BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 40, No. 3, pp. 174–180, 2012

Laboratory Exercise

An Inquiry-based Practical for a Large, Foundation-Level Undergraduate Laboratory that Enhances Student Understanding of Basic Cellular Concepts and Scientific Experimental Design

Received for publication, October 18, 2011, and in revised form, November 21, 2011

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Student-centered education involving research experiences or inquiry have been shown to help undergraduate students understand, and become excited about, the process of scientific investigation. These benefits are particularly important for students in the early stages of their degree (Report and Kenny, http://naplesccsunysbedu/Pres/boyernsf/1998). However, embedding such experiences into the curriculum is particularly difficult when dealing with early stage students, who are in larger cohorts and often lack the background content knowledge necessary to engage with primary research literature and research level methods and equipment. We report here the design, delivery, assessment, and subsequent student learning outcomes of a 4-week practical module for 120 students at the beginning of their second year of university, which successfully engages students in designing cell culture experiments and in understanding the molecular processes and machinery involved in the basic cellular process of macropinocytosis.

Keywords: Cellular biology, curriculum development, general education for science majors, inquiry-based teaching, laboratory exercises.

INTRODUCTION

Laboratory-based practicals are an essential part of the science curricula in higher education, as they engage students in active learning and help students to appreciate how science is conducted in a professional context. Although scientific and educational communities agree that undergraduate laboratories are "where science is done" [1, 2], there have been debates on *how* this should be performed in an undergraduate setting. Traditionally, laboratories have been set up as "recipe-based" or "cookbook" practicals where students follow a preset experimental method to arrive at an expected answer. These laboratories have their advantages as they are relatively inexpensive in both resources and support, and throughout the semester students are exposed to a wide range of basic methods and concepts and therefore acquire a wide range of practical skills and content knowledge. However, as the experimental outcome is already known to the students, they are never challenged to think about how scientific experiments are used to answer specific research questions, let alone develop their own skills in hypothesis formulation and experimental design. Unfortunately, developing advanced professional skills such as problem-solving and critical thinking is often sacrificed. Alternatives to the traditional model have been developed over the last 40 years, such as open-ended, open-induction, inquiry, and investigative models [1]. Using different layouts and assessment requirements, all of these alternatives attempt to lead students toward "how to think like a scientist" and development of cognitive and practical professional skills required for a broad range of science-based careers.

INQUIRY-BASED PRACTICALS

Inquiry-based models of curricula and practicals have been defined by Abraham and Pavelich [3] as:

> "A format... designed to allow each student to work at his/her own intellectual level and to give each student a mini-research experience... This is accomplished by requiring (allowing) the students to define their own Problem Statements, to design their own experiments and to generate their own analysis and explanation of the data collected."

Inquiry-based curricula have been proposed to help students develop advanced critical thinking skills, particularly in the evaluation of evidence in complex settings (e.g. see [4, 5]). Although inquiry-based curricula have been shown to produce superior learning outcomes compared with traditional curricula [6], an extensive review of inquiry-like learning examples revealed that explicit guidance, at a level appropri-

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ate to the learner's skill and knowledge base, is essential for inquiry-style classes to produce learning gains that are superior to traditional "recipe-type" teaching styles [7].

Thus, although inquiry-based classes may help students to gain an appreciation of the process of scientific investigation, there is the danger that such experiences can make it more difficult for students to learn the associated disciplinary content. Furthermore, the hierarchical nature of scientific knowledge means that students often require a solid foundation in a large amount of content knowledge before they are capable of understanding and engaging with research-like experiences [8]. Therefore, there are clear barriers to embedding research-like, or inquiry-based, experiences into the science curriculum at the tertiary level.

This study reports the development, implementation, and evaluation of a 4-week practical module for large numbers of early stage university science students, which has an inquiry-based format and introduces students to the process of macropinocytosis (a cellular uptake process) using mammalian cell culture as an investigative model. Even though these students began this module with little or no background in the experimental paradigm or disciplinary content, they were able to successfully propose a testable hypothesis and an appropriate executable experiment, critically evaluate their findings in light of the experimental approach and relevant scientific literature, as well as gain an in-depth understanding of the process of macropinocytosis.

