

Making Heads or Tails: Planarian Stem Cells in the Classroom †

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Stem cells hold great promise in the treatment of diseases ranging from cancer to dementia. However, as rapidly as the field of stem cell biology has emerged, heated political debate has followed, scrutinizing the ethical implications of stem cell use. It is therefore imperative to promote scientific literacy by educating students about stem cell biology. Yet, there is a definite lack of material to engage students in this subject at the basic science level. Therefore, we have developed and implemented a hands-on introductory laboratory module that introduces students to stem cell biology and can be easily incorporated into existing curricula. Students learn about stem cell biology using an *in vivo* planarian model system in which they down-regulate two genes important in stem cell differentiation using RNA interference and then observe the regenerative phenotype. The module was piloted at the high school, community college, and university levels. Here, we report that introductory biology students enrolled at a community college were able to demonstrate gains in learning after completion of a one-hour lecture and four 45-minute laboratory sessions over the course of three weeks. These gains in learning outcomes were objectively evaluated both before and after its execution using a student quiz and experimental results. Furthermore, students' self-assessments revealed increases in perceived knowledge as well as a general interest in stem cells. Therefore, these data suggest that this module is a simple, useful way to engage and to teach students about stem cell biology.

INTRODUCTION

Stem cell biology is an exciting and expanding area of biomedical research which has the potential to revolutionize clinical medicine on multiple fronts. Stem cells show amazing promise in elucidating the processes governing normal development as well as the mechanisms contributing to a number of human diseases including diabetes, cancer, and Parkinson's disease (9, 17). At their most fundamental level, stem cells have the capability to develop into many or all of the various cell types within the human body during development. These cells are unique because they are unspecialized, renewable, and can be induced to form any particular cell type given the appropriate cellular and extracellular cues. Many tissues, in fact, contain specialized pools of endogenous stem cells that are used to replenish existing cells within that tissue during injury or cellular turnover. The first experiments using stem cells, specifically embryonic stem cells (ESCs), were conducted in the early 1980s. Scientists

were able to derive and genetically alter ESCs from mouse embryos to create new mouse strains that served as models for human diseases and gene function (5, 11). In 1998, these studies were translated to humans as scientists derived human embryonic stem cells (hESCs) from embryos donated from *in vitro* fertilization clinics. The pluripotency of hESCs gives them unlimited self-renewal capabilities and the ability to differentiate into many different cell types. However, despite the numerous therapeutic possibilities, the study of hESCs in particular has generated heated debate resulting in many legislative restrictions on their use (19).

In response to these regulations, researchers have explored other potential sources of pluripotent cells, including adult and umbilical cord stem cells (1). Recently, scientific research has led to methodologies which can alter adult somatic cells to become embryonic-like. These cells, called induced pluripotent stem cells (iPSCs), are formed through the activation of specific genes which revert the host cells to a pluripotent fate. This results in a renewable source of stem cells that are compatible with the donor from which they were originally derived. The implications of these findings led to two Nobel Prizes awarded in Physiology or Medicine in 2012.

In addition to human stem cells, planarians are a useful model system to study cell and tissue replacement as some species display the remarkable ability to regenerate missing

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body structures in as little as one week (5). An excised fragment from their own bodies will reform a complete, perfectly proportionate organism in a short period of time. Planarian regeneration is driven by a group of unique and pluripotent stem cells known as neoblasts (7, 14). During wound healing or regeneration, neoblasts proximal to the wound site will proliferate, giving rise to a regenerative blastema that will differentiate into the missing tissues. These cells are akin to hESCs because they have the ability to become any cell type in the animal during normal development as well as during wound healing and tissue regeneration.

RNA interference (RNAi) can be readily employed to disrupt the function of genes that are important in planarian regeneration. RNAi is an endogenous cellular mechanism used to specifically down-regulate target RNAs in a number of organisms ranging from worms to humans. Since its first discovery in plants, RNAi has been employed extensively to elucidate gene functions and serve as the basis for rational drug design (8). Its utility has become so wide-spread that RNAi clinical trials are currently underway for diseases such as amyloidosis and cancer (2).

