Learning About Cells as Dynamic Entities: An Inquiry-Driven Cell Culture Project

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Abstract: Using cultured fibroblast cells, undergraduate students explore cell division and the responses of cultured cells to a variety of environmental changes. The students learn new research techniques and carry out a self-designed experiment. Through this project, students enhance their creative approach to scientific inquiry, learn time-management and group interaction skills, and communicate their ideas and results in written and oral form. A Likert scale pre/post assessment was administered for three semesters to determine changes in student attitudes.

Keywords: cell biology, cell culture, laboratory project, independent learning, inquiry-based project, fibroblast cells

Introduction

Helping students to understand and visualize function at the level of cells and molecules can be quite challenging. After all, students cannot see or touch a single cell without the aide of technology, nor can they open one up and look inside. As with many biological functions, we are restricted to what we can observe indirectly about cell function to help us understand these essential units of life. In our attempts to help students make the mental leap into the microscopic world of cell function, we have begun to use cultured cells during a sophomore level Cell and Molecular Biology (CMB) course. This paper outlines our approaches and techniques in using cell culture as a teaching tool in the hopes that others may also find it beneficial to their students. Similar approaches have been used in a summer biotechnology program (Lewis *et al.*, 2002) and in teaching apoptosis to advanced students (DiBartolomeis and Moné, 2003). Ledbetter and Lippert (2002) also report using cultured cells in a short-term laboratory project investigating membrane transport.

This laboratory exercise has been used at a liberal arts college with class sizes averaging about 24 students with approximately 12 students per laboratory section, but is appropriate for larger settings as well. As sophomores, most of the roughly 120 students who took part in the project in the last three years are not yet experienced with independent, critical thinking skills in a laboratory setting. They have taken a one semester introductory biology course, at least one semester of general chemistry, and sometimes have completed Genetics. In the sophomore level CMB course, we had two major concerns. First, when the course was initially designed, laboratory time was used primarily as a way to introduce techniques and classroom time emphasized content knowledge. With new instructors in the last several years, the course emphasis has been placed on helping students further their critical thinking skills through problem-solving, discussion, speculation about relationships, and reasoning. The laboratory portion of the course was lagging behind in those changes, still using primarily "cookbook" style labs. Second, students seemed to find CMB to be particularly difficult, apparently because it, along with Genetics, was the first course they encountered that required them to integrate mathematics, chemistry and biology. They also needed to use their imagination as they speculated about dynamic cells and molecules that are too small for them see. The laboratory portion of the course needed to be redesigned to help develop scientific thinking skills and to help students grasp the dynamic nature of living cells.

The specific goal of the project described here was to provide more opportunity for critical analysis, creativity, and independent thought during the CMB laboratory through the use of student-designed experiments with cultured cells. For overviews of reasoning behind the need to involve students in active, inquiry-based science projects as undergraduates, see National Research Council, 2003 and Rothman and Narum, 1999. In addition, we wanted to help students understand that cells are dynamic entities by working with living cells and to develop meticulous laboratory habits through the use of sterile technique and repeated measures. The focus of the project was on the process of doing science in addition to learning content and techniques. Student research teams (see Wright and Boggs, 2002 for another approach to team learning in cell biology) were asked to come up with their own question, design experiments to answer their question, and then report their results to peers and faculty either as a scientific poster or paper. The only given was the mouse fibroblast cell culture model system.

We asked the following questions during the laboratory modification: Is it feasible to permit undergraduate students with no previous experience using cell cultures the opportunity to design and carry out their own cell culture experiments as part of a sophomore level core course in biology? Does the open-endedness of an inquiry-based cell culture laboratory put more responsibility on students to think about what they are doing and thus foster greater autonomy and better learning? In addition we asked: Do students have a better concept of cells as dynamic entities after working with cultured cells for several weeks? We will discuss the feasibility through an analysis of the time and costs involved. Data on attitudes and concepts of cell function were gathered through student surveys administered early and late in the semester as well as through our personal observations (see Angelo and Cross, 1993).

Methods

Overview

The cell culture project is incorporated into the semester beginning sometime between the fourth and eighth weeks of the thirteen week term. At that point, the students have discussed basic cell function, organelles, and the structure and synthesis of the major macromolecules. We are usually beginning to study membrane structure and function at this point in the term and have not yet gotten to the details of cellular respiration or to molecular processing and transport within cells. Working in groups of two to four students, the research groups are taught sterile technique, cell splitting, and counting (for instructional details, please e-mail the author). The groups are then asked to care for and observe their cells for about a week, during which time they should be discussing various options for research questions. Each group must present a short research proposal to the professor that includes a hypothesis, the reasoning behind that hypothesis, an overview of the data collection plans, a predicted outcome, and a list of needed supplies beyond those available to all members of the class. The students are then given three weeks to complete their project. Results are presented either in the form of a laboratory report or a poster.

