# Assignment 9: Cell Trafficking and Molecular Transport

## Cell and Tissue Engineering

## Problems

1. Understanding and analyzing molecular transport mechanism is necessary when designing basic cell culture experiments and perfusion of biomaterials using a bioreactor. Oxygen delivery to cells in culture can by modeled using Fick's Law. Transport of oxygen from the gas phase ($P_g$) to the cell phase ($P_c$) is driven by the oxygen gradient across the height of the medium. The rate of oxygen uptake by cells however can be modeled with Michaelis-Menten kinetics (what we use for facilitated diffusion) (from $P_c$ into the cell).
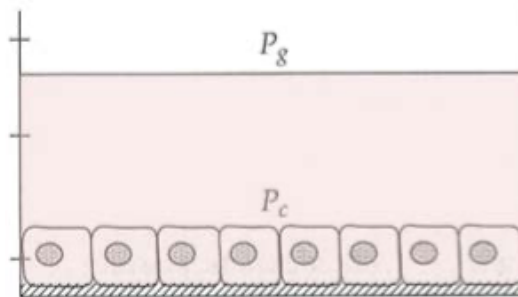


   a) In lecture, we graphed dn/dt as a function of dC/dx for simple diffusion. What is the slope of this line? What do increases or decreases in the slope mean biologically?

   Fick's law: dn/dt = P x A x dC/dx

   Where:
   - P: permeability
   - A: area of the cell membrane where diffusion is occurring

   Since P, and A are constants the rate of diffusion varies linearly with the concentration gradient and dn/dt vs. dC/dx is a line with slope: P x A.
   An increase (similarly decrease) in the slope directly relates to an increase (decrease) in (P x A) which could result from a significant increase (decrease) in P, A or both. Biologically if permeability or surface area of the cell membrane is greater (lesser), the rate of diffusion of the simple diffusion is increased (decreased).

Simple diffusion differs from facilitated diffusion which is subject to saturation and generally follows Michaelis-Menten kinetics.

b) Now assume the concentration gradient is a constant. How does the rate of diffusion (dn/dt) change with the surface area of the cell and the permeability of the diffusing molecule? Graph dn/dt as a function of A or P and describe the function.

Given a constant concentration gradient, $C_0$; as we saw in part a, the rate of diffusion changes linearly with a change in permeability P, or area A. We can then express the rate of diffusion; given a constant concentration gradient to a change in P or A:

$dn/dt = K_1 \times dP/dx$ and $dn/dt = K_2 \times dA/dx$

where $K_1 = f(C_0, P)$, $K_2 = f(C_0, A)$ $K_1, K_2$ = constants

The two plots below show a linear relationship between a change in rate diffusion and a change in A or P.
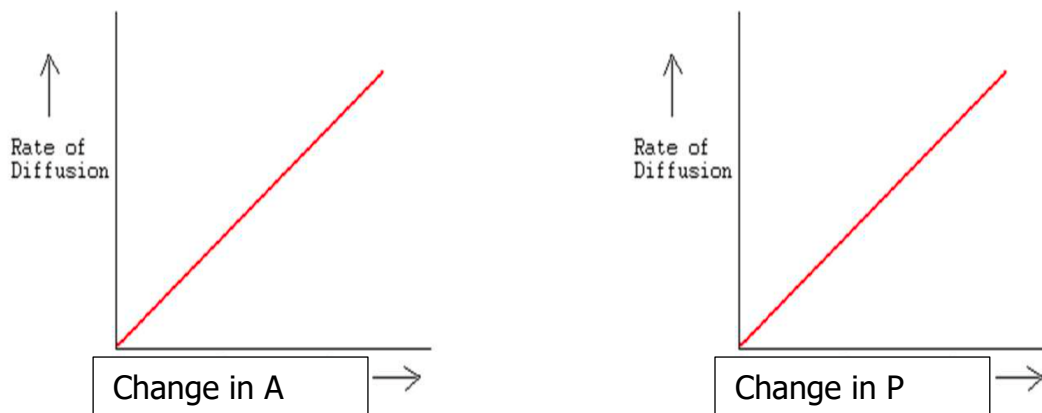


2. Describe 3 principles of bioreactor design used in bioreactors for creating functional tissues – one of your three may be from Dr. Grayson's lecture (improved mass transfer or biostimulation), and the other two you will need to independently research. In each description explain what the principle is, why it is important and some ways that bioreactors are being designed to meet that principle.

- **The perfusion bioreactor**: is a modified version of a Petri dish, containing up to 6 wells where the scaffolds sit. The wells are arranged radially. When the culture medium enters at the center of the system, it is distributed to channels leading to the wells along the interstitial spaces of the porous scaffolds; which contains seeded

cells (e.g., bone cells). It follows turbulent flow paths controlled by the architecture of the scaffolds; exposing the cells to shear stress. The medium is then recycled, reoxygenated and reinjected back into the bioreactor. With this design, the bioreactor provides convective flow through the entire system improving nutrients, oxygen delivery through all the cells within the construct. Number of cells cultured in this bioreactor compared to a static bioreactor; doubles; and depending the flow dynamics form increasing complex tissues.

