Multiweek cell culture project for use in upper-level biology laboratories

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Submitted 8 August 2011; accepted in final form 29 March 2012

Marion RE, Gardner GE, Parks LD. Multiweek cell culture project for use in upper-level biology laboratories. Adv Physiol Educ 36: 154–157, 2012; doi:10.1152/advan.00080.2011.—This article describes a laboratory protocol for a multiweek project piloted in a new upper-level biology laboratory (BIO 426) using cell culture techniques. Human embryonic kidney-293 cells were used, and several culture media and supplements were identified for students to design their own experiments. Treatments included amino acids, EGF, caffeine, epinephrine, heavy metals, and FBS. Students researched primary literature to determine their experimental variables, made their own solutions, and treated cells over a period of 2 wk. Before this, a sterile technique laboratory was developed to teach students how to work with the cells and minimize contamination. Students designed their experiments, mixed their solutions, seeded their cells, and treated them with their control and experimental media. Students had the choice of manipulating a number of variables, including incubation times, exposure to treatment media, and temperature. At the end of the experiment, students observed the effects of their treatment, harvested and dyed their cells, counted relative cell numbers in control and treatment flasks, and determined the ratio of living to dead cells using a hemocytometer. At the conclusion of the experiment, students presented their findings in a poster presentation. This laboratory can be expanded or adapted to include additional cell lines and treatments. The ability to design and implement their own experiments has been shown to increase student engagement in the biology-related laboratory activities as well as develop the critical thinking skills needed for independent research.

METHODS

There have been numerous calls to adjust undergraduate science laboratory experiences away from “cookbook”-type activities, where students are expected to follow standardized protocols to their logical conclusion, to more inquiry-based laboratory experiences (4). Inquiry methods are more student centered and more closely model the scientific research process as well as develop wonder, passion, and curiosity about the natural world (1, 9). These student-centered instructional methods in college laboratories have also been strongly supported by both theoretical (11) and classroom (6) research. One manner in which inquiry-based methods have been implemented successfully in the undergraduate classroom is by having students design and implement their own experiments through active hypothesis formation, variable testing, data collection, and interpretation. The benefit of this type of open inquiry is that it allows for students to negotiate real problems and come up with their own innovative solutions.

Undergraduate student participation in authentic science research has been shown to lead to effective acquisition of learning objectives in the sciences (2) because it provides “situated knowing.” In addition, when undergraduate students participate in authentic science research, there are gains in understanding of the nature of science, scientific content knowledge, confidence and self-efficacy, personal intellectual development, science process skills, satisfaction with science research, communication skills, and willingness to collaborate (1, 9, 12).

Instructors are often resistant to implementing inquiry-based methods in the classroom for a number of reasons. The resistance factors have been summarized by Lawson (5), but one that arises repeatedly is that there is a lack of appropriate equipment and budget for students to properly implement independent research projects. In addition, researchers are often reluctant or unable to accommodate undergraduates in their laboratory. This leads to a lack of opportunities for undergraduates to engage in meaningful research. Solutions to this issue are not always easy, but in the present article, we provide a potential laboratory protocol that overcomes some of these challenges.

In response to these challenges, this article describes an effective cell culture protocol that can be used in upper-level laboratory courses or independent undergraduate research projects. This allows students to gain valuable hands-on experience in cell culture, experimental design, and scientific presentation.

In previous years, our physiology lecture course was an upper-level 3-credit hour course with no accompanying laboratory. It had, and continues to have, ~200 students each semester, which are a mixture of premedical, pregraduate school, and allied health students. In 2009, we developed a new laboratory course as an optional 1-credit course associated with the lecture. Currently, we have 6 sections of 24 students each in the laboratory portion of the course. Students enrolled in the laboratory either require the laboratory for their future career path or major or are interested in the laboratory component of the course.

While the laboratory was being developed, several existing manuals were evaluated. Most were traditional cookbook types with specific steps to follow and a known outcome achieved. While some of these activities were useful in presenting and solidifying basic concepts, there was little opportunity for the independent exploration that we desired our students to engage in. The protocol described below is a 3-wk project that can and has been easily expanded to a longer, more indepth research experience.