A major issue surrounding the stem cell debate is the overall lack of science literacy within the broader public. Therefore, it is imperative as part of standard science curricula to educate the next generation of decision-makers by providing them with the background information necessary to rationally evaluate statements made on “scientific” and ethical grounds (8). Due to the relative deficiency of stem-cell-based laboratory experiences that can be feasibly conducted at the introductory level, we designed a one-lecture and four-laboratory module, aptly named “Stem Cells.” This module introduces students to the scientific and ethical issues surrounding the use of stem cells via hands-on engagement. The lab utilizes planarian flatworms as a simple *in vivo* model system to study the regulation and importance of stem cells by using current molecular biological techniques. In the lab, students elucidate the role of two genes involved in neoblast differentiation and regeneration using RNAi. The laboratory sessions are straightforward and relatively inexpensive to implement such that they can be incorporated seamlessly into standard introductory biology courses.

Intended audience

This lecture and laboratory module is appropriate for use at both the high school and college levels. At the high school level, this module was used at the North Carolina School of Science and Math (NCSSM) with advanced junior and senior science students as part of their anatomy and physiology course. At the college level, this module was used at three separate institutions and courses as follows: 1) North Carolina State University (NCSU), an introduction to biotechnology course for nonmajor freshmen; 2) Wake Technical Community College (WTCC), an introductory biology course for Biology major students, and 3) The University of North Carolina at Chapel Hill (UNC-CH), a

cell and developmental biology course for sophomores and juniors. This module would also be appropriate for use in any class where an understanding of stem cell biology and current biotechnological approaches is needed.

Learning time

The Stem Cells Module consists of one 45- to 60-minute lecture accompanied by four approximately 45- to 60-minute laboratory sessions over the course of three weeks. The lecture material covers general stem cell biology including background, types, derivation, properties, therapeutic potential, and ethical controversy. Students also learn about RNAi technology and the planarian model system as part of the same lecture prior to performing the laboratory. In the laboratory, students work in teams of two to four. During week 1, each team hypothesizes outcomes of down-regulating two genes important in stem cell differentiation during two separate RNAi feeding sessions. The following week (week 2), students perform trunk fragment regeneration assays and observe/record regenerated phenotypes one week later (week 3). A minimum of one week after the trunk fragment regeneration assay is necessary for regeneration to occur prior to final planarian observations.

Prerequisite student knowledge

This module is intended to be used with students who have an introductory knowledge of the central dogma (DNA replication, RNA transcription, and protein translation). Familiarity with mitosis and meiosis would further the understanding of stem cell biology, but it is not a requirement. Instructors may wish to review this topic during the lecture component of this module.

Students who do not have experience using a micropipettor (or if the institution does not have them readily available) can opt to use a transfer pipette.

Learning objectives

The instructors explained the learning outcomes to the students prior to the module lecture and laboratory exercises.

On completion of the module, students will be able to:

1. Describe the properties of stem cells.
2. Explain “potency” as it relates to stem cells.
3. Compare adult, embryonic, and induced pluripotent stem cells.
4. Describe multiple views and provide an example of an ethical debate surrounding the study and use of stem cells.
5. Discuss the different stem cell lineages and which tissues each is derived from.
6. Explain RNA interference and why it is a useful tool to study gene expression.

7. Describe why planarians are a useful laboratory model to study stem cells.
8. Predict experimental outcomes and analyze acquired data. *Note that this learning outcome is achieved when used in conjunction with the laboratory discussion questions, written laboratory reports, and/or poster presentations.*

PROCEDURE

Student instructions

Full student instructions are found in Appendix 1.