EDUCATIONAL CONTEXT

The practical section described in this report was undertaken by a subset of students ($n = 120$) during the first semester of their second year of a Bachelor of Biomedical Science (a 4-year research-focused program) or a 6-year-accelerated medical program (which combines 2 years of a Bachelor of Science with a 4-year postgraduate medical degree), at a large research-orientated Australian university. This practical module was situated within a second level physiology course, which has been designed along with the preceding first year course and the succeeding second semester second level course to form a vertically integrated model of inquiry-based practical curricula [9] that follows the Research Skills Framework [10]. Across these three semesters, students are provided with opportunities of increasing difficulty to develop progressively more advanced technical, cognitive, and communication skills. For example, each semester requires students to take greater ownership of their projects, work with increased autonomy and undertake projects of increasing duration and complexity. As such, this practical is nested within a long-term set of experiences aimed at developing students' ability to 'think, work, and communicate like scientists.

DESIGN OF EXPERIMENT

Background Information for the Practical

Endocytosis is the process of taking the nutrients from the outside of the cell and bringing them inside the cell. Other than nutrient uptake, endocytosis is used by cells for variety of reasons: general housekeeping, downregulation of surface receptors, pathogen entry and immunity. This diverse array of functions is performed by several types of endocytosis, including clathrin-mediated endocytosis (CME), clathrin and lipid raft independent cellular uptake (CLICs), autophagy, phagocytosis, macropinocytosis, and calveolae. Different types of endocytosis are mediated by diverse sets of key molecules that control the uptake of diverse range of cargo molecules. For example, CME will primarily mediate uptake of activated plasma membrane receptors, whereas phagocytosis mediates uptake of solid objects such as bacterial pathogens and latex beads.

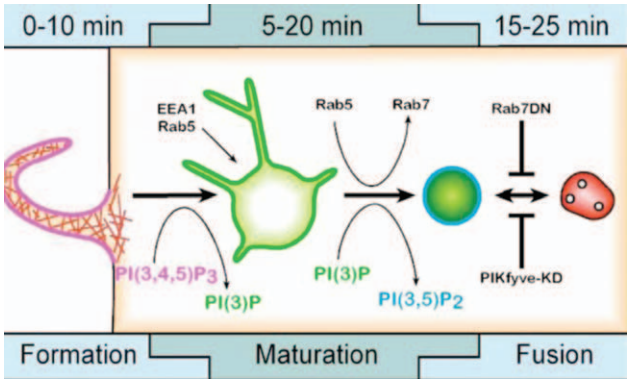

FIG. 1. Simplified diagram of macropinocytosis process. Macropinocytosis is coordinated by the cytoskeleton (red lines), PtdIns (pink, green, and blue), Rab molecules and its effectors (adapted from [11]).

In this practical, students investigated one type of endocytosis called macropinocytosis (Fig. 1). Macropinocytosis is a non-selective "bulk" or "fluid-phase" endocytosis ("macro"—large and "pinocytosis"—cell drinking), which is able to mediate uptake of as many as ~2,500 coated vesicles per minute (~2–3% of cell surface area). A macropinosome is a large (diameters > 0.2 μm) phase-bright endocytic organelle derived from the base of plasma membrane, actin-mediated ruffle (Fig. 1; pink and red) that is readily labeled with fluid-phase markers such as fluorescently labeled dextran or HPTS. Physiologically, macropinocytosis is highly relevant to many aspects of normal cell function and disease states, from sampling of immediate environment by macrophages for antigens to cellular migration and downregulation of signaling from cell surface receptors (for review, see [11]). Several studies have demonstrated that intracellular maturation of macropinosomes occurs via progressive recruitment of early, then late, endosomal, and lysosomal markers, and have identified key molecules that choreograph this process in an extremely tight and timely manner. These key molecules, such as Rab GTPases, cytoskeletal proteins as well as membrane lipids (phosphoinositides [PtdIns]), have been largely identified on the basis of their pharmacological inhibition. The susceptibility of these key molecules to widely available drugs, and easy quantification of the macropinocytosis process were key attributes in the selection of this paradigm for the undergraduate laboratory exercise.

