Experimental analysis of cell function using cytoplasmic streaming

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Abstract: This laboratory exercise investigates the phenomenon of cytoplasmic streaming in the fresh water alga *Nitella*. Students use the fungal toxin cytochalasin D, an inhibitor of actin polymerization, to investigate the mechanism of streaming. Students use simple statistical methods to analyze their data. Typical student data are provided.

Key words: Cytoplasmic streaming, cytochalasin, student t-test.

INTRODUCTION

The development of effective laboratory exercises for large first-year university classes can be a challenging task. We describe here a simple laboratory class that requires students to observe cell structure and to analyze some aspects of cell function. The exercise demonstrates the basic principles of experimental technique and analysis of data.

We have run this experiment for 10 years in our first-year cell biology class using standard laboratory equipment. It can easily be completed in 3 hours by very inexperienced students but also provides interest for students with some previous knowledge of biology. In our classes, groups of 14-16 students are led by a demonstrator (teaching assistant) who is typically a graduate student in a biology discipline.

Cytoplasmic streaming is a phenomenon seen in many large eukaryotic cells in which cytoplasmic particles, such as cellular metabolites, are transported continuously along the cytoskeleton, thus maintaining an even distribution of cytoplasmic contents (Lodish *et al*., 2008). Some freshwater algae such as *Chara* and *Nitella* consist of a branching array of elongated cells (internodal cells) connected at nodes (Casanova, 2009). The process of cytoplasmic streaming can be readily observed in the internodal cells using a light microscope (Wessells *et al.*, 1971; Bradley, 1973). Since the cells are elongated and the cytoskeleton is formed of parallel actin bundles running longitudinally, particles stream in a linear manner along the cell. Consequently, the rate of streaming can easily be measured using an eye-piece micrometer and a stopwatch.

The motive force for cytoplasmic streaming is provided by an interaction between the actin cytoskeleton and myosin-coated endoplasmic organelles (Shimmen & Yokota, 2004). The mechanism of streaming can be studied using cytochalasins. Cytochalasins are fungal products that bind to the plus end of actin filaments, block the interaction between myosin and actin and thus block cytoplasmic streaming (Wessells *et al.*, 1971; Bradley, 1973; Collings, Wasteneys & Williamson, 1995).

In this class, students measure the rate of streaming in cells of *Nitella* and determine the effect of cytochalasin D on streaming. Students analyze their findings using simple statistical analyzes and discuss the importance of controls.

Learning Objectives

After completing the exercises presented in this paper, students should be able to:

1. Describe the process of cytoplasmic streaming.
2. Measure the rate of cytoplasmic streaming in the alga *Nitella*.
3. Explain the mechanism of cytoplasmic streaming.
4. Understand the principles of experimental design, the use of appropriate controls and analysis of data.
5. Use simple statistical methods to analyze experimental results.

MATERIALS AND METHODS

Students work in pairs; two pairs form a group of four.

Materials

Each student needs the following personal protective equipment:

- Enclosed footwear
- Safety glasses
- A laboratory coat
- Disposable gloves
- Material Safety Data Sheets (MSDS) for dimethyl sulfoxide (DMSO) and cytochalasin D

Each pair of students need:

- a compound binocular microscope with an eyepiece micrometer;
- a stop-watch;
- a modified microscope slide made by cutting 0.5cm x 2.5cm sections of a standard microscope slide and gluing them onto another slide to create sidewalls (Figure 1);

- a sample of *Nitella* in pond water; *Nitella* is commonly found in freshwater streams and ponds (Casanova, 2009);
- disposable plastic transfer pipettes.

The supervisors need:

- disposable gloves for handling DMSO and cytochalasin D;
- a solution of 200µM cytochalasin D in 2% DMSO kept on ice;
- a 2% solution of DMSO, kept on ice;
- a micro-pipette suitable for dispensing 50µL.

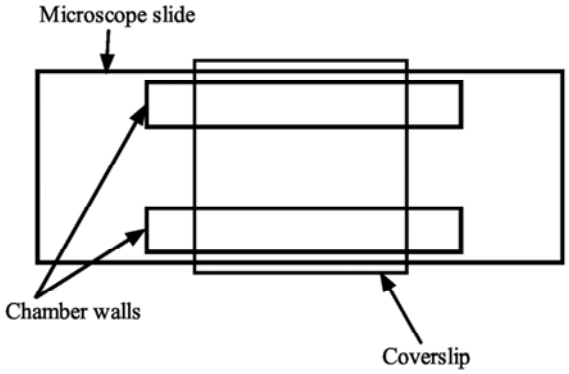

Fig. 1. Diagrammatic representation of the modified microscope slide.

Initial measurement of cytoplasmic streaming

For each pair of students:

A sample of the fresh water alga *Nitella,* containing at least 5 internodal cells, was removed from pond water and placed between the sidewalls of the modified microscope slide. A coverslip was placed over the sample and the chamber filled with pond water (about 300µL filled the chamber). The cells were left for several minutes until streaming resumed.

