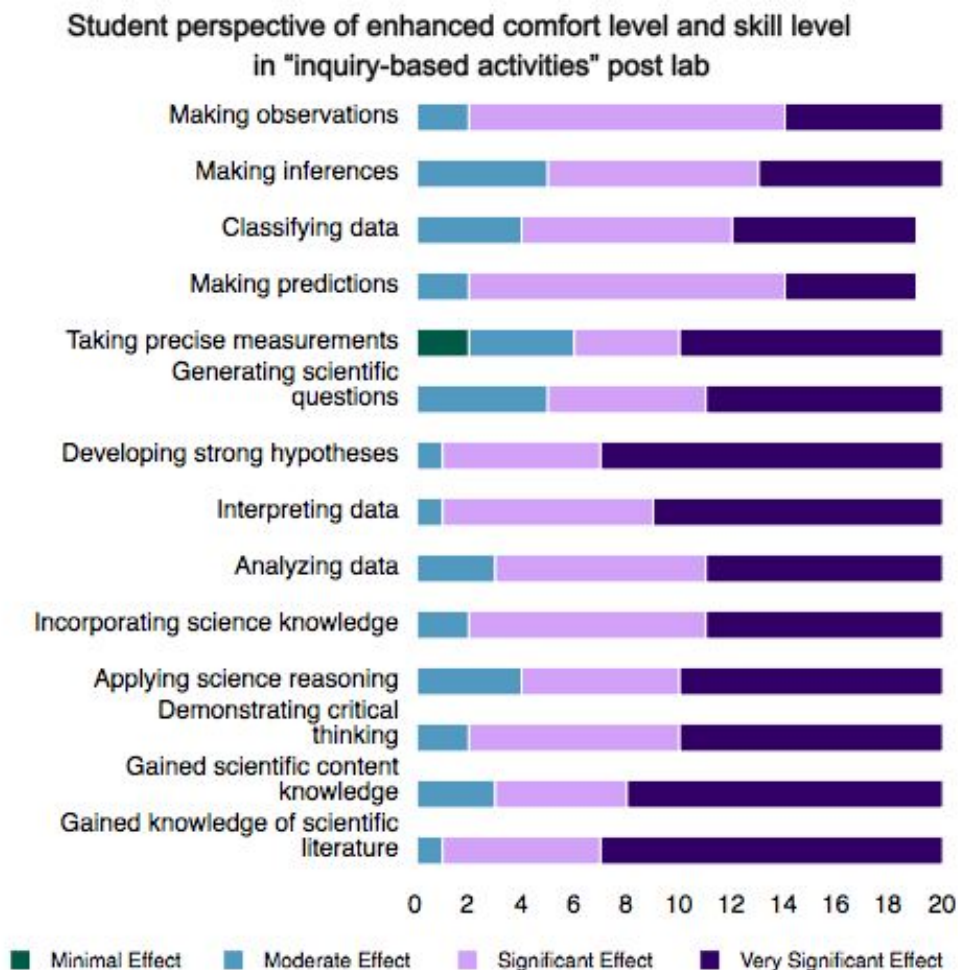


Figure 1. Student perspective of enhanced comfort level and skill level in “inquiry-based activities” post lab

Second, the benefit of this research activity was assessed, as gauged by the 20 participating students, not only with regard to their area of study but to their interest in pursuing research in the future. Ninety percent of the respondents found this activity beneficial (high to very), and the research activity spurred the interest of 85 percent of the students in pursuing active inquiry-based research in this or other areas of study. Figure 2 depicts the results of the questions assessing the benefit of the activity and how it affected their interest in performing additional research.