Experimental Procedures

Equipment—Two commonly used mammalian cell lines were utilized as investigative models of cellular uptake—

Hek293, a human embryonic kidney cell line shown to have high level of macropinocytosis, and HeLa, a human cervical epithelial cell line demonstrated to have low macropinocitic activity. The cells were maintained in DMEM, 10% FBS at 37 °C and 5% CO_2 conditions and used at 1×10^6 cells per experimental or control sample. 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) is membrane-impermeable flourescent pH indicator widely used as a fluorescent dye for quantification of cellular uptake and maturation of endocytic vesicles.

The pharmacological reagents used in the practical include Wortmannin (100 nM), nocodozole (30 μM), and amiloride (3 mM), which inhibit PtdIn formation, microtubular network and actin cytoskeleton, respectively, while EGF (100 ng/mL) and phorbol ester (100 nM) were used as enhancers of macropinocytosis. Note: numbers in brackets represent optimal final concentrations of pharmacological agents listed. Each group of 34 students was supplied with a set of P20, P200, and P1000 pipettes and an Oikspin Qs7000 (Edwards Instrument Company) minifuge for rapid washing procedures.

A FLUOstar OPTIMA Multidetection Microplate Reader (BMG Labtech) equipped with a GFP-A-Basic-OMF (Semrock) filter was used for fluorescent readings (excitation: 405 nm, emission: 520 nm) to determine HPTS levels. The same plate reader was used to record absorbance at 595 nm to determine sample turbidity. The measurements were obtained using Optima Software Version 2.2 and data processed using Excel and Prism software.

Staff Requirements—For the duration of this practical, we retained two full-time technical staff members. Alongside their other duties, this particular laboratory module ran for 4 weeks and took up to 50% of the working week for these two staff members. One staff member was responsible for the mammalian cell culture, including cell maintenance and providing cells plated at the optimal confluency (80–90%) for the practical sessions. The second staff member was responsible for provision of all reagents, maintenance of the equipment, and helping with the measurements students needed to collect upon completion of their experiments.

Monitoring Safety of the Students—As some of the pharmacological agents were hazardous, students were encouraged to familiarize themselves with risks associated with each agent they chose for their experiments. The tutor designated to a specific group of students performed dispensing of the pharmacological agents after a brief safety talk with the students. Four percent PFA/PBS solution was kept in the fume hood at all times and tutor was present when students needed to use it.

Measuring Cellular Uptake Using a Fluorescent Dye in a High-Throughput Assay—Uptake of fluorescent dyes by mammalian cells is not a novel concept and is routinely used by many laboratories around the world to assess different types of cellular uptake. However, a basic technique used to assess this uptake is based on fluorescent microscopy, a technique that is not readily accessible to large cohorts of students. As we were designing a hands-on, inquiry-based, laboratory practical for large groups of early stage undergraduate students, there were several essential considerations in designing an assay for assessing cellular uptake. First, the assay needed to be robust in measuring uptake of the chosen fluorescent dye (HPTS). Second, it needed to be able to dynamically detect changes in the experimental setup depending on the specific research question designed by the students (e.g. will this pharmacological reagent inhibit uptake in this cell line). Third, it needed to be manageable within large student classes. Therefore, we designed a highly reproducible, and robust, high-throughput assay that successfully met all these criteria.