Experimental design. This project is done over the course of 3 wk. The first week is spent going over the proposal assignment and protocol including a brief discussion of how to design an experiment.
Students are given a list of available cell cultures and chemicals. This list can vary from semester to semester or even within class sections. Students are given time to design their experiment in groups of four and present it to the instructor for approval. They must have a rationale for their experiment. Students are then led through a “practice laboratory” emphasizing sterile techniques.

The approved proposal is returned during week 2. Students calculate and make experimental media, seed control and experimental cells, and do initial cell counts. Students are responsible for changing media based on their schedules. Laboratory availability is posted. Pictures can be taken with detachable microscope cameras (Flexcams) for presentation.

During week 3, students make final observations, note any cellular debris and the overall health of culture, and take pictures. They harvest and dye cells and count control and experimental cells using a hemocytometer. Cells taking up the dye are counted as dead; percentages of living and dead cells can be calculated and presented in their data (see Fig. 1 for the timeline).

In addition to a standard CO₂ incubator, Bio-hood, and inverted microscope, supplies include cell culture flasks, micropipettors, growth media, and other cell solutions, as listed in the protocol. Treatment chemicals are generously donated from research laboratories on campus or purchased from Sigma-Aldrich. All laboratory supplies are purchased from Fisher Scientific, although several other companies have comparable equipment and supplies.

**Instructions for students.** The goal of this laboratory is to gain experience in cell culture techniques and experimental design. You will characterize the growth pattern of human embryonic kidney (HEK)-293 cells under varying conditions. The cells you will be receiving are living in a standard growth media of DMEM with 10% FBS. The media will be treated with a mixture of penicillin, streptomycin, and antifungal (1% PSA) that will help prevent bacterial and fungal contamination of your cells. However, it is important that you maintain good sterile techniques throughout the experiment. If at any time throughout your experiment you suspect contamination, have your instructor evaluate your cells immediately. DO NOT put them back in the incubator.

Every object that comes into contact with the culture must be sterilized. It is vitally important that every tube, pipette tip, etc. must be used one time and then disposed to prevent any chance of contamination. Gloves must be worn at all times. The cell culture flasks must be kept under the Bio-hood while you are working with them to prevent contamination. The following website demonstrates the sterile techniques that should be followed during this experiment: http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/cell-culture-videos.html.

**Student proposal assignment and protocol.** Design an experiment that will characterize the growth of the HEK-293 cells. You may do this by manipulating different variables (amino acids, growth factors, alternative media, toxins, differing concentrations or incubation times, etc.) You will be told the stock concentration of your treatment solution and will have to calculate and make your own solution for your experiment (see Table 1). Creativity is encouraged. You will receive cells that have been grown in a standard growth media. You will need to include a brief justification referencing at least one scientific journal article and hypothesis.

Determine your control and treatment groups, including treatment concentrations and calculations, incubation times, media changing.

**Table 1. Available cell treatments for fall 2011**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calculation for 100 ml of Treatment Media</th>
<th>Final Concentration in Media</th>
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<tr>
<td>Varying concentrations of amino acids (see teaching assistant for availability)</td>
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<tr>
<td>Varying concentrations of FBS</td>
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increments, and make a schedule with your laboratory partners for carrying the experiment out over the next 2 wk.

You will receive two tubes, each containing 15 ml DMEM + 10% FBS and 1% PSA (standard media). You will need to adjust your media depending on your treatment plan. Label media tubes with their contents and your group identifier.

**Seeding cells (under the sterile hood).** Directions for seeding the cells are listed below:

1. Label two 25-cm² culture flasks with your conditions (label the sides so that your writing does not interfere with observations under the scope). There will be a 50-ml conical tube of cell suspension in control media under the hood.
2. Invert the tube gently several times to ensure a uniform suspension of cells. Pipette out 5 ml of media + cells from the 50-ml conical tube into the flask. Recap and invert the tube again before seeding the second flask with 5 ml.
3. Invert the tube again, pull out a 10-µl sample, and load the sample onto a fresh hemocytometer.
4. Put the flasks in the incubator on the shelf with your section label.
5. Clean up under the hood with ethyl alcohol, take your hemocytometer to the inverted microscope, and count the cells according to the directions below. Record the data in your notebook.
6. Cells must be incubated overnight to adhere to the bottom of the plate. You may then change the media and begin your experiment.