Faculty instructions and materials

The background lecture on stem cells should precede the laboratory sessions (Appendix 2). During this time, instructors may opt to have their students design hypotheses to test during their subsequent experiments. The laboratory portion of the course requires four separate laboratory sessions. Two lab sessions are required during week 1, followed by 1 lab session per week over the following two weeks, including preparation time in between labs (Fig. 1). If laboratory time is restricted, it would be feasible for instructors to perform the RNAi feedings and have students begin with the trunk fragment regeneration assay. Details regarding husbandry, maintenance, as well as sources of *Dugesia japonica* (*Dj*) can be found in Appendix 3 (13). The assays are conducted as previously described with some modifications (3). Briefly, 4 to 5 worms approximately 8 to 10 mm in length are chosen for use in the assays for each experimental group. Worms are starved at least 5 days before the start of the RNAi feeding protocol (12). Three separate RNAi feeding cohorts were used: 1) *Dj-six-1* (Accession AJ557022.1), a gene important in the formation of the eyespots (10); 2) *Dj- β -catenin-1* (Accession HQ738521.1), a gene important in anterior-posterior polarity (6, 15, 20); and 3) negative control (no RNAi). It is important to note in these experiments that the RNAi constructs have specifically been designed for use in the *Dugesia japonica* species of planarian and will not work for other species. For each RNAi cohort, *E. coli* expressing dsRNA targeting the gene of interest, was prepared in beef liver homogenate (a.k.a. RNAi feeding mixture), which is distributed to each student group consisting of 2 to 4 students. Students feed worms with the respective feeding mixture for 1 hour. Students repeat the RNAi feeding procedure every other day over the course of 5 days. Three days after the last feeding, worms are subject to the trunk fragment regeneration assay. Regenerative phenotypes are scored approximately 1 week later when regenerated structures are visible. For detailed protocols on preparation of the RNAi feeding slurry and the trunk fragment regeneration assay as well as materials with associated costs please refer to Appendices 4 and 5.

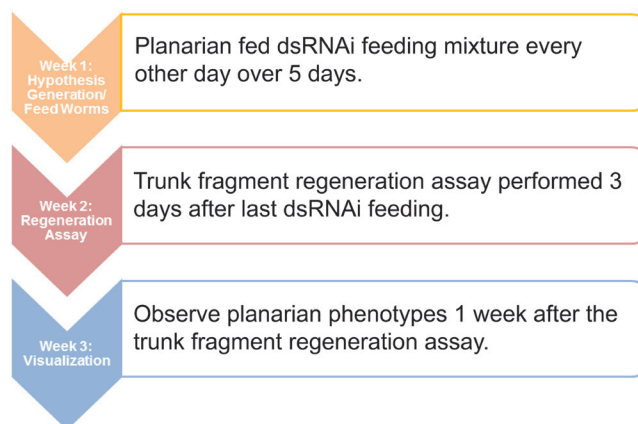


FIGURE 1. Overview of experimental plan.

Suggestions for determining student learning

Student learning outcomes associated with the Stem Cells Module were assessed by an identical pre- and post-quiz (Appendix 6). The quiz consisted of 10 short-answer questions, which addressed learning outcomes 1 to 7 outlined above. Partial credit was awarded based on response. Six questions were knowledge-based, and the remaining questions each individually involved comprehension, analysis, application, or synthesis as classified by Bloom's taxonomy (1). Additionally, to evaluate higher-order thinking (e.g. analysis and evaluation; learning outcome 8), student learning may be assessed upon completion and quality of answers provided for the discussion questions that are found at the end of the laboratory student worksheet (Appendix 1 and 9). Students may also be given the opportunity of presenting a poster or writing a report on their laboratory experiments. Examples of exemplary student work for these assessments and associated grading rubrics may be found in Appendices 8 to 11.

Anonymous survey

To assess attitudes, students were given an optional, anonymous survey before and after the laboratory sequence. Surveys were administered online using Qualtrics Survey Software (www.qualtrics.com). Survey data was collected from a total of 46 students (pre-module survey) and 51 students (post-module survey) over the course of a semester. Students were given a modified version of the Student Assessment of Learning Gains (16), which asked specific questions pertaining to the lecture and lab. Additionally, students were asked about demographic information including gender, major, degree granting program, degree working towards, and current GPA.

The post- survey included open-ended questions such as "What did you like most about the module?" "What did you dislike the most about the module?" and "List any additional skills you gained from this module." The full surveys are available in Appendix 7. Approval to evaluate students

by an anonymous survey (exempt status) was granted by the North Carolina State University Institutional Review Board.