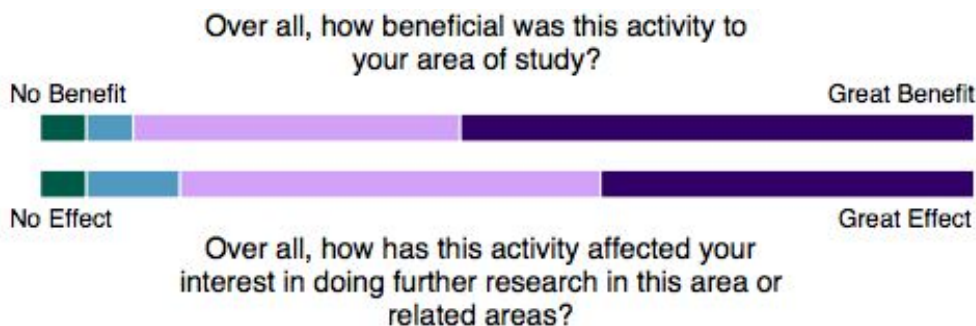


Figure 2. Benefit of the activity and interest in doing further research

Third, student perspectives on multiple aspects of the laboratory activity were collated. The open-ended responses to the questions asking (a) how different the cell culture lab was from other labs completed in the past, (b) impressions of what a scientist does when attempting to answer research questions, (c) specific skills gained as a result of the lab experience, and (d) recommendations for more effective laboratory activities. These responses were grouped into specific skills related to science and performance: communication, content knowledge, new techniques, technology experience, critical thinking, time management, organization, data collection, interpreting data, scientific writing, and experimental design.

The key findings when asked how the cell culture lab was different than other labs experienced were autonomy, authenticity, and new skills and knowledge. In particular, the areas of individualized work, authentic research, and new skills were most cited by the students. In general, the participants appreciated the open nature of the lab and felt it provided them a real-world lab experience. One student stated about the authenticity and the autonomy:

We did almost everything ourselves...from hypothesis, data collection, interpreting results... and coming to lab on a daily basis. It wasn't in and out the door. It felt like we were doing real research.

Another stated a similar comment:

The culture lab contained more hands-on techniques that are helpful for future biology graduates. It provided you with techniques one must know to become decent in cell biology lab research.

Student impressions about what a scientist does when attempting to answer research questions revealed a weakness in preparation for the guided-inquiry-based

laboratory. The students seemed fixated on controlled experiments rather than acknowledging other investigative methodologies within the lab. Participants recognized the importance of grounding the research in literature and observations and of the research question in guiding the investigation. To describe the activity of a scientist and the use of literature review, one student stated:

They create a hypothesis based on knowledge they have and research data from others that was published. They then can find other journals or scientific papers to help support or disagree with their hypothesis. They want to contribute by adding to the knowledge gained by previous researchers.

Student responses did display a number of misconceptions that could be addressed in future studies. One student stated:

They read previous literature; write a protocol which states their hypothesis and methods; then they do a controlled experiment.

Another described the process as:

A scientist conducts an experiment after an initial hypothesis is denoted. He/she then collects qualitative and/or quantitative data, analyzes it, then plots data on corresponding graphs/figures to outline trends. The scientist will then approve or disapprove the initial hypothesis in response to a research question.

Analysis showed 60 percent of the open-ended responses related to skills gained indicated that the students felt they had developed a robust understanding of content related to their research. The participants stated they had gained personal skills, content knowledge, new techniques and technology experiences, and critical thinking skills. Personal skills such as communication, time management, organization, note taking, and writing comprised the more often cited gains. Overall, participants cited experiences with new techniques and time management most often. For example, one participant stated:

[I] gained [knowledge of] background information on how cells grow and generally became familiar with research methods that I did not know about.

These skill gains were notable as the literature cited that a primary concern of teachers is that open-inquiry provides limited opportunities for student to learn content (Munsell & Lederman, 2011). In addition, other high frequency responses included the important role of experimental techniques and equipment use in scientific inquiry (SI); the importance of effective communication in the advancement of scientific inquiry (SI) and scientific

knowledge; the importance of a “guiding question” for SI; and the grounded nature of SI in observation and scientific literature. For example, participants stated:

The process of reading other published papers greatly assisted me in the learning process of how to write like a scientist and how to read and understand accomplished scientists’ findings.

Doing this experiment I became more familiar with lab techniques such as trypsinization and transferring cells. I never really did anything like that so it made me feel more comfortable in the lab. Being able to work under a hood, use pipettes, and micro test tubes are all skills and I feel better about doing it. It also taught me how to put together a real lab report.

The most beneficial aspect to me is that it required critical thinking since it was not a “cookbook” experiment. One had to critically think about the outcome and the reasons behind them.

I found that doing the actual background research was beneficial because it gave me a better understanding of the cells and allowed me to formulate a hypothesis.

I have learned to communicate my findings in a scholarly manner; how to manage my time in the lab; keep a notebook; read papers; and, the appropriate techniques to use when working with live cultures of cells.

