**Osmosis, Plant Cells, Plasmolysis and Microscopy**

*Concept Review: Osmosis and Plasmolysis*

When a plant cell is **turgid**, the central vacuole of the cell is filled with water and the cell contents within the plasma membrane maintain cell shape by exerting outward pressure (**turgor pressure**) onto the cell wall. The inflexible cell wall exerts pressure back onto the plasma membrane and prevents further uptake of water (Freeman, 2011). When plant cells are placed in a hypertonic solution, they undergo **plasmolysis**, in which much of the water exits the cell via osmosis and the phospholipid-rich plasma membrane pulls inward. The relatively rigid cell wall comprised of cellulose, however, retains its shape. Initially you will make a slide of an *Elodea* leaf under baseline conditions (hypotonic). Then you will observe the leaf cells under very hypertonic and very hypotonic conditions.

**PREDICT**: Predict what will happen to plant cells when placed in baseline, very hypertonic and very hypotonic solutions.

Today you will be making a slide of an intact *Elodea* leaf and observing the leaf cells using the compound light microscope. You will be able to see the cell wall, plasma membrane and chloroplasts and possibly the vacuole (an absence of chloroplasts is a good indication of the rough outline of the vacuole).

**Task 1. Making a wet mount of an Elodea leaf in various conditions**

1. Remove one small leaf from the *Elodea* plant.
2. Place the leaf in the center of a glass microscope slide.
3. Take a plastic pipette and remove some of the water from the *Elodea* dish and place one drop on top of the leaf.
4. Carefully place a plastic cover-slip on top of the leaf, making sure to remove all air bubbles.
5. View the leaf under your microscope using all three magnifications and observe the usual appearance of the cells under normal osmotic conditions on high power and sketch a cell or two in your notebook.
6. Record your observations. What cell structures can you see? Neatly label any visible cell structures. Remember to record magnification and estimate the size of the cell and visible organelles if possible.
7. After observing, remove the slide from the stage.
8. Place a small piece of paper towel on the slide, adjacent to cover slip.
9. The paper towel will wick the water from beneath the cover slip.
10. Add three drops of 1M NaCl solution to the slide by placing 2-3 drops at the edge of the cover-slip and letting it wick under the cover-slip.
11. Wait 5 minutes.
12. Observe the slide again. Note any changes in appearance.
13. Repeat procedure with a fresh leaf using deionized water.

**Task 2. Determining threshold for plasmolysis – an inquiry-based lab**

**A note on inquiry-based lab exercises:** In scientific experiments the answers are not known ahead of time. Scientists have to devise appropriate protocols to test their hypotheses. Even if most or all of the procedures involved are standard techniques, scientists still have to determine what sorts of data they want to collect and how data will be interpreted. For most of the lab exercises during the semester, you will first complete a standard directed lab (where you are given a protocol and question), and then in the second portion of the lab you will ask a new question about the same system and come up with a method to test your question.

**MISSION**: Your task is to determine what concentration of NaCl causes plasmolysis in Elodea leaf cells.

You have now seen what *Elodea* leaf cells look like when placed in a concentrated salt solution; but at what external NaCl concentration do the cells begin to lose significant amounts of water?

Consider the following when designing your protocol:

- How will you determine when a cell has experienced plasmolysis? What will it look like?
- How will you determine the difference between plasmolyzed cells and flaccid cells?
- How long will you allow the cells to sit in the salt solution before making your observations
- How many leaf preparations will you make at each concentration?
- How will you account for variability in leaf size/shape?
- How will you record data?
- How will you present your results graphically?

Get ready for the experiment:

- Discuss your strategy with your lab partners
- Jot down a protocol in list form
- Make data recording tables

**STOP**: Show your protocol to your lab instructor. Once you've discussed your protocol with your instructor, complete the experiment with your lab group.

**Post-lab Discussion and Analysis**

Each lab group should prepare to share the following information with the rest of the class:

- What concentration of NaCl triggered plasmolysis
- What your methods were for determining the point of plasmolysis

**Include the following** in your lab notebook

- Clear, repeatable protocol for your experiment – may be written in list format
- All data tables and graphs (label axes!) and explanatory captions
- A clear indication of what concentration of NaCl caused plasmolysis in your lab group's experiment
- Why you think different groups had varying estimates of concentration
- What physiological implications you think changing salinity in the water has for this plant and for other aquatic organisms
- What would have happened if you put an animal cell in hypo- and hyper-tonic conditions? Contrast with plant cells.