**Changin media (under the sterile hood).** Directions for changing the media are listed below:

1. Get the cells out of the incubator and check under the inverted microscope for cell attachment and relative cell numbers (estimate the percent coverage and look for possible contamination). Record the data in your notebook.
2. Warm control and experimental media to 37°C in the water bath. (This takes 3–5 min.)
3. Carefully pipette off the old media and place it in the waste beaker under the hood. DO NOT disturb the cells.
4. Pipette 5 ml of experimental media into one flask and 5 ml of control media into the control flask. Make sure that the flasks are labeled.
5. Return the cells to the incubator. Return media to the refrigerator and wipe down the hood with ethyl alcohol.

**Remove and count cells after confluence.** Directions for removing and counting the cells after confluence are listed below:

1. Retrieve the cells from the incubator and check under the inverted microscope for cell attachment and relative cell numbers (estimate the percent coverage). Cells and cellular debris floating in the media have died or are dying. Note the presence and relative amount of debris in the media and record those in your notebook.
2. Warm trypsin-EDTA, PBS, and media to 37°C in the water bath.
3. Discard media from the flasks.
4. Add trypsin-EDTA. Add enough (~2 ml) to cover the cell culture. Rock the flask back and forth for ~10 s. (Adding this solution will allow the cells to separate from the plate and prevent clumping.)
5. Gently discard trypsin into the waste beaker.
6. Rinse the flask gently with 1 ml of PBS, rocking it back and forth, and then discard PBS.
7. Add 5 ml of PBS* to each flask. Hit the flask with the palm of your hand to knock the cells off of the bottom of the flask. Pipette PBS + cells from the same growth conditions into a 14-ml Falcon tube. Rinse each well with 1 ml of PBS and add it to the Falcon tube.

*If cells were being saved to be frozen or replated, FBS should be used instead of PBS to prevent excess trypsinization and destruction of the cells.

**Determining health of cells (after confluence).** Directions for determining the health of the cells after confluence are listed below:

1. Spin the Falcon tubes with PBS + cells on low speed (2,500 rpm) for 5 min.
2. Carefully pipette off most of the PBS (leave ~2 ml to avoid disrupting the cell pellet).
3. Pipette up and down with a 1,000-µl pipette slowly to resuspend each cell pellet. Take 40-µl samples of experimental and control cells and place them in separate labeled 1.5-ml bullet tubes.
4. Add 20 µl of 0.4% trypan blue dye solution to the samples in each bullet tube. The dye will stain only dead or injured cells.
5. Take a 10-µl sample from each tube and count it with a hemocytometer. Record the data in your laboratory notebook and calculate the percentage of dead or injured cells.

**Classroom management.** The proposal assignment and protocol can be posted online before week 1 or handed out the previous week. Week 1 should be devoted to group discussion of the project and working out the preliminary ideas for the treatment. If time permits, students can begin a literature search using PubMed and calculations. A division of labor should be established within each group to make sure that the proposal is completed and approved by the instructor by the beginning of laboratory week 2.

In addition, we’ve found that practicing good sterile technique is an excellent activity to get students used to the manipulations of cell culturing. This can be done with nonsterile flasks, pipettors, 50-ml tubes, and alcohol flames on laboratory benches. Let students practice opening and closing flasks, flaming, keeping the pipette tip from touching the table or rim of the flask, loading hemocytometers, etc. The instructor should circulate and help students see some of the pitfalls that could lead to bacterial or fungal contamination when actual cells are used under the hood. Behind the scenes during week 1, instructors should be growing stock cultures for the following week and making sure treatment chemicals are available and the equipment is sterile.