Sample data

Students who participated in the Stem Cells Module developed their laboratory skills by performing hands-on experiments with the planarian *Dugesia japonica*. Students down-regulated the expression of two genes important for neoblast differentiation, *Dj-six-1* and *Dj- β -catenin-1*, by using RNAi. At the beginning of the first lab period, students were asked to formulate a hypothesis based on the background information provided during lecture. Students were then required to critically consider experimental outcomes prior to starting the lab.

In all sections of the course conducted at WTCC, about 33% of groups observed planarian phenotypes after regeneration consistent with the successful knockdown of the target genes tested. Planarians fed the dsRNAi construct *Dj-six-1* produced worms with smaller/reduced eyespots compared to control worms following the trunk fragment regeneration assay (Fig. 2A vs. 2B). RNAi targeted to *Dj- β -catenin-1* produced bipolar planarians following trunk fragment regeneration (Fig. 2C). Although not part of the present study, freshman life science major and nonmajor students enrolled in an introduction to biotechnology course at NCSU also took part in the Stem Cells Module. The instructor of the course reported about 86% success rates for *Dj- β -catenin-1* RNAi (based on the two-headed phenotype) and a 43% success rate for *Dj-six-1* RNAi (based on the lack or reduction of eyespots). The majority of these students had no previous laboratory experience.

Overall the experiments were successful. However, there were two main problems associated with the implementation of the lab: 1) loss of dissected worm fragments and 2) lack of RNAi-induced phenotype. The first issue occurred after the trunk fragment regeneration assay, as a subset of students noted a loss in planarian fragments when observed during the next lab session. While cannibalism has been documented in planarian species, it is likely that students did not allow for proper wound closure (i.e. ~2–3 min) after dissection prior to placement back in the dish. If the wound formed after dissection does not have sufficient time to seal after cutting, the worm fragment will disintegrate. The second problem encountered by a subset of students was a lack of an obvious *Dj-six-1* RNAi phenotype. There are a number of reasons as to why this may have occurred. Since some instructors varied the feeding schedule to accommodate class meeting times, there may not have been adequate time for knockdown to have taken place prior to the trunk fragment regeneration assay, as each RNAi construct has specific conditions for optimal efficacy. Although the feeding schedule is somewhat flexible and can be modified, any deviations should be piloted prior to the implementation of the lab. Furthermore, the success of RNAi knockdown rests on how much of the RNAi feeding mixture the planarians

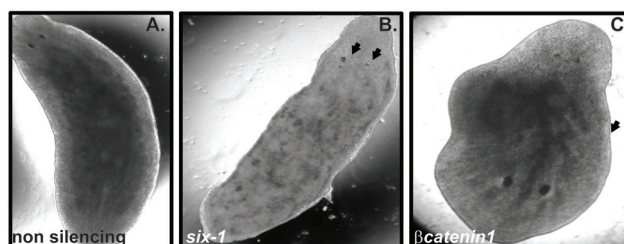


FIGURE 2. Trunk fragment regeneration assay. Images were taken 7 days after excision. A) Image of a planarian fed a control dsRNAi construct. B) Reduced eyespots formed by *Dj-six-1* RNAi. C) Bipolar planarian produced by *Dj- β -catenin-1* RNAi. Note the formation of a secondary head structure and posterior eyespots. In A–C, the original anterior end of the worms is oriented to the bottom. Arrow indicates site of excision and double arrow denotes reduced eyespots. Representative students' images of planarian with an observed phenotype are shown.

consume. If the worms are not sufficiently starved, they will not eat as much of the mixture and knockdown of the gene of interest may not occur or may not be sufficient enough for the formation of an observable phenotype. Moreover, if the worms are disturbed excessively during feeding they will regurgitate their food resulting in lower gene knockdown.

Safety issues

The experiments described herein should be conducted using standard safety practices for BSL1 microorganisms. Further details on proper biosafety guidelines in the teaching laboratory can be found in a previous issue of the *Journal of Microbiology & Biology Education* (4).

DISCUSSION

Field testing and evidence of student learning

The Stem Cells Module was designed to provide undergraduate students with a hands-on learning experience with which to introduce them to concepts in stem cell biology and how it relates to real-world issues. The module was designed to fit in one lecture period and four laboratory sessions in order to be easily incorporated into an existing course. Using planarians as a model system, students down-regulated expression of genes important in stem cell differentiation via RNAi. Upon regeneration, the resulting phenotypes are striking evidence as to the power of stem cells and the genetics that modulate them. We therefore wanted to determine if this module enhanced student learning and attitudes on the subject.