The assay began with students trypsinizing the mammalian cells to detach them from the plastic plate, washing the trypsin using growth media and leaving the cells to recover in a plastic tube for 10 minutes. The uptake was performed by addition of HPTS fluorescent dye (8 μg/ml) to cell suspension, and reaction was incubated with gentle rocking at 37 °C for up to 30–40 minutes, with samples taken every 10 minutes. Cells in each time point sample needed to be thoroughly washed by gentle 5-second spin in a minifuge followed by a resuspension in PBS at least four times to remove ALL extracellular dye. Samples were then transferred into a fume hood and fixed using 4% PFA/PBS for 10 minutes. Half of the sample was transferred into each of the two wells of a 96-well dish. Once all time points have been collected, the 96-well dish was transferred into a plate reader and two parameters were recorded for each well: fluorescence with excitation at 405 nm and emission at 520 nm, and absorbance at 595 nm. Fluorescence measured the increase in uptake of fluorescent dye whereas absorbance value was used to measure turbidity of the sample, and thus control for the cell number in each well. To quantify cellular uptake, students needed to divide value obtained for fluorescence reading by the value obtained for absorbance reading for each well (fluorescence/absorbance).

PRACTICAL MODULE IMPLEMENTATION

Approximately 120 students were divided into two practical groups. The practical was held over four consecutive weeks with three-hour sessions per week and one optional "help session" between Sessions I and II (Table I). Sessions I, II, and III were designed as practical, hands-on sessions whereas Session IV was designated as a critical reflection and data-analysis session. The practical was delivered with help from four tutors for each practical group (each tutor attended to three to four groups of three to four students, depending on the student numbers). Tutors were all biomedical scientists, from PhD students to postdoctoral scientists.

TABLE 1
Outline of practical sessions with assessment tasks marked in bold

Week 1	Session I	Development of high throughput assay
	"Help Session"	**Research Proposal**
Week 2	Session II	Inhibition of macropinocytosis
Week 3	Session III	Enhancement of macropinocytosis
Week 4	Session IV	Critical data analysis
		Research Report

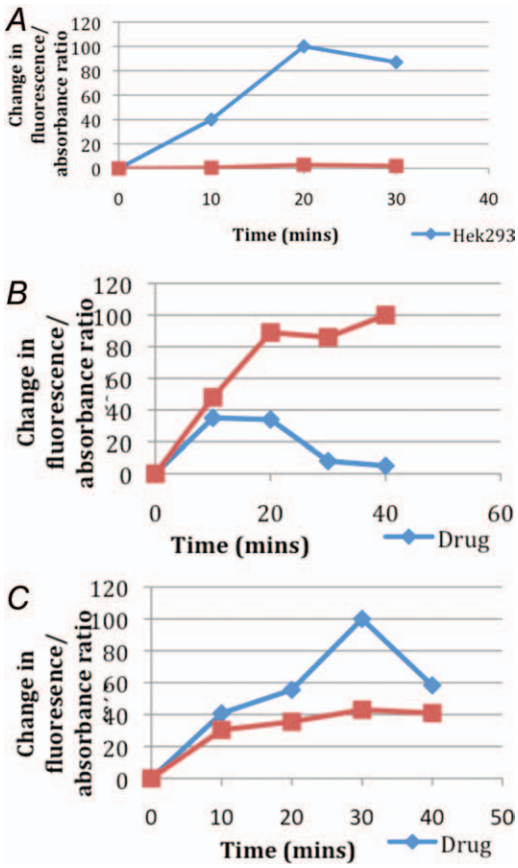

FIG. 2. Example of student data. Measurements of fluorescent dye uptake into HeLa and Hek293 cells (*A*) with differences observed in Hek293 cells upon amiloride inhibition (*B*) and in HeLa cells following EGF stimulation (*C*). Graph in (*A*) was obtained in Session I where students needed to ascertain which cell line to use for inhibition or enhancement of the uptake process. Students then designed and executed these experiments and data obtained is depicted in (*B*) and (*C*), respectively. Square symbols in (*A*) represents uptake process in HeLa cells. In (*B*) and (*C*) panels, square symbols represents "no drug" control dye uptake in Hek293 and HeLa cells, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Session I