**Showing students how to recognize contamination is important; pointing out bacterial or fungal contamination on images from the internet is done during the first week. Impress on the students that it is critical to alert the instructor if they suspect contamination. Bacterial cells are much smaller and rounder; they don’t have the typical adhesion or extending processes. Fungal contamination can be filamentous, as black or dark threads throughout. If contamination is found, separate the cell flasks from the others and, if time permits, have students reseed and begin their experiments again. Make sure that the other cell flasks are not contaminated, clean the incubator and Bio-hood thoroughly with alcohol, and monitor the cultures closely. Since introducing the sterile technique practice laboratory in week 1, we have been fortunate to have very few contaminations, and they have been contained to a few flasks. If contamination does occur in a laboratory section, make it a learning process for the entire class, by having the students go through their laboratory notebooks and determine where they think the contamination occurred and how that might have affected any results if the contamination had persisted.**

**Week 2** was devoted to having students make their solutions and seeding and counting their cell stock. This is labor intensive, and the instructor should be near the Bio-hood to address any questions as students are working. Make sure all students in the group are present at the hood as they will all need to work with the cells to change media at some point during the next week. Work out a laboratory schedule with each group to make sure that the laboratory is open during the week for them to check cells, change media, and take pictures and notes. Have students make an appointment to minimize teaching assistant time in the laboratory.

The last week of the experiment was devoted to having students take their final pictures and observations of the cells. They were able to note cell debris floating in the media and the percentage of cell growth (whether the cells had reached confluence) and cell quality after dyeing and harvesting cells. Groups should then get together and discuss the significance of their data and work out a division of labor for their research paper and poster presentation. Posters were presented in a mock symposium format; each student in the group described a part of their experimental design, results, and significance.
to the laboratory. Approximately 5–10 min of question and answer time was allotted per group. Posters were displayed in the lobby until the end of the semester.

RESULTS AND DISCUSSION

Student comments were extremely positive about their laboratory experience. Sample student comments were as follows: “The lab helped me understand the materials we learned about in class” and “I really enjoyed the ‘different’ lab. Usually anatomy and physiology labs are all dissections but I feel that this lab helped me understand more about the physiology of the body.” In addition, the poster session presented an opportunity for students to discuss their project with various faculty members in a conference-like setting. This gave them valuable experience in presenting and interpreting data and fielding questions from experts. Several students ended up receiving offers from the faculty members to work in their research laboratories.

Students often see laboratories as a 3-h march through a standard procedure with a predicted outcome. They go through the motions, memorize the results, and have, at best, a neutral opinion of their experience. Providing a sense of ownership, through a series of decisions make about the direction of their experiment, engages and excites them. They come in throughout the week to check on their cells. They want to know how their experiment is progressing.

We are hoping that laboratory experiences like this will become more common in the undergraduate curriculum. In the next year, we are hoping to develop a cell biology laboratory similar to the one described by Howard and Miskowski (3) and move this series to that course. We have added new cell lines donated by researchers to increase the variability of the experiments. Treatment options can vary each semester according to availability and resources. More and more universities are seeing the value of inquiry-based laboratories and seeing the benefits of increased student involvement and retention of material (7, 8). Allowing a larger number of students to gain meaningful laboratory experience will benefit the students and university alike. Students will be more qualified and more readily accepted into research laboratories and become potentially more marketable for employers in a difficult economy. Ultimately, we don’t want to educate a generation of memorizers. We want and need our students to be problem solvers and critical thinkers.

ACKNOWLEDGMENTS

The authors thank the BIO 426 students and teaching assistants in the fall of 2009 who took part in the development of these procedures, Dr. Scott Witherow, who generously donated the cell cultures, and Dr. Damian Shea (Biology Department Head), who approved and supported the development of this laboratory.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.E.M. performed experiments; R.E.M. and G.E.G. drafted manuscript; G.E.G. analyzed data; G.E.G. and L.D.P. edited and revised manuscript; L.D.P. conception and design of research; L.D.P. prepared figures; L.D.P. approved final version of manuscript.

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