The Stem Cells Module was conducted with students who were enrolled in BIO 111 General Biology I for science majors at Wake Technical Community College (WTCC), Raleigh, NC. A total of 75 students enrolled in the course during the spring 2013 semester. The prerequisites for the course were introductory Algebra, English and Reading. The data were collected from three separate sections of

the course taught by three different instructors over one full semester. The majority of students' self-reported GPAs within the "B" range.

Assessment of student learning was determined by administration of a pre- and post-quiz consisting of 10 short-answer questions. The same quiz was administered both before and after the lecture/lab and categorized by learning outcome. Students' written responses were graded for correctness according to the following scale: 1 point (correct answer) 0.5 point (partial correct) or zero points (no or incorrect answer). Figure 3 summarizes the findings of the pre- and post-quiz results in conjunction with the specific learning outcomes each question addressed. The results of the pre-quiz confirmed that most students did not have a solid understanding of stem cell biology or RNAi. However, after completion of the Stem Cells Module, post-quiz data and experimental results suggest that students had considerable gains in a number of the learning outcomes (Fig. 3). In addition to WTCC, the Stem Cells Module was also conducted at The University of North Carolina at Chapel Hill (UNC-CH). Students at this institution were of sophomore and junior status and enrolled in the course BIO 205 Cell and Developmental Biology. Results from the pre- and post-quiz administered to this cohort of students ($n=27$) also showed learning gains (Fig. 3). Compared to WTCC student scores, those from the UNC-CH students were considerably higher, with an average across learning outcomes of 87% vs. 52%, respectively. The differences in achievement between these two cohorts may be indicative of a higher level of preexisting knowledge in the UNC-CH students. The WTCC students were more naïve as, for many students, this was their first science class, while the UNC-CH students had taken previous science courses. It is also important to note that this assessment was performed for the purposes of this study, and although scores did not factor into student grades, completion of the quiz was offered as extra credit. Therefore, it is feasible that students did not put forth their best efforts, thereby reducing overall scores.

In addition to instructor-based assessments, students' self-assessment results indicated a high degree of satisfaction with the module (Fig. 4). WTCC students were encouraged to complete a pre- and post-questionnaire that asked them to respond to a series of questions pertaining to the module using a 1–5 rating scale, where 1 was strongly disagree and 5 was strongly agree. The vast majority of student attitudes shifted after taking the Stem Cells Module. Figure 4 shows the questions asked as well as the student responses from both before ($n=46$) and after ($n=51$) the module. The results indicate that students convey a high degree of perceived satisfaction with the course as well as the topics it encompasses. Notably, when asked, "How much did you gain in the following as a result of taking the Stem Cells Module?" a large number of students agreed/strongly agreed that taking the course helped them understand: planarians as a model system (94%), RNAi (84%), properties of stem cells as well as the impact of stem cell research (84%) (Fig. 4). These results

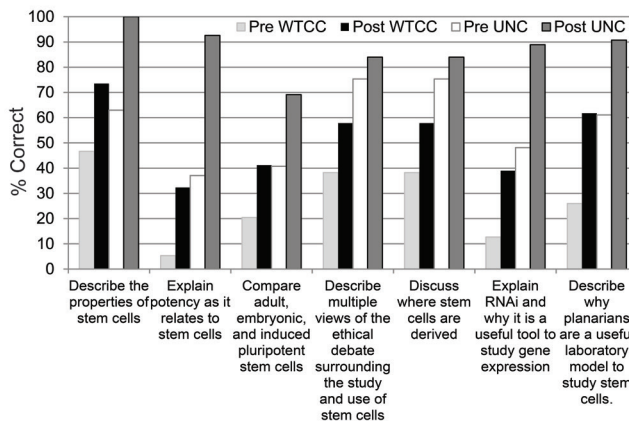


FIGURE 3. Students demonstrated gains in learning after taking the Stem Cells Module. Questions from the pre- and post-quiz were grouped based on learning outcomes they primarily addressed. Shown are average student results from both Wake Technical Community College (WTCC) and the University of North Carolina at Chapel Hill (UNC-CH).

were very encouraging as these were key topics pertaining to the learning outcomes for the module, and suggest that the hands-on learning approach is crucial for teaching and engaging students (18). Many students commented that the hands-on nature of the activity was the most enjoyable aspect of the module in the post-questionnaire.