All three of these concepts—difference from prior experiences, scientific processes, and new skills—were key components of the National Science Education Standards on SI (NRC, 1996 & 2000). Overall, the participants stated that they gained personal skills, content knowledge, new techniques and technology experiences, and critical thinking skills.

The participant responses also offered some insight for instructors concerning the implementation of guided and open-inquiry research experiences for their students. In particular, the majority of students cited that they greatly appreciated the real-world, authentic research experience that the guided-inquiry research project provided them. For example, one participant stated that:

[I] explore[d] new topics and activities that are real-world and applicable to society, and that can increase or better a student’s learning experience in a field.

The participants also stated that they felt that they learned a great deal from the autonomy they had during the project which allowed them to learn from their own mistakes, and helped them develop their organizational skills, time management, communication, etc.

[Research experiences] should be more hands-on and allow the students to be more independent and learn by the mistakes you make, but steer them in the right direction.

We got to bring our own flavor to the lab. It wasn't just reading instructions and repeating tasks. It made us think and allowed us to be creative.

I have learned to communicate my findings in a scholarly manner; how to manage my time in the lab...

Limitations

Participants were students who had enrolled in Biology 230W during the semester. This study also served as a pilot study of the guided-inquiry-based laboratory. The outcomes of this study represented this case study and informed changes to the directions given to the students. Observations were not formally recorded for analysis. The journal-like scientific paper served as an evaluative assessment to measure the students' learning and as an educative assessment to provide feedback to the students. The paper can serve as a more formative evaluation in future studies. The formative nature for this study resulted in limited information related to how the students were presenting their outcomes and ideas in a journal article format as determined by general journal guidelines and not a specific journal's formatting and reference requirements.

Conclusion

Scientific inquiry was a powerful way of understanding science content. Students learned how to ask questions and use evidence to answer them. In the process of learning the strategies of scientific inquiry, students learned to conduct an investigation and collect evidence from a variety of sources, develop an explanation from the data, and communicate and defend their conclusions. Based on survey responses, this guided research project not only exposed students to scientific inquiry while building critical technical skills, it also allowed them to think like a scientist and understand how scientists study the natural world, which is in keeping with the NSTA Position statement, 2012. This contradicts the findings of Abd-El-Khalick and Lederman (2000) concerning explicit versus implicit teaching of science concepts. Abd-El-Khalick and Lederman (2000) found that students are highly unlikely to assimilate concepts that are not explicitly taught by the instructor while this study indicates a diametrically opposite view

in spite of these aspects not being explicitly addressed. Changes to the guided-inquiry-based laboratory and a more extensive study were planned as a result of this research.

Additionally, this guided inquiry research project aided in informing better teaching practices. As outlined in *Vision and Change*, questions were asked in ways they have never been asked before in the experiences of the students. In general, the students recommended that instructors provide future students with authentic research experiences that allow for autonomy and individual exploration of the related concepts and experimental design process. According to the participants, this approach allowed them to learn more content, gain new skills, and better understand the process of SI. According to Herron (1971), these experiences are best created for students when high-level, open-inquiry experiences were offered. One key weakness of this study highlighted in the participant responses was that the participants seem fixated on controlled experiments, rather than acknowledging other investigation methodologies. This could be addressed through a more explicit approach as recommended by Abd-El-Khalick and Lederman (2000) or by providing multiple open-inquiry experiences that utilize various methodologies.

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Appendix

Life and Death in the Cell Culture: An Undergraduate Laboratory in Cell Biology

Missy Coyle, Swathi A. Kumar, and Jacqueline S. McLaughlin

1. Objective:

The key objective in this lab is to have you critically think about, and understand, “life at the cellular level.” The cell is as fundamental to biology as the atom is to chemistry. Truly, everything an organism does, occurs, fundamentally, at the cellular level. Think about yourself, for example. When you exercise, red blood cells are transporting oxygen to all of your body cells for aerobic cellular respiration, myocytes are working to shorten their sarcomeres so that your heart beats faster and your skeletal muscles contract, pancreatic beta cells are regulating glucose concentration in your blood by way of insulin secretion, and neurons are secreting neurotransmitters to stimulate your sweat glands to cool off your body.

