Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays

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Abstract

Dopamine (DA) is a neurotransmitter that controls motor movement. When the neurons that release DA are deficient, patients become afflicted with Parkinson's disease (PD)[1]. Current therapies for PD lessen symptoms by supplementing the lost dopamine; this improves patient quality of life but does not treat the underlying disease mechanisms[2]. Neural stem cells (NSCs) have the potential to regenerate lost DA neurons and restore healthy dopamine levels in PD patients due to their ability to differentiate into all types of neurons [3]. Differentiation of NSCs is primarily regulated by the cellular microenvironment, which is in part determined by the localization and concentration of certain growth factors and other soluble molecules[4]. Microfluidic devices are advantageous for examining cell responses because they allow for high-throughput analysis with reduced costs, efficient manipulation of fluids, and certain fluid characteristics, such as laminar flow[5]. We adapted and fabricated a microfluidic device, originally devised by Jeon *et al.*, that uses seven Christmas tree structures to generate concentration gradients of soluble molecules[6]. By integrating this system to flow gradients over a cellular microarray, the effects of various factors on NSC differentiation can be tested in a high-throughput manner. This will enable efficient identification of mechanisms related to DA neural differentiation and ultimately produce a homogenous population of neurons for regenerative medicine.

Introduction

Motivation

- Neurodegenerative diseases result from loss of neuron structure and function
  - Typically, mature neurons have limited proliferation capacity[3]
  - Parkinson's disease: loss of dopaminergic neurons for movement control[1]
    - ~ Prevalence: 500,000 individuals in the United States[1]
- Current therapies treat symptoms, not underlying cause[2]
  - Research suggests neural stem cells can generate all neuron types[3]
    - ~ Knowledge of factors required to promote differentiation into specific neurons could lead to production of cells to replace damaged or dead neurons[3]

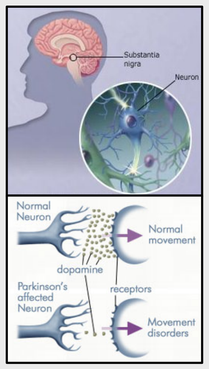


Figure 1: Primary Cause of Parkinson's Disease[7].
A: Parkinson's disease results from a loss of function in the dopaminergic neurons of the substantia nigra, located in the hindbrain. **B:** These neurons lose the ability to release dopamine, resulting in the symptoms commonly seen with Parkinson's disease.

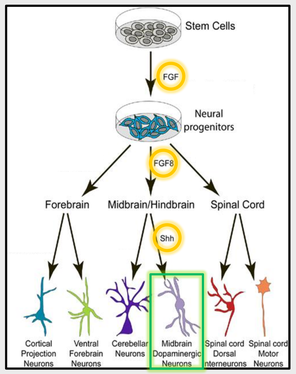


Figure 2: Stem Cell-based Regenerative Medicine[8].
Neural stem cells can differentiate into all types of neurons. Differentiation is partially dependent on the localization and concentration of growth factors present in the cellular microenvironment. FGF: fibroblast growth factor. Shh: sonic hedgehog.

Background

- **Advantages of microfluidics[5]**
  - Generate accurate gradients *in vitro*
  - Minimize reagent volumes
  - Allow for high resolution and sensitivity
  - Permit laminar flow and low shear stress
  - Facilitate high-throughput analysis
- **Temporal gradient investigation[9]**
  - Gradients form during embryonic development
  - Stem cell fates are directed by varying concentrations of soluble molecules
- **Clonal microarrays[10]**
  - Cytophilic islands patterned on gold-coated glass
    - ~ Islands separated by cytophobic regions
    - ~ Cells seeded on device at clonal densities
  - Enable high-throughput screening
  - Need a high-throughput method for establishing different colonies

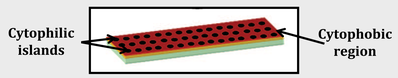


Figure 3: Cellular Microarray[10].
Cells can be patterned on glass in a defined arrangement, making microarrays beneficial for high-throughput biological assays.

