The Effect of Membrane Permeability of Plant Cell Wall Inspired Structures

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Abstract

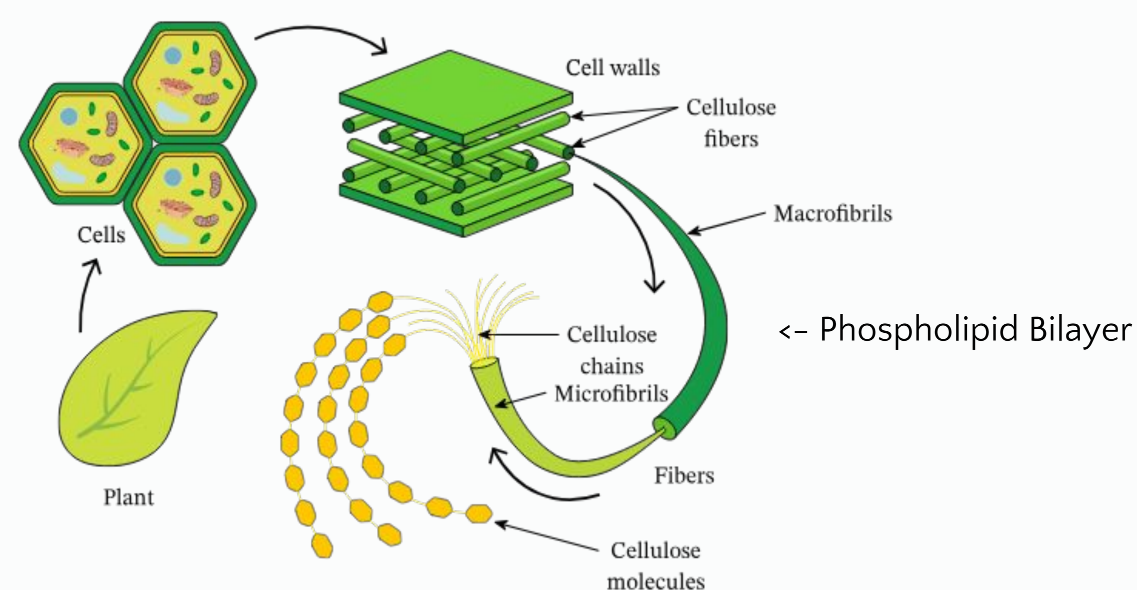
In this poster we conduct experiments to determine whether we can replicate the barrier properties present in the plant cell wall using ordinary cellulose paper and purified solutions of lipids. To determine the barrier properties we develop a membrane diffusion cell system that allows us to track the movement of a small fluorescent FITC molecule across the membrane. We also experiment with various setups such as traditional falcon tube transfer and hydrostatic pressure. After testing diffusion, we used the fluorescent plate reader to detect the fluorescence levels. We confirmed the results with the calibration curve. For visual insight, we made a bar graph, allowing us to continue our lipid studies. However, higher fluorescence in filter-paper DOPC solution reveals unsuitability due to larger pores, unlike plant cell membranes. We find that the lipid bilayer on ordinary cellulose paper may be leaky and thus expect to continue a following experimental phase which is to test tracing paper because we assume that the pores will be smaller, and would work better to support the lipid bilayer structure required to block diffusion of small molecules.

Introduction

The phospholipid bilayer is a fundamental component of cell membranes, consisting of a double layer of phospholipid molecules. This arrangement forms a selectively permeable barrier that controls the movement of substances in and out of the cell.

To mimic the structure of the plant cell membrane we combined together a mixture of Di water and nanocellulose to create a clear, cloudy solution that would eventually dry to the texture of thin sheet paper. Out of the tested weights of the nanocellulose paper, we found the most success within the range of 0.1-3% weight. Once the paper was fully dried, it was used in a timed two-falcon tube set-up experiment to test the permeability of the paper using Di water and food coloring as an indicator.

