Exercise 2: Diffusion and Permeability Measurements

Transport in Biological Systems

Fall 2015

Overview

In the first section of this course, you will develop your ability to manipulate and understand key approaches to diffusive processes. We will start by considering a widely-used experiment to measure the permeability of endothelial cells to macromolecules, first in steady state and then dynamically.

One of the many roles of endothelial cells lining blood vessels is to regulate the passage of molecules (and white blood cells) between the blood and surrounding tissues. During inflammation, cytokines are released by the injured cells. This results in endothelial activation as well as an increase in endothelial permeability. Endothelial permeability is a function of both cell-matrix adhesion and also cell-cell adhesion, and appears to be regulated by changes in the cytoskeleton. Thus, defects in molecules involved in adhesion or cytoskeletal regulation can cause changes in permeability. For example, loss of the Ena/VASP family of proteins in mice is embryonic lethal and these mice show severe edema during development, which appears to be due in part to increased endothelial permeability (C. Furman, A. L. Sieminski, *et al.* Ena/VASP is required for endothelial barrier function *in vivo*. **J Cell Biol**, 179(4):76175, 2007).

Reading Assignment

Read Chapter 6 in *Transport Phenomena in Biological Systems* by Truskey, Yuan, and Katz.

Steady State Solutions for 2 Membranes

To investigate the molecular-level mechanisms underlying permeability maintenance and regulation, various permeability assays are used. The simplest one is shown in Figure 1. In this setup, endothelial cells are grown on a porous membrane in cell culture media. After they cover the whole membrane, a labeled

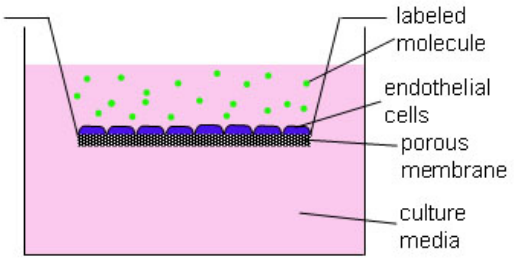

Figure 1: **Experimental setup for endothelial permeability measurements.**

molecule is placed in one chamber at a known concentration and its concentration in the other chamber is monitored over time. Fluorescently-labeled dextran is frequently used because it is inert and can be purchased in a variety of molecular weights. Assuming there is 1) no convection (assured by making sure the liquid levels are the same in top and bottom) and 2) no active transport of molecules through the body of the endothelial cells, this process is a purely diffusive process and we can calculate a permeability from measurements of fluorescence in both baths. In practice, we measure the permeability of the membrane alone and then with cells on it. Thus, for the whole system of cells plus membrane, we calculate a so-called "effective" permeability. What we want is the permeability of the cells alone. Here you develop the equations to describe this.

In class we discussed how to describe the steady state concentration profile within a single membrane. In this case, it turns out that the cells and the membrane can be treated as two membranes adjacent to each other. Now, using the same approach as we did in class and as seen in Chapter 6 of Truskey:

1. Draw and label a schematic of the system (including key parameters and a coordinate system). We are just interested in the membranes, which are in contact with the two baths.
2. Write the governing equations for the concentration profiles within the membranes.
3. Take the first step in writing a general solution for the governing equations (if you didn't do that as part of step 1) assuming the concentrations in the chambers are constant, that the chambers are well-mixed, and that steady state has been reached in the membrane (we'll consider time-dependent solutions shortly).
4. Write down the boundary conditions (Hint: Based on the previous step, you should know that you need 4 of them!)
5. Finish your solution, solving for unknowns.
6. Comparing your solution to the single membrane solution, write an expression for the effective permeability in terms of parameters related to both membranes.

Time-Dependent Solutions for 2 Membranes

Thus far, we have been considering only the membranes and assuming the concentrations in the chambers are constant. The reality of this experiment is that they are not (since you monitor the changing concentration in the bottom chamber). It is usually assumed that the relatively thin membrane comes to steady state very fast relative to the rate of change of concentrations in the chambers, also known as the quasi-steady state assumption. We also typically assume the baths are still well-mixed. The goal of this section is to assess whether the quasi-steady state assumption is reasonable. That is, do the membranes come to steady state faster than there is a small change in the bath concentrations?

Things to think about:

1. You are to consider the changing concentrations in the 2 chambers and develop an analytical solution for the concentration in the baths in time.
2. In these experiments, the chamber volumes are typically different so your solution should account for 2 different volumes.
3. Also, please be aware – this is one of those places where you need to pay attention to moles, not just concentration.
4. You will solve for the concentrations of the baths as a function of time, using the intitial conditions. You should get an expression relating the two baths.

5. In doing so, the transfer of material from one bath to the other should clearly depend upon the properties of the membrane so it should appear in the solution.
6. Write your final solution to describe the concentration in the bottom bath (which initially has nothing in it) as a function of time.
7. Note that if the volumes in the two chambers are the same, this should look a lot like the solution from section 6.8.4 in the book! Use this general approach to guide yours.
8. Use your understanding of this problem to set up a time-dependent COMSOL model of this system. Assume the partition coefficient is one.

Finally, let's put this all together to answer some questions.

1. About how long does it take for two membranes (cells and membrane) to reach steady state? What about the membrane alone? You may wish to take into account some useful experimental details from experiments by Furman, *et al.* including:
   (a) the permeabilities of a membrane and endothelial cells plus a membrane (1 cm^2)were $4.5x10^{-3}$ cm/s and $1.8x10^{-3}$ cm/s, respectively,
   (b) the top and bottom chamber volumes were 525 μl and 1475 μl, respectively,
   (c) the initial concentration of 70 kDa Texas red dextran in the top chamber was 2 mg/ml,
   (d) the experiment was performed for 4h, and
   (e) the rate of dextran transfer between chambers was 5.0 and and 2.1 $\mu g/h$ for the membrane only and cells+membrane, respectively (A.L.S., unpublished data).
   (f) You can assume that the cells are 2 μm thick and the membrane is 10 μm thick.
2. Now, How long does it take to see a significant change in the chambers?
3. How do the relative time scales compare? Is the quasi-steady state assumption reasonable??

Objectives and Deliverables

The objective of this course is for you to apply concepts and skills in modeling and simulation to problems in biological transport. The objectives of this particular exercise are to gain practice setting up diffusion problems and manipulating the governing equations analytically and then to further analyze the problem using COMSOL. I recommend that, as an individual, you develop your knowledge and abilities in all aspects of the course. Towards this objective, I believe that there is tremendous benefit in working with other people. For this exercise you should work out your own solutions but you are permitted to work collaboratively with other students.

We will work on this problem in class on September 24th. Your results are due in class on October 5th. For the steady-state analytical section, please turn in any derivations you generated (hand-written is fine). Do not forget to check that your answer makes sense. For your time-dependent work in COMSOL, turn in a brief write up about your findings including any relevant graphs or figures.