Design Criteria

- Integrate cellular microarray and microfluidic platform
- Generate concentration gradient
  - Fick's law – adequate diffusive mixing[11]: $(\boldsymbol{v}\cdot\nabla C) = D\nabla^2 C$
    - ~ Maximum allowable input flow rate: 1.0275 µL/min
  - Low fluid shear stress on cells[12]: $\tau_{max} = 12\mu Q/h^3$
    - ~ Maximum shear stress generated by device: 0.021 dynes/cm^2
- Support flow in multiple regimes
  - Peclet's number – convection vs. diffusion[13]: $Pe = vh/D$
    - ~ Convection dominates diffusion for $Pe >> 1$
  - Peclet's number/Damkohler number – convection vs. reaction[13]: $Pe/Da = v/k_{on}R_s$
    - ~ Convection dominates reaction for $Pe/Da >> 1$

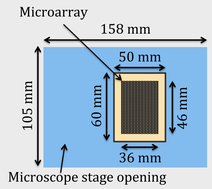


Figure 4: Platform Dimensions.

Variables			
$\boldsymbol{v}$	Velocity vector	Q	Flow rate
C	Concentration	h	Height
D	Diffusivity	v	Average velocity
τ_{max}	Maximum shear stress per cell	k_{on}	Ligand binding-on rate
μ	Viscosity	R_s	Receptor density

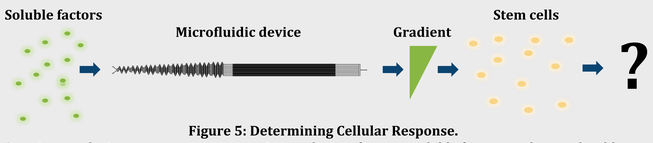


Figure 5: Determining Cellular Response.
By using our device to generate concentration gradients of various soluble factors, we hope to be able to determine the influence of these factors on neural stem cell differentiation.

Final Design

- **Christmas Tree Microfluidic Design[6]**
  - 7 separate Christmas tree gradient generators
    - ~ Each tree produces 10 concentrations
    - ~ Each concentration covers 1 cell row
  - Material: polydimethyl siloxane (PDMS)
  - Glass with cellular microarray is removable

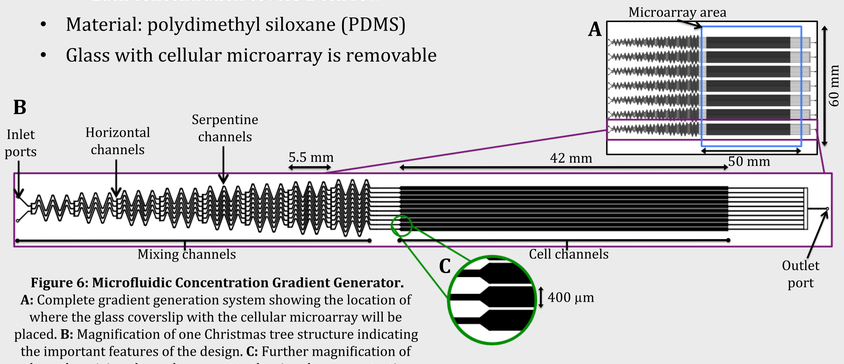
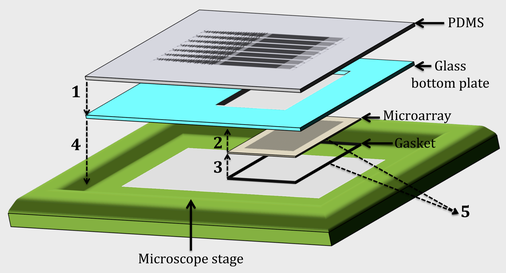


Figure 6: Microfluidic Concentration Gradient Generator.
A: Complete gradient generation system showing the location of where the glass coverslip with the cellular microarray will be placed. **B:** Magnification of one Christmas tree structure indicating the important features of the design. **C:** Further magnification of where the mixing channels convert to the singular concentration channels that will flow fluids over the cellular microarray.