Fluorescein Isothiocyanate or FITC, an orange-yellow powdered fluorescent dye, commonly used in molecular biology as a labeling compound for antibodies and proteins, was found to be the best indicator for our group. The ideal concentration of the FITC solution was found to be 0.1g/mL. To create a model that best replicated the plant cell membrane, we created a vertical set up with one falcon tube consisting of our FITC solution and one consisting of water with tracing paper in between the two acting as the permeable layer. Then scanned under a fluorescent plate reader to compare data from different concentrations. It turns out that the setup is invalid because the gravity will affect the amount of solution and the speed the solution goes through. That means we have to come up with another setup, we chose to apply hydrostatic pressure in our setup to make it fair and consistent.



<- Phospholipid Bilayer

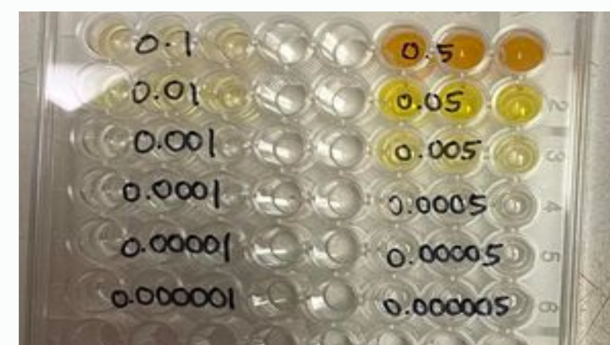
Methodology

Dilution

Formula: V1C1=V2C2

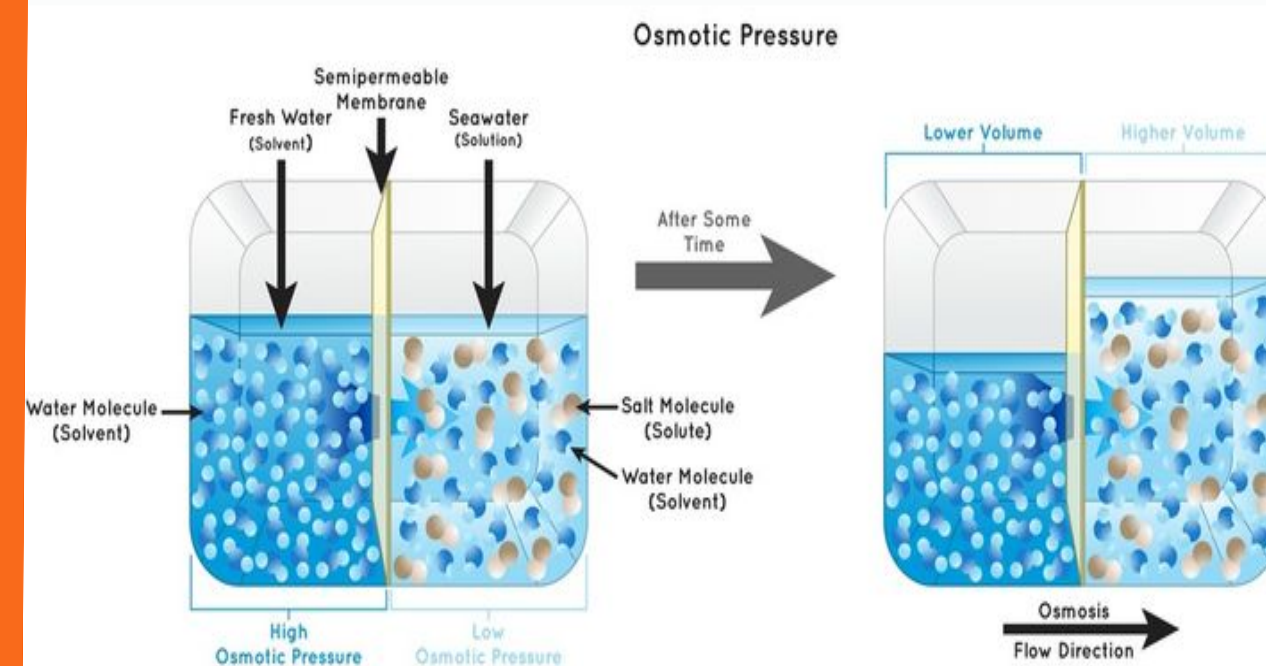
V is the volume of the sample and

C is the concentration, We found that the ratio should be 1:10 for example, if we start from 0.1 and we want 0.01, then it should be 1 μL of 0.1 and 10 μL of water



