Preparation of Biomolecular Gradients on Patterned Hydrogel Surfaces

*Joshua Lewis, Fereshtehsadat Mirab, Dr.Shereen Majd**

UNIVERSITY of HOUSTON
CULLEN COLLEGE of ENGINEERING
Department of Biomedical Engineering

Introduction:

➢ ***Significance of this research:***

Gradients of chemotactic biomolecules, present in extracellular environments, have been shown to provide biochemical cues needed to initiate cellular migration. If these cellular response mechanisms to chemotactic gradients can be reproduced within an in-vitro setting it can lead to substantial implications on regenerative medicine studies involved in cell guidance and differentiation.

➢ ***Goals:***

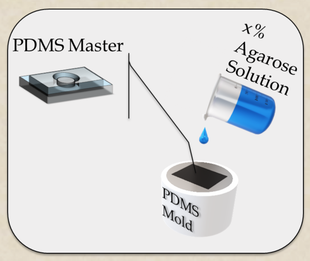
In this study, our lab aims to develop a method that:

- ❖ Yields predictable time dependent molecular gradients on patterned hydrogel stamps
- ❖ Investigates reproducibility of fabricated molecular gradients through agarose hydrogels
- ❖ Examines the effect that changing pore size has on molecular gradient profiles within agarose hydrogels.

➢ ***Why use this method?:***

- ❖ Materials used are relatively inexpensive
- ❖ Versatility inherent within the design of hydrogels
- ❖ Potential use in direction of cell growth/migration using chemotactic molecular gradients in cell culture.

Methodology:

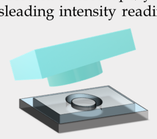


Gel preparation using replica molding:

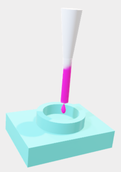
1. Create PDMS Master with desired design for hydrogel stamp and place within PDMS mold.
2. Calculate the necessary amount of agarose solute and DI H_2O needed for specific (w/v) gel concentration.
3. Heat is then added to mixture for thermodynamic gelation of agarose solution.
4. Heated solution is transferred into the PDMS Mold.
5. Poured solution is then degassed using vacuum pump.
6. Solution is allowed to gel for ~45 minutes at 4° C.

Hydrogel Removal & Inking procedure:

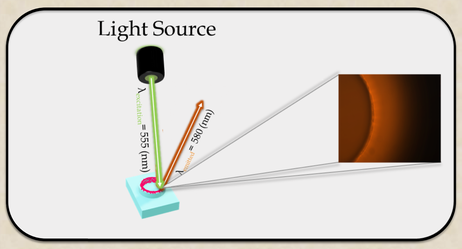
Step 1: Gel Removal
Gel is carefully removed from PDMS master. Improper removal of gel can lead to deformation in gel which causes an edge effect that, when imaged using the fluorescent microscope, yields misleading intensity readings.



Step 2: Gel Inking
Approximately 3µL of Tetramythlrhodamine (TMR, 70,000 kDa) is applied directly to the center of the hydrogel.



Step 3: Allow for molecular diffusion
The TMR absorbs through the surface of the well and begins to diffuse radially through the porous matrix of the gel. As time progresses, the gel will consist of different gradient profiles as a result of this radial diffusion.



Quantitative Fluorescence Imaging:

The diffused TMR molecules, when excited by a light source with the excitation wavelength of 555 (nm), will emit an orange fluorescent light with a wavelength of 580 (nm). The intensity of these emissions that are then recorded and quantified by the microscope.

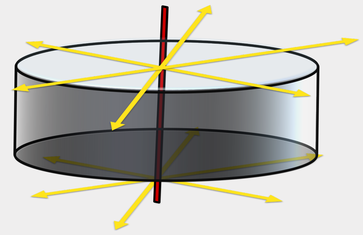
Results:

Diffusion of Molecules Theory:

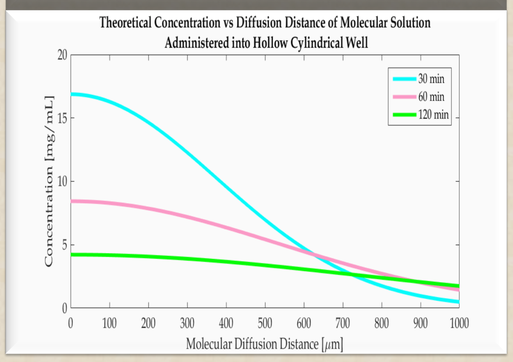
Fick's Second Law:

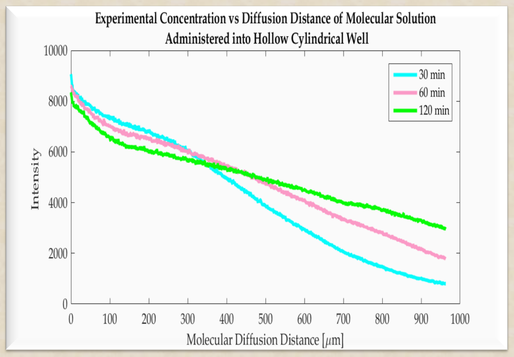
$$C(r,t) = \frac{Q}{4\pi Dt} e^{-\frac{r^2}{4Dt}}$$

C=Concentration of molecule radially diffused at given radius (r) from line segment in an amount of time (t) [mg/cm²]
Q=Amount of molecule per unit length of line segment [mg/cm]
D=Diffusion coefficient of molecule through medium (x% agarose hydrogel) [cm²/s]
t=time of diffusion [s]
r=radial diffusion distance from line segment [cm]

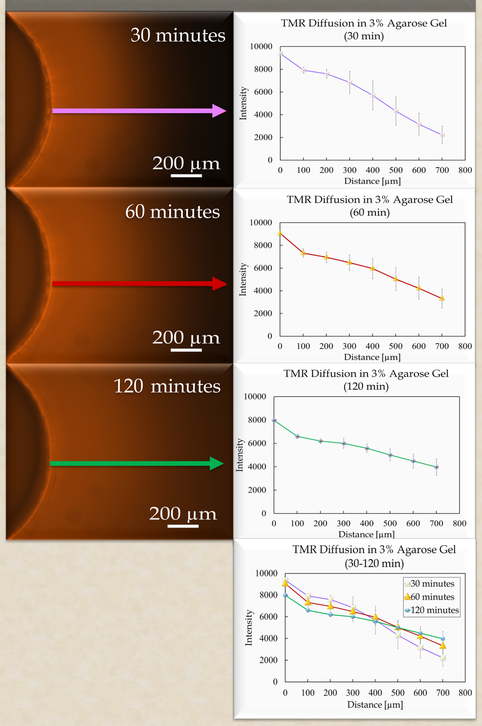


Theoretical vs Experimental

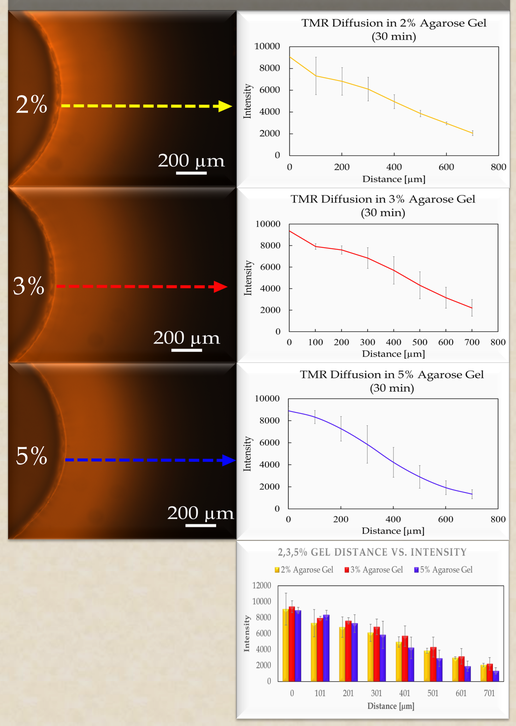




Time dependent diffusion gradients (3% Gel)



2,3,5% gel comparisons



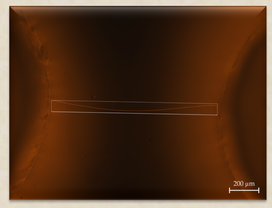
Conclusions:

- ❖ Theoretical and experimental comparisons show trending behavior, but is not yet a full-bodied predictive model for biomolecular diffusion gradients.
- ❖ Time dependent gradients have been successfully observed using this approach.
- ❖ Results are reproducible, but limiting factors such as PDMS master geometry might impact results due to "edge effect".
- ❖ Indiscernible differences in variance between 2, 3, & 5% gel gradient profiles; higher concentrations, higher molecular size, or more samples may yield more conclusive results

Future Works:

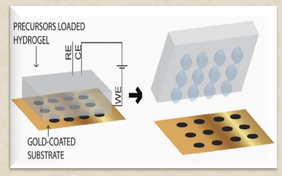
2-Directional Gradient Preparation:

This study will investigate the potential use of two different biomolecules in hopes of creating multi-layered gradients on one hydrogel surface.



Conductive Polymer Film Gradient Preparations:

This study attempts to functionalize biocompatible CP films with gradients of biomolecules using all the methods described thus far.



Depiction of Conducting polymer electropolymerization from SooHyun Park's "Hydrogel-Mediated Direct Patterning of Conducting Polymer Films with Multiple Surface Chemistries" paper

References:

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