In the post-questionnaire, students were also given an opportunity to respond to open-ended questions asking what they disliked most about the lab. Although the comments were quite varied, some themes were apparent. Many students did not like working in groups with other students who didn't take ownership for the project, while others wished more time was spent on the experiments.

Possible modifications

The Stem Cells Module was integrated seamlessly into a preexisting introductory biology course at Wake Technical Community College. The lecture was incorporated as part of the mitosis/meiosis chapter complemented by four laboratory periods, which were about 45–60 minutes in length. Students learned about stem cells during the lecture and then generated and tested their hypotheses during the laboratory sessions, where they used RNAi to down-regulate the expression of genes important in neoblast differentiation in the regenerating planarian.

This module was specifically designed to be easily adapted and assimilated into standard courses. The laboratory sessions created are inexpensive to conduct and do not require specialized instrumentation. Additionally, this module is instructor malleable and more (or less) student assessment can be added to augment the module. For example, although the primary data shown in this manuscript represent the findings from one cohort of students at the

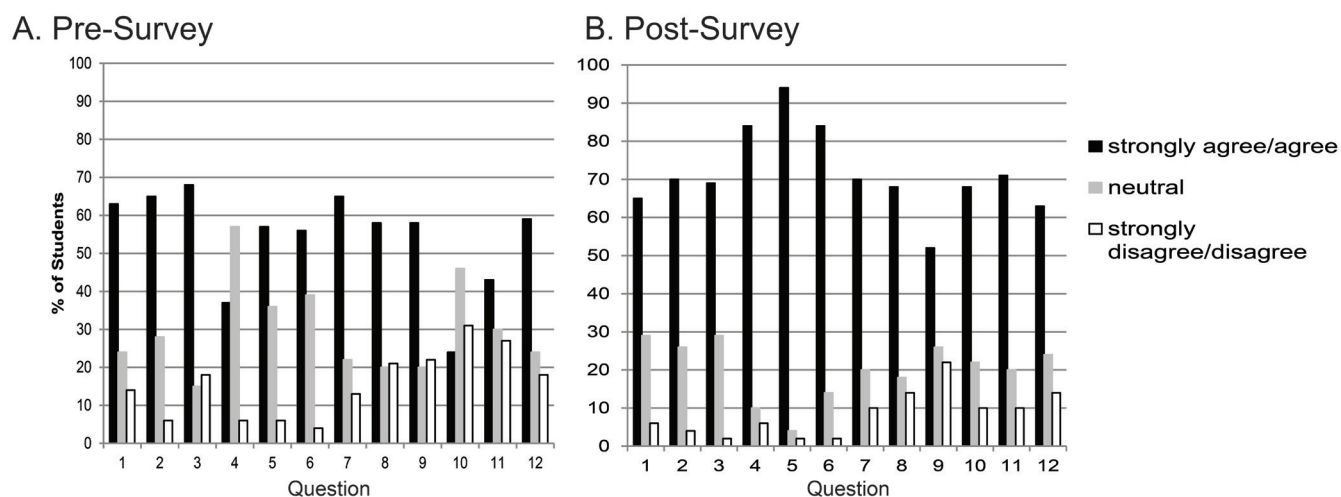


FIGURE 4. Student self-assessment of learning gains. Ratings from the A) pre- and B) post- questionnaires for each semester were scored and averaged. Students were asked to rate their current levels of competence or knowledge for each conceptual or technical skill listed on a scale of 1 to 5, with 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, and 5 = strongly agree.