Student Projects

As they consider their individual projects, most student groups discuss various ideas with the professors beforehand. We try to point out if a project is too ambitious or costly to carry out within the constraints of the class, if the students have a serious lack of control in the proposed experiment, or if the students have not considered how they will collect and analyze the data to draw reasonable conclusions. The greatest challenge is overly ambitious ideas, but we remind students that they have only three weeks to complete the project and that this is just one of the classes they are taking. Students also often need reminders that anything added to the medium must be sterile. By one week after the initial instructional laboratory session, each student group must turn in a short written proposal documenting their plans. That proposal includes a hypothesis and the reasoning behind that hypothesis, a list of any supplies needed including the source and cost, a summary of the research techniques including the number of flasks or wells to be repeated for each point in the dataset, what data will be gathered (visual observations, cell counts, viable cell counts, or some other variable), and predicted results, preferably in graphic form. The laboratory assistant helps the students in looking up items in biological and chemical supply catalogs and orders the things they have requested upon approval by the instructor.

During the three weeks of the project, no other formal laboratory sessions are held. Students frequently ask for assistance in determining if their cell cultures have become contaminated, in making and sterilizing things they wish to add to the medium, in determining how to use the 24 well plates, etc. Occasionally, a student group contaminates their cultures. The instructor splits a backup set of cells every few days to have a new stock available in those cases. The instructors and laboratory assistant also monitor how well the students are doing at keeping the work areas clean and whether more disposable supplies are needed. Our greatest challenges have been students failing to clean and put away the hemocytometers and students trying to keep **all** of their cells when splitting rather than just keeping a few flasks for use (ending up with as many as 20 flasks in the incubator).

Assessment

One concern students often have is "How will I be graded?" We try to be clear with our students that we are grading them on a variety of factors, but whether they get the "right" answer from their experiment is not one of them. We do assess our students' group interaction, cooperation, and effort through a combination of our own observation and student surveys given later in the term. We also grade them on their experimental design and techniques, looking for an answerable but creative question, good controls, repetition, and a logical approach to data analysis. Finally, we grade them on their ability to present the results and to see how the results of their small experiment would modify how they approached the same question again and would generalize to broader issues in cell biology. See Walvoord and Anderson, 1998 and Allen and Tanner, 2006 for discussions of the development of grading rubrics. The grading rubrics used for poster and laboratory report presentations are included in Appendix A.

In addition, we wanted to assess whether the cell culture project was achieving the goals we had for it as laid out in the introduction. We administered an eleven question Likert scale survey to the students before and after the project (Appendix B) during three semesters. We conducted one tailed Mann-Whitney U tests (Avery, 2007) on before and after Likert data. These data give an indication of student opinions about their learning and confidence.

Supplies

Table 1 lists the major supplies used for the cell culture project, including vendors, catalog numbers, and cost estimates. The total cost of running the cell culture project for about 24 students in one semester is approximately \$1500. Other items used that are assumed to be readily available in the laboratory are a funnel and flask for the disposal of liquid wastes, microscopes for counting cells using the hemocytometer, a 37˚ degree incubator with 5% oxygen and 95% carbon dioxide, micropipetors and tips, test tube and microfuge tube racks, an inverted microscope for viewing the cells in their flasks, sodium chloride, sodium phosphate, sodium bicarbonate, potassium chloride, potassium phosphate, distilled water, balances, stir bars, flasks, a pH probe, an autoclave, and sterile media bottles. Details for making the solutions are available from the authors.

Table 1. Supplies needed for the cell culture laboratory.

Product	Use	Size	Vendor	Cost estimate
Disposable lab coats	Worn whenever working with cells and left in the lab	Various; 30/box	VWR (80076-732)	\$154
Gloves	Worn whenever working with cells or chemicals	Various	Dash; 100/box	\$4
Cidecon	Disinfection of lab surfaces	1 gallon	Fisher (04-355-64)	\$30
Nonsterile gauze sponge	To line a funnel for a liquid waste disposal flask	4000/box	Fisher (22-415-496)	\$71
McCoy's medium	For growing cells	1 liter (10X concentration)	Sigma (M4892)	\$30
Newborn calf serum	Added to the medium	100 ml	Sigma (T8154)	\$16
Pen/Strep solution	Added to the medium and trypsin to kill bacteria	Stabilized; 10,000 units Penicillin; 10mg Streptomycin); 6 x 100 ml	VWR (45000-652)	\$67
Trypsin	To loosen cells from the flask	10 g	Sigma (T4799)	\$53
EDTA	Added to the trypsin	Tetrasodium salt; 100 g	Sigma (ED4S)	\$26
Culture flasks	Cell growth	25 ml and 50 ml; 100 per case	Fischer (08-772-1E and 10-126-9)	\$135
24 well plates	Cell growth	100/case	ISC Bio (T-3026-1)	\$79
Conical tubes	Alloquots of solutions for student use	15 ml (700/case) and 50 ml (500 per case)	ISC Bio (C-3317-2W and C-3317-3)	\$89
Glass pipets (sterile)	Measurement of solutions	1 ml (500/pkg), 5 ml (250/pkg), 10 ml (200/pkg)	ISC Bio (P2830-1, P2830-5, P2830-10)	\$53, \$42, \$37
Microfuge tubes	Alloquots of trypan blue and cells solutions	500/pkg	ISC Bio (C-3269-1)	\$9
Hemacytometer	Cell counting	1 slide with coverslip	VWR (48300-476)	\$82
Hemacytometer cover slips	Cell counting	12/pkg	VWR (15170-321)	\$29
Trypan blue	Determining cell viability	100 ml	Sigma (T8154)	\$11