You, the cell biology student in this class, must appreciate that cells are alive! Today’s cell biologists are the researchers who are devising the newest medicines against cancer cells, cardiovascular disease, and hypertension; developing vaccines against viruses that invade normal, healthy cells; and growing stem cell lines in cell culture to differentiate neurons, blood cells, and muscle cells to help fight specific diseases—to name just a few of research undertakings cell biologists are presently involved in. The crucial point being made here is that today’s scientist must be able to critically think about the ever-so-intriguing “life” of each and every cell they are investigating.

[Video: Stem Cells Breakthrough](#)

Herein, you will grow a specific **cell line** in **cell culture** and plot its **growth curve** in order to investigate cellular life. The cells under investigation will be placed in fresh nutrient-rich media on day 0, and then allowed to grow in culture without media change for eight days. Your task is to: 1) hypothesize what will happen to these cells over the allotted time in cell culture given limited nutrients, growth factors, buffering capacity, and space; 2) design and carry-out an experiment to test your hypothesis utilizing cell culture techniques; and 3) interpret, then present your results in the form of a research paper.

2. Background Information:

Animal or plant cells, after removal from specified tissues, will continue to grow if supplied with the appropriate nutrients and conditions. When carried out in a laboratory, the process is called **cell culture**. It occurs *in vitro* (‘in glass or plastic’) as opposed to *in vivo* (‘in the multicellular organism’). The culture process allows single cells to act as independent units that “live” off of the nutrients supplied in a liquid or semi-liquid growth medium.

Milestones of Cell Culture

The history of cell culture dates back to early twentieth century, when Ross Harrison, in 1910, published his findings on the growth of nerve fibers (axons and dendrites) *in vitro*. Using expert surgical and aseptic technique developed from successfully operating on tiny frog embryos, Harrison explanted embryonic frog neural tube fragments into a drop of fresh frog lymph on a sterile coverslip. Once the lymph clotted, he inverted the coverslip over the well in a glass depression slide creating a hanging drop culture, a technique often used by microbiologists for studying bacteria. He then watched the development of frog nerve fibers *in vitro* from the neurons in the explanted tissue.

[Read: Cell Culture Solves a Problem](#)

Many scientists have stated that his paper, “**The outgrowth of the nerve fiber as a mode of protoplasmic movement,**” is likely the most important paper ever published in the *Journal of Experimental Zoology* (Harrison, 1910). Others have said that it is also among the most important papers published in the field of neuroscience during the first half of the 20th century. In a single stroke Harrison invented the method of cell culture, and then used it to support the neuron doctrine.

Following Harrison’s simple, but elegant experiments, cell culture evolved and with the development of antibiotics in the late 1940s to the early 1950s contamination problems were avoided. Other breakthroughs included: the development of enzymatic techniques to remove adherent cells from culture vessels so as to continuously grow **cell lines** (such as HeLa cells); and, the development of standardized, chemically defined **culture media** that made it far easier to grow cells for research.

Types of Mammalian Cultures

Freshly isolated cultures of cells from mammalian tissues are known as **primary cultures** until sub-cultured. At this stage, cells usually represent their parent cell types as well as the expression of tissue specific properties. When the cells in the primary culture vessel have grown and fill up all the available culture substrate, they must be **subcultured** (aka “**passed**”) to give them room to continue growth. For cells grown in media, this is usually done by removing the cells from their culture flask, centrifuging them into a pellet, re-suspending and simultaneously diluting them in fresh culture media, and then placing them in a new flask and back in the incubator. After several sub-cultures into fresh media, the cell is considered a **cell line**.

Importantly, two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated substrate (**monolayer or adherent cell culture systems**) or floating free in the culture medium (**suspension cell culture systems**).

[Video: Sterile technique](#)

[Video: Passaging cells](#)

Cell Culture Conditions

To grow cells in cell culture is the complex process. Cells are typically maintained at an appropriate temperature and gas mixture (typically 37°C and 5% CO₂ for

mammalian cells) in a carefully calibrated, and frequently checked, incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the chosen **culture media**. Recipes for so-called “**media**” can vary in pH, glucose concentration, growth factors, buffers, electrolytes, amino acids and ion composition, and vitamins and minerals. Moreover, growth factors may be used to supplement media and these are often derived from animal blood, such as fetal or calf serum. To cell culturists, an ideal cell culture environment is one that does more than just allow cells to increase in number by undergoing cell division (mitosis). Even better, is an environment that allows for mitosis and cellular expression of *in vivo* physiological and cellular functions, such as hemoglobin expression in red blood cells or secretion of insulin by beta, pancreatic cells.

