**Cell Membrane Permeability**

**Guide to Instructors**

**Introduction**: Because membrane permeability is such a nebulous process, this lab is designed to encourage students to link concepts in chemistry to concepts in biology, forming hypotheses about whether chemical compounds will be able to cross a red blood cell membrane. They also have the opportunity to think about osmosis, because if the solutes in solution are too big to cross the membrane and at a concentration greater than the concentration of the cell interior, then water will move and they will be able to see the cells shrink. This aspect of the lab is helpful to students to understand the distinction between iso-osmotic solutions and iso-tonic solutions.

There are a number of factors that influence whether a material will cross a biological membrane. Because of the lipid bilayer, nonpolar materials (hydrophobic) are more likely to cross than polar (hydrophilic) materials. Because of this feature of membranes, charged molecules are extremely unlikely to cross. This is a great opportunity to discuss functional groups and polarity, such as hydroxyl groups, because you can vary the number of hydroxyl groups on a molecule and affect the ability of the material to cross the membrane. Size also plays a role, because the smaller the molecule, the more likely and faster it will be able to get through a membrane. Gasses such as oxygen and carbon dioxide, however, readily cross the membrane, as well as water.

**Standards covered using the lab**: Below is an outline of the purposes of the lab and a partial list of standards completed with the lab. The list may change depending on which aspects of the lab you choose to focus on.

1. Scientific Practices in the Lab
	1. Model Lesson using SC.912.L.18.3 (Describe the structures of fatty acids, triglycerides, phospholipids, and steroids. Explain the functions of lipids in living organisms. Identify some reactions that fatty acids undergo. Relate the structure and function of cell membranes) and SC.912.L.14.4 (Compare and contrast structure and function of various types of microscopes.)
	2. Specifically we are developing a model lesson that incorporates both standards to illustrate a simple, inexpensive lab activity. It is essential that students understand microscope function and standard SC.912.L.18.3 is difficult for students to grasp.
	3. We will be modeling the lesson using the SM2 lesson template and will specifically hitting on:
		1. Hook/Motivation for lesson – may be an application problem
		2. Use of multiple representations
		3. Hands-on activities
		4. Use of discovery learning techniques – give students opportunity to make and test conjectures and to learn from each other
		5. Assessment to determine if students have mastered the objective (Assessment can take place throughout the lesson.  It can be formal or informal.)

**Materials Needed**:

1. Compound microscopes (can be done with one scope and a projection camera as a demo)
2. Slides
3. Cover slips
4. Stopwatch
5. Red blood cells (we used aseptic blood designed for blood typing, Carolina Biological order number 700168; however, the lab can be done with any kind of blood (I have also used defibrinated horse blood)
6. Transfer pipets
7. Very small containers for solutions; eyedropper bottles to be passed around the lab would work too, and would reduce the need for transfer pipets
8. Small pieces of kimwipes or paper towels, to use to pull the solutions under the coverslips to mix with the blood sample
9. Solutions below, plus distilled water
	1. Solution preparation: The following solutions will need to be prepared in the week prior to lab. Instructions are provided for 0.5 liters of stock solution, which would go a long way considering each student or student group would only need about 5 ml of each solution for even repeated experiments.

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| --- | --- | --- | --- | --- |
| **Concentration** |  **Compound** | **Mol. Wt.** | **M/L** |  **\_\_\_g/0.5 L** |
| 0.3M | Methanol | 32.04 | 0.3 | 4.81 |
| 0.3M | Sodium Laureth Sulfate (dishwashing liquid) | 420.0 |  |  |
| 0.3M | Ammonium Sulfate | 132.14 | 0.3 | 19.821 |
| 0.3M | Acetic Acid (vinegar) | 60.05 | 0.3 | 9.0075 |
| 0.15M | NaCl (table salt) | 58.44 | 0.15 | 4.383 |
| 0.3M | NaCl | 58.44 | 0.3 | 8.766 |
| 0.3 M | Ethylene Glycol (antifreeze) | 62.07 | 0.3 | 9.3105 |
| **0.3 M** | **Glycerol (glycerine – available in bakery aisle)** | **92.09** | **0.3** |  |
| **0.3 M** | **Isopropanol (rubbing alcohol)** | **60.1** | **0.3** |  |
| 0.3 M | Dextrose | 180.16 | 0.3 | 54.05 |
| 0.3 M | Sucrose | 342.3 | 0.3 | 102.7 |
| 0.3M | Butane | 58.12 | 0.3 | Remove from lighter |

**Procedure**: Students should work in pairs for the exercise, to get the maximum benefit from discussing their hypotheses based on the molecules to be studied and from comparing their observations of the behavior of the red blood cells.

1. Instructor should discuss prior to laboratory experience membrane structure and function, and what molecules cross without help vs. which would need transporters and/or ATP to cross.
2. Students should then be handed the sheet of chemical structures, and be asked what the behavior of these substances in water (dissolved around a cell) would be. Would they ionize like a salt or an acid or base? Are they polar or non-polar? Would they dissolve in water? What functional groups do they have? Would they dissolve the membrane itself? If size might be important, then a good group to look at would be the sugars, which are for the most part different numbers of C-H2O atoms connected together. After a discussion to introduce this part of the lesson, students should explicitly write out their hypotheses as to what they think will happen to the cell (cell will shrink, cell will swell, cell will burst, membrane will dissolve, cell will not change) and why (molecules are: polar/charged and won’t be able to cross; non-polar/hydrophobic and will cross; molecule will cross and will cause cell to burst; etc.)
3. Then students will need to test their hypotheses. This works best with a projector showing a sample of red blood cells (RBC’s) that don’t have any solutions on them to serve as a visual control for what students are seeing through their microscopes. To test their hypotheses, students will need to:
	1. Obtain a set of microscope slides. Place a drop of blood on the center of a slide, and gently place a coverslip on it. Gently treat the RBC’s because the membranes will rupture if they get shaken too violently, heated up to far, etc.
	2. Place the slide on the microscope, and focus at the lowest magnification, then and the next level, and then at the highest magnification. RBC’s are very small, so this is best done at the highest magnification.
	3. While focused at the highest magnification, with one lab partner watching, the other one should place a drop of the solution to be tested on the edge of the coverslip. **To get the fluid to move under the cover slip and mix with the blood cells, a kimwipe or a paper towel can be used on the opposite side of the coverslip to wick the fluid under it (great demonstration of the effect of capillary action – you are basically using the kimwipe/paper towel as a straw to pull the fluid under the coverslip!).**
	4. Students should then describe their observations. RBC’s are plate shaped, with a clear indentation on one side in their normal state, so if they look very round (like a ball) or shriveled, then water or solute has either moved into them or out of them. If you see clear cell membranes in pieces, you know that the cells have broken open (lysed) or been dissolved (in this last case, the membranes tend to congregate all together in clumps surrounded by the material that dissolved them).
	5. Students should run each solution at least twice, with an opportunity for each student in the group to watch the RBC’s respond to the chemical solution.
4. Cleanup and storage: Coverslips and slides can be washed thoroughly with water and re-used in subsequent classes. Solutions except for the sugars (dextrose and sucrose) can be stored capped at room temperature. Sugars can be stored capped in the refrigerator for weeks.