As students had not been introduced to the concept of cellular uptake in any of the lectures prior to the practical, a short introductory lecture was provided at the beginning of the first practical session. The lecture covered the basics of endocytosis, focusing on macropinocytosis as one type of cellular uptake. Students were presented with a short summary of the key molecular players in this process using visual tools, including videos of cells taking up fluorescent dye. The students were then introduced to the high-throughput assay developed for the evaluation of macropinocytosis they would use in their practical. Students were informed that they needed to design two experiments that tested the molecular machinery they thought to be important for the cellular uptake using the high-throughput assay and two pharmacological reagents that they needed to choose from their analysis of primary literature over the next week. The students were also briefed on the assessment tasks associated with the practical, which consisted of a research proposal (20% of the module mark) and research report (80% of the module mark).

After this short introduction, students were asked to form groups of 3–4, assigned a tutor and allocated to a bench space in the laboratory. The students were provided with the research question for this session ("Does the rate of cellular uptake differ between two mammalian cell lines (Hek293 and HeLa)?") and a protocol for the assay written out in a "recipe" style. This was done for two reasons. First, literature suggests inquiry coupled to a completely novel concept (cellular uptake) without appropriate guidance leads to confusion and stress and diminishes learning [7]. Second, by answering the posed question students had acquired one critical piece of information needed for subsequent session (i.e., which cell line is more appropriate for each of the two pharmacological agents they would choose to use in their experimental design).

Help Session

This was the only voluntary session in the module. It was run between Sessions I and II and designed as a student-directed consultancy session, and staffed by only the Head Tutor (first author). Therefore, students were expected to come prepared with data obtained from Session I (for an example, see Fig. 2*A*) and have found and read scientific literature on relevant pharmacological reagents. Attendance was surprisingly high; although some groups sent one or two students as representatives, others attended the entire session as a whole group, choosing to write their first assessment task as a team during the session, resulting in an attendance by ~75% of the cohort. The Head Tutor acted as a facilitator to guide the students through difficult concepts, how to use the reagents and how to design experiment in a way that it will produce meaningful data (e.g. how to use proper experimental controls). Importantly, the Head Tutor was not involved in the experiment design, which was the responsibility of each individual group and part of the assessment. At the end of the session students needed to have a clear idea of both the practical requirements needed to design successful experiments for Sessions II and III and the subsequent data analysis. Two days after the "Help Session" students were required to submit their group research proposal, consisting of a set of dot-points to structure the introduction content (and guide them in writing of this section for the final research report), and a detailed experimental plan (including the two pharmacological reagents with appropriate effective concentrations they had chosen from the scientific literature to use in Sessions II and III).

Sessions II and III

These classes were devoted to students conducting the experiments they had designed. Students employed the assay they had learnt in Session I, this time using pharmacological reagents (one per session) to further

investigate the process of cellular uptake (e.g. see Figs. 2*B* and 2*C*). The tutors provided guidance on any technical points the students struggled with, while the students were solely responsible for ensuring they obtained the data they required for subsequent analysis to test their hypotheses.

Session IV

This class was designed as a "critical reflection" session, providing students with an opportunity to analyze primary data they obtained, and to reflect on their results both as individuals and as part of a group. In our view, this is the most important session for two reasons. First, focusing students on analyzing their own results put emphasis on learning to formulate their own conclusions about cellular uptake process, as well as learning to critically analyze their own data in relation to primary literature. Second, students realized that the conclusions *they* drew from their data, was not necessarily what *others* in the group saw. This was an important aspect of working in a collaborative group environment, and the tutors were instrumental in encouraging—and where necessary, instigating—debate amongst the students. One week after the completion of Session IV students were required to submit a final research report (four-page limit excluding references) written in a style of a journal article.