Results

Student projects

Students have tried a variety of projects since the inception of the cell culture labs. Examples include variations in the amount of time cells are exposed to trypsin, variations in the temperature of the trypsin, various dilutions of the medium with PBS, and variations in incubation temperature. The latter can be quite challenging since we have only one incubator which is kept at 37˚C. To try other temperatures, students must also consider gas concentrations, thus realizing that they are manipulating more than one variable. Other students have tried exposing cells to ultraviolet light of various intensities and durations. Many students like to try adding something to the medium. Examples include additional glucose, chemicals known to solubulize membranes, proteinases, salts, and viruses. One group even tried incubating the cells in various dilutions of Gatorade™. With these projects, most student groups confront several experimental design challenges. These include framing a simple, clear question, the use of proper controls, determining a method for data gathering that will be consistent for all group members, determining how to analyze data in such a way that it will answer the question asked, and considering how to manage their time to gather truly reliable results.

Assessment of student attitudes and learning

Although the results were all statistically significant, it was somewhat difficult to measure changes in student perception about confidence and learning through the attitude survey we administered because the students showed great confidence in themselves and their knowledge even before they began the project. That confidence and knowledge is not particularly consistent with our informal observations based on classroom discussions, test results, and discussions with the students during office hours. Transylvania students, however, were often some of the best students in their high school classes, so they tend to enter college with a rather high level of self-esteem.

The combined results from the attitude surveys given in the winter and fall terms of 2005 and the winter term of 2007 to 58 students are shown in Table 2. P values from one-tailed Mann-Whitney U tests on before and after Likert data are shown in the last column. The exact questions asked are shown in Appendix B. The results indicate that despite mild anxiety to begin with, most students were glad they had the opportunity to work with the cell cultures (Question 11). They also show that they felt like they were involved in the scientific process (Question 8) and that the project helped them understand the interactions of cells (Question 3). They also indicate that students felt more confident in their experimental design abilities (Questions 4 and 10) and that they felt like they had developed skills through repetition (Question 5). Finally, the results indicate that students felt that the lab project helped them understand concepts and relationships presented throughout the course (Questions 2 and 9).

Table 2. Average Likert scale scores from the student survey (n=58).

Question	Pre-lab survey	Post-lab Survey	Difference	P
1. Visual image	4.28	4.69	0.41	.0037
2. Concept understanding	3.91	4.25	0.34	.0123
3. Cell interaction	3.81	4.46	0.65	.0000
4. Experimental design	3.53	4.12	0.58	.0002
5. Repetition	3.57	4.25	0.69	.0004
6. Time and groups	4.24	4.59	0.35	.0038
7. Decision making	3.88	4.27	0.39	.0123
8. Real science	3.67	4.56	0.89	.0000
9. Relationships	3.78	4.39	0.61	.0000
10. Outlook on independence	3.57	4.19	0.63	.0010
11. Anxiety/Gladness	3.90	4.19	0.30	.0413

The students ranked themselves amazingly high on time management and group interaction skills before beginning the project (Question 6), something the professors would have ranked quite low. Despite the high starting perception, students felt that their skills improved during the project. The professors noted many groups struggling with time management, work allocation, and responsibility during the project. With this and the many other group projects that are included throughout a Transylvania education, informal observation of the faculty would indicate a large improvement in these skills throughout their college experience. Given the many mistakes that the groups made and learned from, it is pleasing to note that student confidence in their decision making ability rose significantly during the project (Question 7). In fact, this project showed many students that they had overestimated their initial abilities.

Question 1 addressed one of our central goals for this project, helping students understand cells as dynamic entities. In addition to the survey results, informal observations of the professors are consistent with an improvement in this aspect cell biology. In the discussions students had with us while studying for exams and while discussing their projects, we noticed more students considering cells as changing, dynamic entities than before we began the project. In responses to open-ended questions accompanying the survey given after the project, students often indicated that they had learned a great deal about time management, independent learning, and group interaction skills. The following is one student's analysis of the experience.