community college level, this course was also incorporated at various other institutions. Instructors at the North Carolina School of Science and Math used the Stem Cells Module as part of their high school Human Physiology Course, and instructors at North Carolina State University (NCSU) used the module in their Introduction to Biotechnology class. Both undergraduate and high school students at these institutions were required to perform literature searches on *Dj- β -catenin-1* and *Dj-six-1* and explain the pathways they modulated to their classmates. Having researched this background information, students generated hypotheses regarding the down-regulation of *Dj- β -catenin-1* and *Dj-six-1* prior to performing the laboratory portion of the course. They then discussed cases surrounding the political/ethical debate associated with the use of embryonic stem cells. To address higher order learning outcomes, “Predict experimental outcomes and analyze acquired data,” students at NCSU were graded on their responses to the laboratory discussion questions (Table I) and written laboratory reports (Appendix 10). Overall, students were able to accurately analyze experimental data based on written responses to the discussion questions (Table I) and laboratory report grades (e.g. average 90.3%). However, some students had difficulty evaluating experimental outcomes that did not work as expected (Table I; Questions 3 and 4). This proved to be a good opportunity for instructors to model critical thinking, by leading those students through a discussion of how to think through possible reasons for the unanticipated results. Additionally, UNC-CH students were also required to summarize their laboratory findings in the form of a written lab report or poster/oral presentation (Appendix 11). In general, the feedback from students and instructors at these three institutions was extremely positive. Since the lecture and lab components were easy to implement and inexpensive to execute, the Stem Cells Module will be conducted again at all institutions (*personal*

communication). Furthermore, future courses will utilize the Stem Cells Module as an inter-disciplinary inquiry-based lab where students from courses spanning microbiology, biology, and physiology will collaborate together on various components of the module.

CONCLUSION

We found this module to be useful in creating an engaging environment for learning about various aspects surrounding stem cells including their biology and ethical implications. Students have the hands-on experience of working with planarians, while utilizing cutting edge scientific technologies to heighten their understanding of stem cell physiology. Furthermore, the Stem Cells Module is easy to incorporate into existing curricula and is inexpensive to implement. Student-generated data, instructor-based assessment and student survey results suggest that students achieved gains in learning after completion of the Stem Cells Module. Moreover, students and instructors alike enjoyed the course and learned from the experience on a multitude of levels. Therefore, these findings support the use of this module to enrich existing courses.

SUPPLEMENTAL MATERIALS

- Appendix 1: Student experimental protocol
- Appendix 2: PowerPoint of lecture slides with instructor notes
- Appendix 3: *Dugesia japonica* husbandry
- Appendix 4: RNA interference feeding mixture protocol
- Appendix 5: Estimated planarian husbandry and experimental costs
- Appendix 6: Student pre-/post-quiz with answers
- Appendix 7: Student pre- and post-self-assessment surveys

- Appendix 8: Example of student work – pre-/post-quiz
 Appendix 9: Example of student work, answers, and grading rubric – discussion questions
 Appendix 10: Example of student work and grading rubric – laboratory report
 Appendix 11: Example of student work and grading rubric – poster and oral presentation

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TABLE I.

Average student responses to the laboratory discussion questions.

Q1. What happens to planarian neoblast cells during regeneration? Where do they go, and what do they become? To answer this question, draw a picture of a planarian with an amputation to illustrate the where and the what.

- 86% of students provided a correct response
- 14% of students provided a partially correct response
- 0% of students provided an incorrect response

Q2. Would regeneration occur if there were no neoblast cells?

- 86% of students provided a correct response
- 14% of students provided a partially correct response
- 0% of students provided an incorrect response

Q3. Did all the planarian body segments each regenerate into a complete organism? Did they all regenerate organisms with a normal body plan?

- 79% of students provided a correct response
- 21% of students provided a partially correct response
- 0% of students provided an incorrect response

Q4. Were there any planarians that didn't regenerate a normal body plan? If so, can you describe why? (Hint: think about what they ate)

- 43% of students provided a correct response
- 57% of students provided a partially correct response
- 0% of students provided an incorrect response

Q5. Describe RNA interference and why it is a useful scientific tool to study gene expression.

- 43% of students provided a correct response
- 57% of students provided a partially correct response
- 0% of students provided an incorrect response

The laboratory discussion questions involved data interpretation and analysis. Students evaluated their own data and had to apply their knowledge to make conclusions based on experimental outcomes. Each discussion question is listed along with the percentage of students who generated appropriate responses.

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