REFLECTIONS

Recipe-style practicals are designed and re-designed over a number of years with a simple message—everything student touches will work first and every time. Depending on individual instructor's perception this can be perceived as both an advantage and disadvantage. Students leave these practicals with acquisition of a specific practical skill but are robbed from asking a question, designing an experiment to answer the question or contributing scientifically as an individual to any aspect of the practical. On the other hand, inquiry laboratories coupled with initial expert guidance have been shown to have strong positive effects on learning outcomes that encompass both theoretical and practical aspects of a specific discipline. Designing an inquiry laboratory is challenging for both the student and the instructor and as such several important points need to be considered—is the practical extending student's ability to understand the theoretical background, is the method(s) used sufficiently simple and easily adjustable to student's requirements and is data analysis appropriate to answer the questions posed. If the answer to all of these questions is YES, one can consider designing an inquiry-based practical that embodies "being a scientist."

From educational point of view, Session IV in the present practical played a key part in the module in guiding students to develop a deeper understanding of the reality of the process of scientific investigation. For example, some students realized that some of their experimental data failed to support, and in some cases went as far as disputing, their research hypothesis. This became an important "A-HA moment" for students—many realized that failure is part of success in science and commented on *how* to improve their experimental designs as well as expressing desire to implement these changes. Therefore, the students themselves were actively contributing to improving the laboratory practical in ways that have now become discussion points for expansion in the future. Thus, the aim of encouraging students to think critically and creatively about experimental design and primary data, in the ways that scientists do, has been realized with this inquiry model.

An extremely important aspect of this practical was choosing and training tutors, as it requires knowledge of a very specific and relatively narrow biological field. Tutors needed to have the ability to "think on their feet," both in a practical sense, interpreting the data from several different pharmacological reagents used at differing concentrations, and in an educational sense by encouraging discussions among students in a way that is respectful and ensures development of student's critical thinking. At times, this required tutors to ask leading questions to the quieter group members and lowering contributions from the more vocal ones ensuring similar learning outcomes for all students.

From a technical perspective, the point that students soon discovered was crucial for success of their experiments was appropriate and extensive washing of cells at each time point. If the extracellular dye was not removed with great care or if the cells underwent necrosis in the process of washing, these flaws were easily detectable in the data analysis as outliers, and the measurements would therefore be unusable.

Treatment with pharmacological agents can in itself cause cell necrosis, but students were not able to control for this during this iteration of the practical. The most convenient method of measuring cell death in this setting would be subjecting a proportion of each sample to a trypan blue staining, prior to fixation and subsequent to cell washing. This is an inexpensive and quick method, which requires a transmitted light microscope; however, students did not have access to this equipment during the practical. As some of the students commented that cell death might have had an impact on the data they obtained during their discussions in Session IV and in their research reports, this additional aspect of the experimental design will now be incorporated into the practical sessions in future years.

STUDENT LEARNING OUTCOMES

In the final assessment piece, the research report, students clearly demonstrated that they had gained an in depth understanding of the molecular processes underlying macropinocytosis. From a randomly selected sample of 28 reports, 19 reports showed clear descriptions of the macropinocytosis at the level described in the literature, with some students not only describing the process but also introducing descriptions of specific molecules involved in macropinocytosis (Fig. 3*A*). For example:

> "Macropinosomes are self-organized structures that form spontaneously, yet can be stimulated by growth factor receptors, at sites of membrane ruffling which have diameters between 0.2 to 5.0μm (17, 18). Cell surface assembly of actin filaments form plasma membrane extensions