The cells were observed for the appearance of cytoplasmic streaming with a light microscope, using a 10x objective. One eyepiece was fitted with an eyepiece micrometer. The time taken (in seconds) for a particle to move the length of the micrometer scale (approximately 1mm) was measured 5 times in 5 different cells. Following a discussion on the possibility of cell size affecting the rate of streaming, students selected cells of varying lengths to study and measured the length of each cell using the eyepiece micrometer.

It is important in these experiments that the microscope light is turned off between measurements. In some cases, the rate of cytoplasmic streaming increased slightly as the experiment proceeded. It is most likely that this is attributable to a warming of the incubation fluid by the microscope light. We have not found this to impact significantly on the results obtained, but it can lead to confusion for some students.

Effect of cytochalasin D

Before starting this part of the experiment, teaching staff and students discussed the importance of controls and what would constitute a suitable control when testing the solution of cytochalasin D used in this experiment.

The addition of cytochalasin D was used to study the mechanism of streaming. Cytochalasin D was dissolved in dimethyl sulfoxide (DMSO) to make a 10 mM solution (5mg cytochalasin D in 1ml DMSO). The stock solution was stored at –20°C. The stock solution was diluted with pond water to make a 200µM working solution.

DMSO may readily penetrate skin and so it is essential that gloves are worn when handling the DMSO or the solution of cytochalasin D in DMSO. Only the experienced teaching assistants are permitted to handle the working solution of DMSO to further reduce any potential risk. Technical staff prepared the working solutions of DMSO and cytochalasin D before the class and these were given to the teaching assistants.

For one pair of students/group:

A 50µl aliquot of 200µM cytochalasin D was added to the chamber by the teaching assistants and mixed by gently pipetting up and down (giving a final concentration of cytochalasin D of approximately 30µM). Cytoplasmic streaming was sequentially measured in each of the five cells at 5 minute intervals after the addition of cytochalasin D. After being treated with the drug for 15 minutes, the cells were washed extensively with several changes of deionised water by using absorbent paper to draw the liquid out from underneath the coverslip and then replacing with deionised water. The chamber was then refilled with pond water. Cytoplasmic streaming in the cells was measured in each cell at 5-10 minute intervals for a further 60 minutes.

For the second pair of students/group:

The above procedure was performed in a separate group of cells using 50µL of 2% DMSO in place of the cytochalasin D solution. The results from this pair of students formed the control for the experiment.

Each group then had two sets of data: an experimental group of cells which had been exposed to cytochalasin D and a control group of cells which had been treated in an identical way except that the DMSO solution contained no cytochalasin D.

Analysis of data:

The rates of streaming (in µm/s) were calculated and statistical analysis of the data was performed. The means and standard deviations were calculated for each time-point. Student's t-tests were used for analysis and differences were considered significant when $p<0.05$.

The class coordinator collected multiple sets of results from each session. These were used in a post-lab workshop to demonstrate consistency in the effect

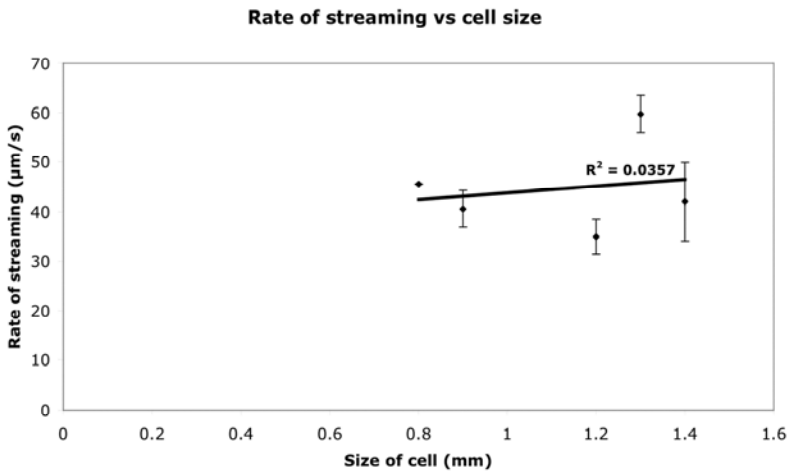

Fig. 2. Rate of cytoplasmic streaming versus cell size (length of cell) in *Nitella.* Results for each cell are presented as mean±S.D. The horizontal line is a linear regression of the means; the slope is not significantly different from 0 ($R^2 = 0.0357$).

of the cytochalasin D on the rate of streaming. There were typically 40-50 different groups each year and the findings were consistent in the vast majority of these.

RESULTS

The results presented here are representative student results.

Effect of cell size on rate of streaming:

There was no consistent difference in the rate of streaming in cells of different lengths from 0.8 to 1.4 mm. (Figure 2). These findings indicate that there is no need for students to find similarly sized cells to carry out this experiment.

Effect of addition of cytochalasin D on the rate of streaming:

The rates of cytoplasmic streaming were measured in 5 different cells of *Nitella* before the addition of cytochalasin D or DMSO and then at 5-minute intervals after the addition of cytochalasin D or DMSO. The cells were washed at t=15 minutes and the rates of streaming were measured subsequently at 5-10 minute intervals (Figure 3).