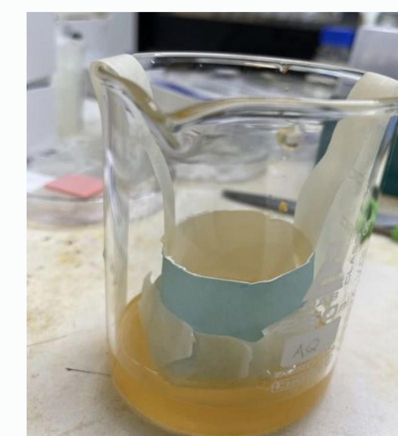
^ what our set of diluted wells looked like

^ solutions we started with



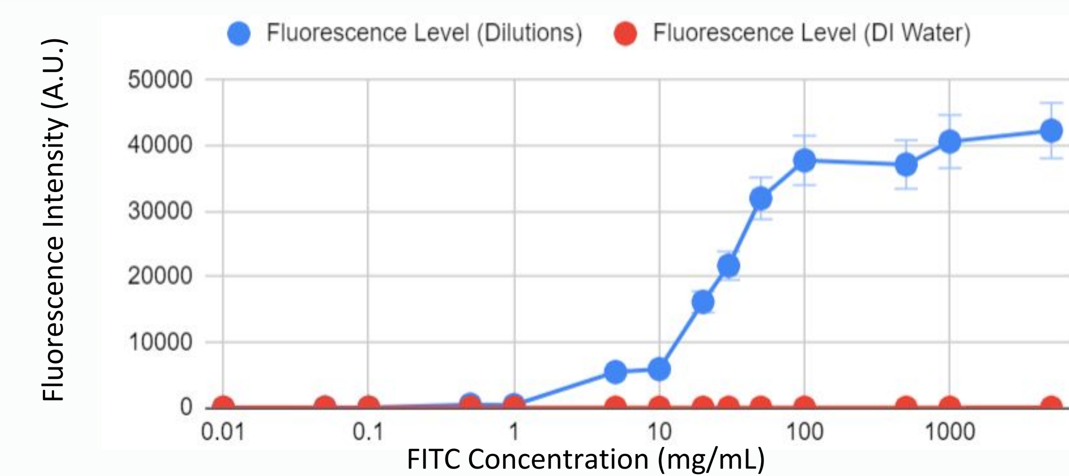
Hydrostatic Pressure setup through a membrane

A pressure exerted by a fluid (in this case, FITC solution) due to the weight of fluid in stationary state



Starting with using a paper cup and a beaker, the level of liquid has to be the same in both containers, Both opening of the smaller container has to be open, so the cup does not touch the bottom of the beaker. Even though we get more surface area from the cup setup, we want to use the same amount of solutions everytime to keep it consistent, which means that we have to keep the smaller cup at the same level. To solve this problem, we switched to the other setup which has a clip on the top of the smaller cup, it makes sure that the height will be the same every time.