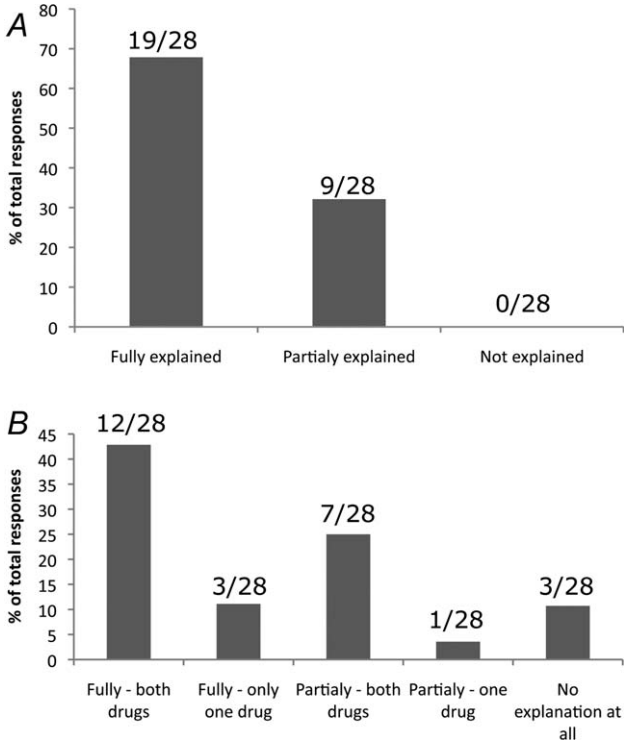

FIG. 3. Student learning outcomes. *A*: The Introduction sections of a sample of student reports ($n = 28$) were analyzed for the depth of description of the process of macropinocytosis under investigation studied and plotted as percentages of the total reports analyzed. *B*: The Discussion sections of a sample of student reports ($n = 26$; see Student Learning Outcomes section) were analyzed for the depth of interpretation of the student's own data in relation to the relevant scientific literature. The descriptors were plotted as percentages of the total reports analyzed. Numbers represent actual number of reports in each descriptor category; an additional two students did not relate their findings to their hypothesis (or the scientific literature) but instead focused on technical faults when discussing their data.

> called ruffles, which extend, curve and recede into the cytoplasm creating endocytic cups after closure... The enzymatic activity of phosphoinositide 3-kinase (PI3K) has been associated with the regulation of actin cytoskeleton ... Different isoforms of PI3K, classes I, II, and III enzymes, mediate this mechanism via catalyzing the phosphorylation of phosphoinositides, which act as essential second messengers..."

A further nine reports showed partial understanding of the process, where only a limited description of the process was included. For example in the quote below, the description is sketchy and does not name the key molecules involved in the process of macropinocytosis:

> "Cells uptake media from their surroundings by way of actin-mediated cell surface extrusions called ruffles. With this feature, macropinocytosis diverges from other forms of endocytosis because it is not defined by interaction between target and receptor molecules (Kerr & Teasdale, 2009). Researchers have exploited this feature in order to elucidate the mechanism of action of the macropinosome (Kerr & Teasdale, 2009; Falcone et al., 2008)."

The research reports also revealed that students in this cohort were at several different levels in their ability to interpret the data they obtained from their experiments in relation to the knowledge they acquired from literature (Fig. 3*B*). At the extreme end of this variation, only two reports (of the 28 reports analyzed) were excluded from the analysis shown in Fig. 3*B* because the students did not relate their findings to their hypothesis (or the scientific literature) but instead focused on technical faults when discussing their data. For example:

> "The fact that our results do not support these findings may be a result of insufficient EGF concentration or incubation period... In addition, successful experiments have used amiloride concentrations of 3mM, which is greater than the 1mM concentration used in this study (West et al., 1989)."

A small proportion of students (3/26) did not offer any explanations of the data they observed but simply described the data more broadly:

> "The cell line that was submitted to the inhibition phase also seemed to support the hypothesis with the control having what looks like a significantly higher rate of extracellular fluid uptake. The treatment which had the drug amiloride added to it appeared to be successfully inhibited. These results are backed up by the literature which all came to the same conclusion (Swanson & Watts, 1995; Koivusalo et al., 2010)."

Importantly, most students (23/26) demonstrated an accurate appreciation of how the experimental approach they employed allowed them to investigate the process of macropinocytosis in the context of cell culture, and applied their acquired knowledge fully or partially to the data obtained (Fig. 3*B*). This was most evident, where students critically evaluated their findings in relation to the scientific literature in the Discussion sections of the reports, for example:

> "Acidification caused by amiloride would adversely affect the pH-sensitive signalling pathways require for the activation of cdc42, rac1 and their effector proteins (Koivusalo et al., 2010). Ultimately, this would affect the formation of membrane ruffles and thus reduced HPTS uptake. Koivusalo et al. (2010) also showed that impairing NHEs by removing Na^+ reduced macropinosomes formed and hence confirmed that amiloride inhibits macropinocytosis. There was a 30% drop in the change in fluorescence/absorbance ratio between 20 to 30 minutes. Due to longer incubation time, amiloride caused massive pHc reduction, which inhibited the formation of most membrane ruffles. Consequently, there was a huge decrease in cellular uptake of HPTS and hence massive decline in the change in ratio."