In cells treated with cytochalasin D, the rate of cytoplasmic streaming was reduced substantially (Figure 3). At 15 minutes after the addition of cytochalasin D, Student's t-test analysis demonstrated there was a significant decrease in the rate of streaming ($p<0.01$) compared with the rate before the addition of cytochalasin D. The cells were washed 15 minutes after the addition of cytochalasin D. The rate of streaming did not change initially but, by 30 minutes after the cytochalasin D was washed from the cells (45 minutes after cytochalasin D was originally added), the rate of streaming had increased and reached a rate similar to the original rate ($p>0.1$).

In cells treated with DMSO there was little change in the rate of streaming over the course of the experiment from time = 0 when the incubation was first set up, through the addition of DMSO, the washing out of the DMSO, to the end of the experiment. Student's t-test analysis showed that the rate of streaming did not significantly vary throughout the experiment; specifically, such analysis showed there was no significant difference ($p>0.1$) in the rate of streaming 15 minutes after the addition of DMSO at a time when cytochalasin D had had a major effect, or at the end of the experiment (45 minutes after addition of DMSO and 30 minutes after DMSO had been washed out of the system, during which time cytochalasin D-treated cells had recovered).

These results demonstrate clearly that cytochalasin D inhibits cytoplasmic streaming in *Nitella* and that DMSO, the solvent for cytochalasin D, has no effect. The results shown here are from one typical student experiment. In many cases, cytochalasin D completely blocked streaming and the recovery from blocking was more rapid than that shown here. It is, however, routine for students to get qualitatively consistent results from group to group and from year to year.

DISCUSSION

This experiment provides a simple and technically undemanding way of meeting the Learning Objectives set out earlier in this paper. First year students find it relatively easy to see cytoplasmic streaming occurring and to measure its rate. The class can be structured in a number of ways depending upon the abilities of the students. If the aim of the class is to allow students to observe and measure the rate of cytoplasmic streaming, then very detailed instructions of all steps to be performed can be given. Alternatively, if the aim is also for students to explore experimental design, teaching assistants can lead small group discussions to allow students to shape the experiment. For example, questioning whether cell size affects the rate of streaming requires cells of different sizes to be measured. A discussion of the importance of sample size for statistical analysis requires students to consider the

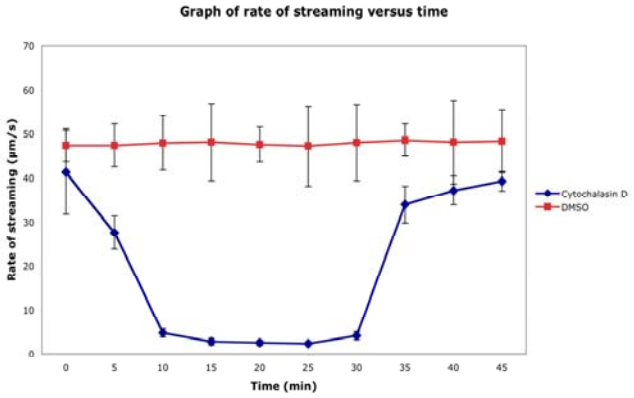

Fig. 3. Rate of cytoplasmic streaming versus time for cells of *Nitella* treated with cytochalasin D or DMSO.

number of cells to be measured. Consideration of appropriate controls can be explored. Students can also determine whether the length of time that the preparation has been set up has any effect upon the rate of streaming and they can analyze their findings using a variety of statistical techniques. We routinely use Student's t test to analyze the results but we have also used non-parametric tests such as the Mann-Whitney U test which enables us to discuss the important difference between parametric and non-parametric tests.

In the above account, we have used cytochalasin D dissolved in DMSO as the effector with an equivalent solution of DMSO as the experimental control. Other cytochalasins (e.g. cytochalasin B) also interfere with actin-myosin interaction while some (e.g. cytochalasin C) have little or no effect (Foissner & Wasteneys, 2007). This option for variability has permitted us to alter the protocol slightly from year to year to avoid the direct passing on of data and discussion from students who have previously completed the unit. This is a common occurrence in our experience, especially between students in student residences. In some years we have changed the alga being studied (*Nitella/Chara*), used cytochalasin B instead of cytochalasin D or used a solution of cytochalasin C, an agent which does not affect the rate of cytoplasmic streaming, in DMSO in place of DMSO.

The use of cytochalasin C in place of DMSO raises the question of whether it is an appropriate control and, if not, what controls should be used. In comparing the effect of cytochalasin D dissolved in DMSO with cytochalasin C dissolved in DMSO, students found that cytochalasin D + DMSO inhibited cytoplasmic streaming while cytochalasin C + DMSO did not. In the ensuing class discussion, nearly all students agreed that they demonstrated that cytochalasin D inhibited cytoplasmic streaming whereas cytochalasin C did not. When they reached this conclusion, we asked the students if they thought that the experiment was adequately controlled. Most thought it was. However, an equally valid interpretation of the data is that DMSO inhibits cytoplasmic streaming but the inhibitory effect is blocked by cytochalasin C but not by cytochalasin D. Therefore, cytochalasin C + DMSO is not an adequate control. We found this to be an effective way of showing students the importance of controls.

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