- **Integration Steps**
  1. Plasma oxidize PDMS to glass bottom plate
  2. Align microarray and insert underneath
  3. Insert gasket and sealant
  4. Insert into microscope stage and run experiments
  5. Remove microarray, gasket, and sealant

Figure 7: Microfluidic Platform Integration.
Imaging of the device is possible due to an opening in the microscope stage where it can placed and viewed with an objective from underneath. The PDMS component is oxidized to a glass bottom piece, but they are separated in the figure for ease of viewing. This glass has a section removed to allow for integration of the gold-coated glass piece that contains the cellular microarray. The microarray will be affixed to the PDMS prior to imaging and removed afterwards to allow for further experimentation with the cells.

Results

Simulation

- COMSOL: finite element analysis software
  - Simulated design functionality

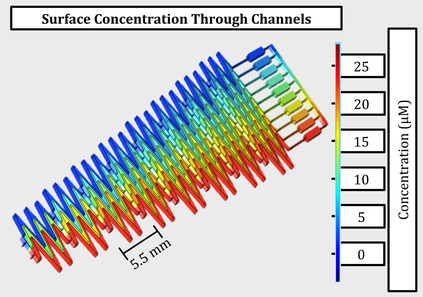


Figure 8: COMSOL Analysis.
Concentrations of 0 µM and 25 µM were input into the two inlets of one Christmas tree structure. Creep flow was used to simulate the flow of fluids through the channels. The resulting concentrations in the cell channels are shown above.

Flow Regimes

- Convection-dominated flow

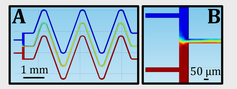


Figure 9: COMSOL Analysis.
Input concentrations: 25 µM and 0 µM; Pe=74, Pe/Da=1254.

- Reaction/diffusion-dominated flow

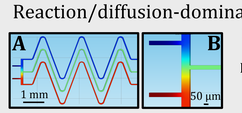


Figure 10: COMSOL Analysis.
Input concentrations: 25 µM and 0 µM; Pe=0.05, Pe/Da=1.

Experimental

- Gradient generated in experimental device

Figure 11: Experimental Device Performance Verification.
Fluorescence testing with 25 µM dextran-fluorescein isothiocyanate (FITC) and ultra-filtered deionized water (0 µM) input into the inlet ports at a rate of 1 µL/min.

Comparison

- Quantitatively compared results from COMSOL analysis and experimental device
  - Determined accuracy of experimental device
- COMSOL parameters
  - Flow rate: 1µL/min
  - Inlet 1 concentration: 25 µM
  - Inlet 2 concentration: 0 µM
- Experimental device parameters
  - Flow rate: 1 µL/min
  - Inlet 1 concentration: 25 µM
  - Inlet 2 concentration: 0 µM
- Compared concentrations in each channel (normalized to maximum concentrations)

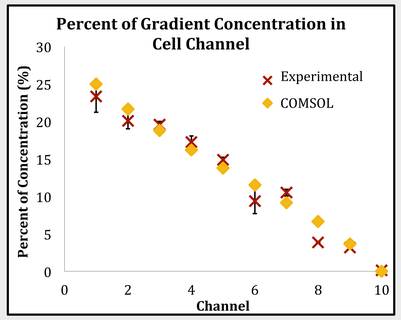


Figure 12: Comparison of COMSOL Analysis and Experimental Device Performance.
Solutions of 25 µM dextran-FITC and ultra-filtered deionized water (0 µM) were input into the two inlet ports at a flow rate of 1 µL/min. Fluorescence was measured at three locations in each of the cell channels and averages were compared to the expected results based on COMSOL analysis.

Future Work

- Integrate microarray
  - Confirm adequate seal is formed
  - Confirm removal without damage
- Incorporate cells
  - Ensure viability
  - Determine response to factors

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