The depth of understanding is clear in the way this student has detailed the specific molecules involved in the process from the literature. In addition, not only have they explicitly and clearly indicated that their findings were consistent with those in the scientific literature, they have also provided a quantitative comparison of the magnitude of change in their experimental outcomes compared with the study they are citing, and used this to form an argument about the relationship between drug

dose and cellular effect. This level of detailed integration of findings with literature is a key learning objective of the curriculum in which this laboratory module was situated, and this example demonstrates the level to which students are achieving the goal of thinking like a scientist. The following example also highlights the depth and detail present in the 15/28 reports judged as achieving a full level of detail for one or both drugs (Fig. 3*B*):

> "Wortmannin also affects the activity of actin polymerization, responsible for membrane ruffling, which is mediated by Rho GTPases that acts on phosphoinositide kinases such as PtdIns(4,5)P_2. An important protein that drives this polymerization by reacting with actin is the WASP/Scar complex. Wortmannin inactivates this complex which prevents the binding to PtdIns(4,5)P_2, actin and another protein complex Arp2/3. It inactivates a small Rho GTPase, Cdc42, which activates the Arp2/3 complex and plays a small role in activating the PI-3 kinase as well (Tolias, Cantley & Carpenter, 1995). It also prevents Rho proteins to be phosphorylated and without the bound GTP, these proteins wouldn't be able to enhance the synthesis of PtdIns(4,5)P_2 which produces PtdIns(3,4,5)P_3 to promote the closure of vesicles from the plasma membrane and ready for the uptake of any bulky extracelluar fluid (Seastone et al., 2001). Overall, an inhibition to these kinases and protein complexes would prevent the aggregation of an actin molecule to the existing growing actin branch which then averts the plasma membrane to extend outwards to form ruffles which produces macropinosomes and eventually to vesicles (Seastone et al., 2001). This may explain why there was an inhibition of vesicle uptake in Hek293 cells with the presence of this drug."

In this particular example, the student clearly indicates that this process "may explain" their findings, but without direct evidence that these actually were the molecules involved they can only speculate on the involvement of these molecules with the support of the scientific literature. Appreciating this fine line between conclusions and speculations is also a key learning objective of the curriculum in which this laboratory module is nested.

STUDENT EVALUATIONS

Upon completion of the practical session after the final reports have been submitted students were asked to provide feedback on what aspects of the overall session they enjoyed, what they learned and what they would change. In general, responses were positive with students enjoying working with mammalian cell lines in a "real-life" situation where they were given "independence of design." Student's perceptions of their learning achievements included acquisition of practical skills (e.g.: "centrifuge, washing cells, pipetting" and "How to write good papers") in addition to reinforcing lecture content (e.g.: "appreciation for the complexity of endocytosis"). Overall, students commented on changing the length of the practical so that time for troubleshooting is included, an aspect of the practical which is now being taken into consideration.

CONCLUSIONS

Overall, the laboratory module described here was designed as a student-directed model of learning, focused on providing students with opportunities to practice and develop their skills in critical thinking within a specific scientific context. It is clear from our reflections, student feedback and the assessment outcomes, that this laboratory module is both feasible on a large scale with early stage undergraduate students, and effective in encouraging students to pose ideas and critically evaluate those in a "real-life" research setting, while gaining a high level of detailed content knowledge.

Acknowledgment— Authors would like to thank Dr Markus C Kerr for helpful discussions and expert advice. Also, we would like to acknowledge all the tutors involved with running of this practical as well as students for all their hard work and helpful feedback.

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