Vero cells

Vero cells are epithelial cells that were derived from the kidney of a normal adult African green monkey in 1962 by Y. Yasumura and Y. Kawakita at Chiba University in Japan. Although these cells were derived from a normal kidney, they present with an abnormal chromosome number, or aneuploidy (ATCC, 2012). Because these adherent cells can be grown as a continuous cell line, they are commonly used in microbiology and cell biology research. Some of the more common applications include virology studies, viral vaccine production, toxicity studies, and propagation and study of intracellular bacteria and parasites (Ammerman, Beier-Sexton, & Azad, 2008).

3. Laboratory Assignment:

1. Learn select experimental techniques including the following: basic cell culture techniques; Vero cell culture conditions and passage; and cell counting (viable and non-viable cells) technique using a hemocytometer.
2. Devise a hypothesis which explains the changes (life versus death) in freshly passed Vero cells at 10^5 cells/mL that remain in culture for an eight day period without media change when all other culture conditions are kept constant.
3. Envision a research strategy based on the above hypothesis that includes these components: Purpose (which includes background information, hypothesis under investigation, and scientific reasoning), Materials, Procedure, Data Interpretation, and References (using a standard “protocol” format). Importantly, on each day over an eight-day period, microscopic observations of Vero cell density and morphology, and counts of viable and dead cells must be noted.
4. Undertake the experiment as per research design, collect data, and present research findings in the form of a scientific paper.

[Website: Scientific Writing](#)

4. Techniques

I. Vero Cell Passage

Materials and Equipment

- Vero cells, confluent monolayer, in tissue culture flask
- Growth Media, RPMI 1640 (1X), supplemented with 5% fetal bovine serum (FBS), 1% penicillin-streptomycin
- Trypsin-EDTA (1X) or cell scrapers
- Phosphate Buffered Saline (PBS) (1X), without calcium or magnesium
- 15mL conical tubes, sterile
- 25cm² tissue culture flasks, sterile (T25)
- Serological pipettes, sterile
- 70% ethanol solution (used for decontamination of hood and objects brought into the hood)
- Waterbath, setpoint 37°C
- CO₂ incubator, setpoint 37°C and 5%CO₂
- Inverted light microscope (used to check cell morphology)
- Biological safety cabinet (hood)

Procedure:

1. Remove growth medium from monolayer of Vero cells.
2. Wash flask 2X with PBS (no Ca²⁺ or Mg²⁺). Use 2mL for each wash making sure to wash the bottom of the flask. Decant wash into waste beaker.
3. Add 2ml trypsin to the flask.
4. Rock the flask back and forth for 60 sec. Put the flask in the 37°C incubator for 1-2 minutes.
5. Strike the flask sharply against the palm of the hand to dislodge the cells and then look at the flask under the microscope. If the cells remain attached, warm the flask in the palm of the hand for 30 sec. and then strike the flask again.
6. Add 4.5mL fresh media flask (the FBS in the growth media inactivates the trypsin). Mix with pipette, then add the media to a separate 15mL centrifuge tube.
7. Centrifuge for 5 minutes at 500rpm.
8. Remove and discard supernatant.
9. Re-suspend cells in 5mL RPMI with 5% FBS.

10. Prepare desired dilution of cells in RPMI with 5% FBS and add to 25cm² (T25) cell culture flasks.

11. Incubate flasks in 37°C incubator with 5%CO₂.

Note: Monitor cells daily or every other day. Change media every few days. When cells reach a 70-90% confluent monolayer, passage cells again.