> *I enjoyed this lab. It allowed us to apply the knowledge we have gained about the nature of cells to design our own experiment. This knowledge gave us better understanding of what occurred in our experiment. This lab made us think about what we were doing and understand it. We weren't given a road map. Typically in labs we get step-by-step instructions of procedures so it's easy to thoughtlessly follow directions. With this lab, the instructions were our own; therefore, we had to understand why and how every step was to be taken. We learned responsibility in this lab. We learned to rely on each other. We visited the lab every day and 99% of the time, it was all three of us, each with a different task to complete. We alternated each time so everyone got to learn new lab skills and hands on experience. Work in the hood made us consider every potential source of contamination and take extreme care in avoiding it. Everything we did was carefully monitored and done with precision, so as to avoid mistakes and contamination. We had to absolutely focus on our every move. This lab gave us many new skills and much more careful and precise technique. Learning to use hemocytometers was amazing. [Unreadable section] This has probably been the most interesting, valuable, meaningful, tedious, long, informative lab I've ever done. I would love to do it over. As I look back, it is amazing how much we have all learned from it.*

As this above passage indicates, to gather better data on such the cell culture project's impact on attitudes and learning, it would probably be a good idea to conduct interviews of students before and after the experience.

Challenges

Another observation made by the professors is that many students struggled with considering the role of controls and repeats in experimental design. Their initial proposals often included confounding variables that they were not even aware of. In addition, they often failed to consider the importance of staggering times of well set-up to prepare for the time needed for data gathering at the end. In other words, they would start many wells at the identical time, but then discover that counting cells took many hours. Therefore, some wells had incubated for much longer than others.

The presentation of the project in the form of a poster or written report revealed many experimental errors to the students. They often indicated a desire to have more time during the term to repeat the experiment more carefully. Although more time was not available during CMB class, Transylvania biology students get many more opportunities to do independent projects in later classes, so the impact of this learning experience is seen in other settings.

During one semester, one of the professors who supports this project was on sabbatical and the other had part-time administrative duties which often required her to be out of the building. During that semester, some students indicated frustration with lack of access to an "expert" to consult when a problem arose. Based on that experience, we would recommend that this project be undertaken when the professor and/or laboratory assistant can have a high level of visibility to students throughout the term.

Discussion

In summary, students seem to benefit greatly from inclusion of the cell culture project in CMB. Their ability to manage time, design experiments, work with a group, and imagine cells as dynamic, interactive entities appears to improve. In addition, most students report that they enjoy the independence of asking their own questions. There are, of course, a few exceptions. Some students prefer a more "cookbook" approach because it is simpler, takes less time, and does not require that they depend on others. Students who have traditionally gotten very high grades by working alone and in a more regimented fashion sometimes find the cell culture project uncomfortable. The project does require some intense time by both the professors and the laboratory technical assistant, particularly during the training sessions, but the cost is not prohibitive and the benefits seem to be high.

We began this project in an attempt to more actively engage sophomore level students the scientific process as a part of CMB class. In doing so, we asked whether it is feasible to permit undergraduate students with no previous experience using cell cultures the opportunity to design and carry out their own cell culture experiments as part of a sophomore level core course in biology. The answer to that is clearly affirmative. The time and money expenses invested are not unreasonable. The most expensive items are a laminar flow hood, which we have shown is not essential, and an incubator. Disposable supplies are not insignificant, but are reasonable (less than $75 per student). One of the greatest challenges was getting the students to work with 24 well plates for their experiments after teaching them the techniques using flasks. In the future, we plan to try to teach the students to observe and split cells directly in the 24 well plates rather than ever working with flasks.

In addition, we asked whether the open-endedness of an inquiry-based cell culture laboratory put more responsibility on students to think about what they are doing and thus foster greater autonomy and better learning. We also asked if students got a better concept of cells as dynamic entities after working with the cell cultures. Survey results seem to indicate that the answers to these questions are also affirmative. Our informal observations definitely indicate greater autonomy and responsibility on the part of the students. To further foster student learning, we would like to more strongly link the cell culture project with many of the subjects discussed in a CMB class. For example, how could the cells be used to specifically study membrane transport? Could they be used to study respiration, energetics, or organelle function? Could their structure be examined through microscopic techniques? If the model system was used not only by the students in one project of their own design, but also in other experiments designed by the professors, it might assist the students even more in demonstrating relationships between cell structure and function.

In conclusion, we recommend that others try working with cultured cells early in a biology education for undergraduate students. This model system provides an opportunity for students to gain a variety of scientific skills and to have fun doing so. It can provide a foundation for further class-based research projects as students advance through the major.

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