- **Bioreactor to support long-term culture of lung tissue**: Petersen et al. (Petersen et al.) have developed a bioreactor that allows in vitro culture of a whole rodent lung for a week. In vivo, breathing is accomplished via negative pressure ventilation which is obtained in the bioreactor, using a syringe pump withdrawing air from the main bioreactor. This negative pressure is compensated by medium flowing into the lungs through the trachea from the tracheal reservoir. For exhalation, the same pump pushes air back into the main bioreactor, causing medium to flow back into the tracheal reservoir. In the "loop" configuration, the medium follows a different path during inhalation and exhalation, delivering fresh medium with each breath. Passive vascular perfusion of medium in and out of the lung vasculature enable survival and differentiation of endothelium.

- **A Rotating-Shaft bioreactor (RSB) system for two-phase cultivation of tissue-engineered cartilage** (Chen et al.): consists in a rotating-shaft bioreactor connected to a medium reservoir for medium perfusion and a $CO_2$ incubator for gas perfusion. In the reactor, chondrocyte scaffold constructs are attached to needles joined to a shaft. Half of the reactor is filled with medium. By rotating the shaft, the scaffolds are alternatively exposed to air and medium. The medium reservoir is stirred and temperature is controlled at 37 $^0$C. A two-stage culture is necessary to have the optimal density in both GAG and collagen contents which consists in maintaining a 10-rpm speed in the first 3 weeks to 2-rpm in a final week. RSB confers efficient oxygen transfer and periodical mechanical stress to modulate chondrogenesis and growth of tissue-engineered cartilage.

3. In the paper Engineered cell homing, by Sarkar et al. researchers describe a new method to enhance homing of mesenchymal stem cells (MSCs) to inflamed tissue. In 250 words or less please compare this method to adoptive cell therapy. What are the pros and cons of each?

Adoptive cell therapy (ACT) has been successful in tumor regression (50% of patient with metastatic melanoma (Rosenberg et al.)), and eradication. In ACT, a small number of a patient's own T lymphocytes; lowering the risks of immune response; with the appropriate properties are expanded in the lab. A chimeric antigen receptor; which helps the T cells to attach to a specific cancer cell antigen, is added. Prior to be

reinjected, the patient can be conditioned through lymphodepletion to increase infused cell persistence. ACT technics challenges are:

- It is personalized for each patient slowing down development process and delaying treatments for patient in critical needs.

- Target selection is difficult with risk of insufficient targeting efficacy or off-target effects (Magalhaes et al.).

- It requires rigorous quality control during production, and choice of cytokines during cell culture is critical for T cell potency in vivo (Magalhaes et al.).

- Cells can persist for a long time in the host requiring long patient monitoring.

Mesenchymal stem cells (MSCs) are characterized to home towards cancer cells. MSCs can be modified to over express cytotoxic proteins against tumors after specific homing. Mediated cell rolling with adhesion ligand can enhance MSC homing (Sarkar et al.). However, MSC integration with inflamed endothelial cells and migration mechanisms are not fully understood (Vicinanza et al.). Its role towards cancer cells, is controversial, favoring metastasis, promoting drug resistance or counteracting cancer expansion (Vicinanza et al.). Compared to ACT, manufacturing requirements for clinical grade production have yet to be defined and honed.

References:

Chen, H. C., et al. "A Novel Rotating-Shaft Bioreactor for Two-Phase Cultivation of Tissue-Engineered Cartilage." *Biotechnology Progress*, vol. 20, no. 6, Dec. 2004, pp. 1802–09. *DOI.org (Crossref)*, https://doi.org/10.1021/bp049740s.

Magalhaes, Isabelle, et al. "Facing the Future: Challenges and Opportunities in Adoptive T Cell

    Therapy in Cancer." *Expert Opinion on Biological Therapy*, vol. 19, no. 8, Aug. 2019,

    pp. 811–27. *DOI.org (Crossref)*, https://doi.org/10.1080/14712598.2019.1608179.

Petersen, Thomas H., et al. "Bioreactor for the Long-Term Culture of Lung Tissue." *Cell

    Transplantation*, vol. 20, no. 7, Aug. 2011, pp. 1117–26. *DOI.org (Crossref)*,

    https://doi.org/10.3727/096368910X544933.

Rosenberg, Steven A., et al. "Adoptive Cell Transfer: A Clinical Path to Effective Cancer

    Immunotherapy." *Nature Reviews Cancer*, vol. 8, no. 4, Apr. 2008, pp. 299–308.

    *DOI.org (Crossref)*, https://doi.org/10.1038/nrc2355.

Sarkar, Debanjan, et al. "Engineered Cell Homing." *Blood*, vol. 118, no. 25, Dec. 2011, pp.

    e184–91. *DOI.org (Crossref)*, https://doi.org/10.1182/blood-2010-10-311464.

Vicinanza, Carla, et al. "Modified Mesenchymal Stem Cells in Cancer Therapy: A Smart

    Weapon Requiring Upgrades for Wider Clinical Applications." *World Journal of Stem

    Cells*, vol. 14, no. 1, Jan. 2022, pp. 54–75. *DOI.org (Crossref)*,

    https://doi.org/10.4252/wjsc.v14.i1.54.

## Rubric

| Question | Component | Total Point Value |
|---|---|---|
| 1 | a | 4 |
| | b | 4 |
| 2 | | 12 |
| 3 | | 10 |
| | Total Points | 30 |