II. Vero Cell Counting Using a Hemocytometer

Materials and Equipment

- Hemocytometer
- Weighted coverslip
- Trypan Blue, 0.4% solution
- PBS (1X)
- 70% ethanol solution (to clean slide and coverslip)
- Lens wipes
- Microscope
- Pipette and tips
- Biological safety cabinet (hood)
- Centrifuge

Procedure

1. Decant media from Vero cell stock flask into 15 mL centrifuge tube.
2. Wash flask 2X with PBS (no Ca²⁺ or Mg²⁺). Use 2mL for each wash making sure to wash the bottom of the flask. Decant wash into waste beaker.
3. Add 2ml trypsin to the flask.
4. Rock the flask back and forth for 60 sec. Put the flask in the 37°C incubator for 1-2 minutes.
5. Strike the flask sharply against the palm of the hand to dislodge the cells and then look at the flask under the microscope. If the cells remain attached, warm the flask in the palm of the hand for 30 sec. and then strike the flask again.
6. Add 4.5mL fresh media flask (the FBS in the growth media inactivates the trypsin). Mix with pipette, then add the media to a separate 15mL centrifuge tube.
7. Centrifuge for 5 minutes at 500rpm. Pipette off supernatant (discard) and re-suspend pellet in 1mL growth media. Transfer 1mL cells to microcentrifuge tube for cell count.

8. In a fresh dilution tube, mix 400 μ l PBS 1X (or fresh cell growth medium), 50 μ l trypan blue, and 50 μ l cell suspension. This constitutes a ten-fold dilution (dilution factor = 10).
9. Carefully load 10 μ L to both sides of the hemocytometer (counting chamber). Take care not to flood either side of the slide.
10. Count and record the total number of live (clear) and dead (blue) cells in five squares. Repeat the process for the cells loaded on the other side of the hemocytometer.
11. Calculate the number of cells per ml, the number of live cells per flask, the number of dead cells per flask, and the percent viability.
12. Dispose of all cell culture flasks and pipettes in a biohazard bag.

Calculations

$$[\text{cells/mL}] = \frac{\# \text{ cells in squares}}{\# \text{ squares counted}} \times 10^4 \times \text{dilution factor}$$

$$\% \text{viability} = \frac{\# \text{unstained cells (live)}}{\text{total \# cells}} \times 100$$

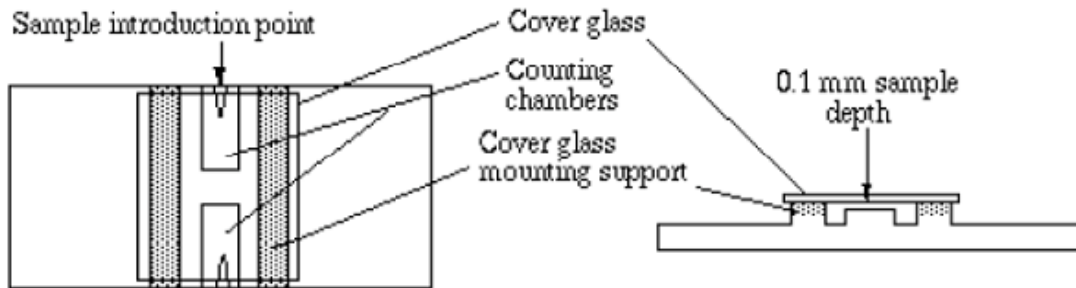


Figure 1. Diagram of hemocytometer (Counting cells with a hemocytometer protocol, 2006)

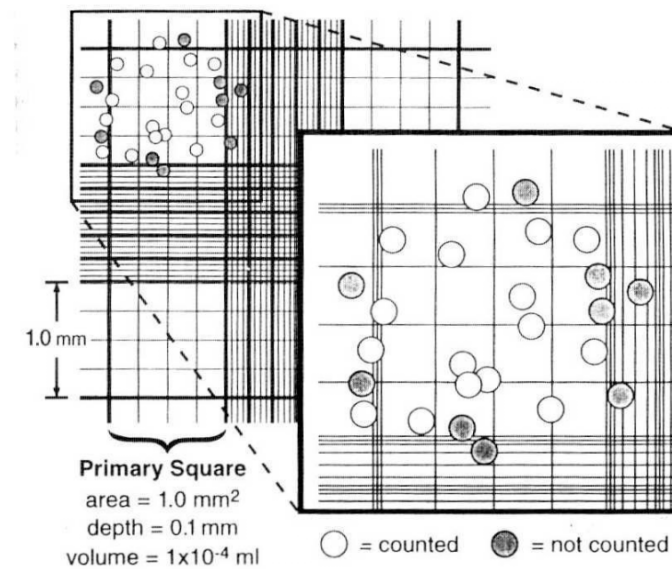


Figure 2. Counting cells and grids of a hemocytometer (Counting cells with a hemocytometer protocol, 2006)

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