Data



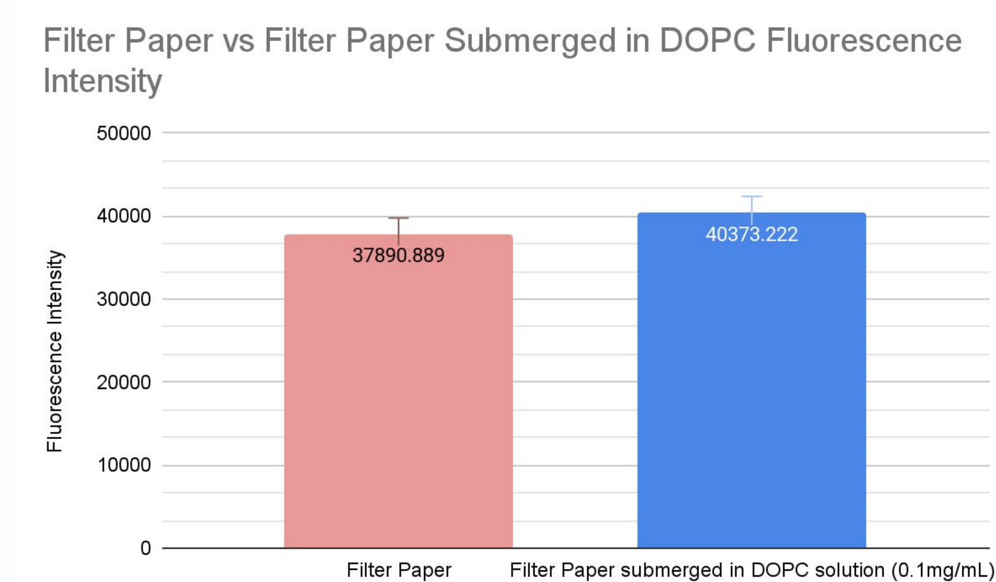
Graph 1 - Calibration curve we put together after using the plate reader to read the fluorescence level of the solutions we got after diluting

Sample Label	Data - How High the Fluorescent level is
Filter Paper Sample 1	36188, 37273, 37996 Avg: 37152.333
Filter Paper Sample 2	35402, 38467, 38726 Avg: 37531.667
Filter Paper Sample 3	39480, 39026, 38460 Avg: 38988.667
Filter Paper submerged in DOPC solution (0.1mg/mL) sample 1	40162, 38123, 40435 Avg: 39573.333
Filter Paper submerged in DOPC solution (0.1mg/mL) sample 2	40925, 40952, 40983 Avg: 40953.333
Filter Paper submerged in DOPC solution (0.1mg/mL) sample 3	39623, 41396, 40760 Avg: 40593

Table 1 - Table of data we collected from reading the solutions of different samples after diffusing for an hour.

Results

From the calibration curve, we will use the beginning of the plateau on the right side, which is 100 mg/mL or 0.1 g/mL FITC solution.



Graph 2 - FITC Paper vs FITC Paper Submerged in DOPC (0.1mg/ml) for Fluorescence Intensity. Error bars of standard deviation included.

From the bar graph, we can compare and see the results fit what we expected from the calibration curve. It should be around the top of the curving part which is around 38000 to 40000. Since the results were stable for the 3 samples, we decided to move on and use lipids. As we can see on the bar graph, the results of the filter paper submerged in DOPC solution is higher, which is not ideal, so this proves that filter paper won't be the best choice for working with lipids.

Conclusion

Since we know that from the results the fluorescence level is very high, the pores are big in filter paper. This makes sense because the purpose of filter paper is to filter and is it easier for the liquids to go through when the pores are bigger.

What we want is that the pore should be mimicking the small openings in the plant cell/ plant cell membrane, but the membranous pores are not as big as the pores of filter paper due to the plant cell wall being sturdy. Therefore, using filter paper is not a ideal choice to work with.

The higher fluorescence levels exhibited by the filter paper-submerged DOPC solution (38,000 to 40,000) show its poor suitability for studying lipids because of its large pores in contrast to smaller pores resembling plant cell membranes. It is important to choose appropriate structures.

This leads to the next step of our lab, working with tracing paper. We assume that tracing paper will better support a lipid bilayer that we expect could control the diffusive transport of the FITC molecule similar to the plant cell wall.

Future Studies

- Test permeability of tracing paper
- Dilute fluorescein to find the best ratio and repeat what we did to filter paper on tracing paper
- Using fluorescence microscope to check the tracing paper
- Try out other kinds of paper like nanocellulose

Bibliography

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