Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays

Sarah Reichert, Anthony Sprangers, Alex Johnson, John Byce

Client: Randolph Ashton, Ph.D., **Advisor:** John Puccinelli, Ph.D.

Department of Biomedical Engineering, University of Wisconsin – Madison

Abstract

Parkinson's disease affects 500,000 Americans and is caused by the loss of function in dopamine-releasing neurons[1]. Current therapies only lessen symptoms by replacing lost dopamine (DA), a neurotransmitter that controls motor movement. This improves patient quality of life, but does not treat the underlying disease mechanisms[2]. Neural stem cells (NSCs) maintain the ability to differentiate into all types of neurons and stand to replace lost DA neurons and restore healthy dopamine levels in Parkinson's patients[3]. Differentiation of NSCs is primarily regulated by the features of the cellular microenvironment; one such characteristic is the localization and concentration of certain growth factors and other soluble molecules[4]. Microfluidics is an advantageous technique for examining cell responses because it allows for high-throughput analysis with reduced costs, efficient manipulation of fluids, and certain fluid characteristics, such as laminar flow[5]. We designed and fabricated a microfluidic device that uses six Christmas tree structures to generate concentration gradients of soluble molecules[6]. By adjusting this system to flow gradients over a cellular microarray, specific factors capable of instructing NSC differentiation can be tested in a high throughput manner. This will allow for 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 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]

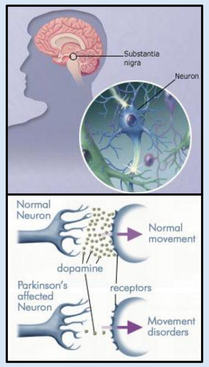

Figure 1: Primary Cause of Parkinson's Disease[7].
A: Parkinson's disease results from a loss in function of the dopaminergic neurons of the substantia nigra, located in the hindbrain. **B:** These neurons lose the ability to release dopamine, resulting in the symptoms commonly seen with Parkinson's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

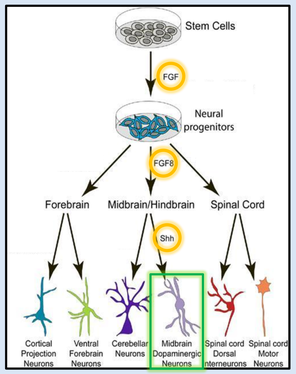

Figure 2: Stem Cell-based Regenerative Medicine[8].
Neural stem cells can differentiate into all types of neurons. Differentiation is partially dependent on the localization and concentration of growth factors present in the cellular microenvironment[8].FGF: fibroblast growth factor. Shh: sonic hedgehog.

Background

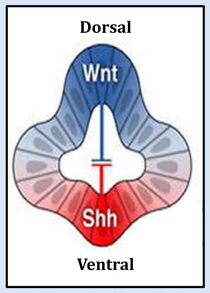

Figure 3: Soluble Factor Gradients during Embryonic Neural Development[9].
Cell fate in neural tissue development is directed by concentration gradients of soluble factors within the neural tube. Sonic hedgehog (Shh) and Wnt induce variable gene expression in neural progenitor cells along their concentration gradients to dictate differentiation fate[9].

- **Microfluidics[5]**
  - Generates accurate gradients *in vitro*
  - Reduces quantities of reagents needed
  - Allows for high resolution and sensitivity
  - Permits laminar flow and low shear stress
- Compatible with a cellular microarray

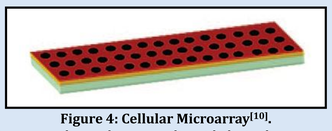

Figure 4: Cellular Microarray[10].
Cells patterned on a glass coverslip with desired arrangement, often used in high-throughput biological assays.

Design Criteria

- Integrate cellular microarrays with a microfluidic platform that:
  - Is compatible with a standard microscope stage (158 x 105 mm, < 0.5 kg)
    - To enable live-cell imaging and high-throughput analysis
  - Generates a gradient across the microarray (40 x 60 cell spots)
  - Creates a water-tight seal with a microscope coverslip
  - Is reusable for multiple microarrays
- Initial focus: generate concentration gradient
  - Adequate diffusive mixing in serpentine channels – Fick's law[11]: $(\mathbf{v}\cdot\nabla C) = D\nabla^2 C$ (Concentration; Velocity vector; Diffusivity constant)
  - Low fluid shear stress on cells[12]: $\tau_{max} = 18\mu Q/h^2 w$ (Maximum single cell shear stress; Flow rate; Channel width; Viscosity; Channel height)

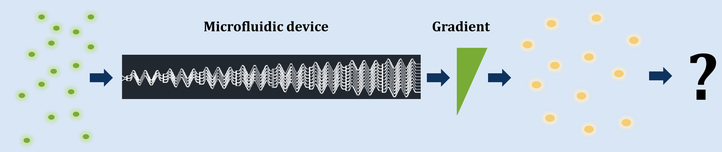

Figure 5: Determining Cellular Response.
By using our device to generate concentration gradients of various soluble factors, we hope to be able to determine the influence of these factors on neural stem cell differentiation.

Final Design

- **Christmas Tree Microfluidic Design**
  - 6 separate Christmas trees
    - ~ Each tree generates 10 concentrations
    - ~ Each concentration will cover 1 cell row
  - Material: polydimethyl siloxane (PDMS)
  - Glass coverslip with microarray is removable

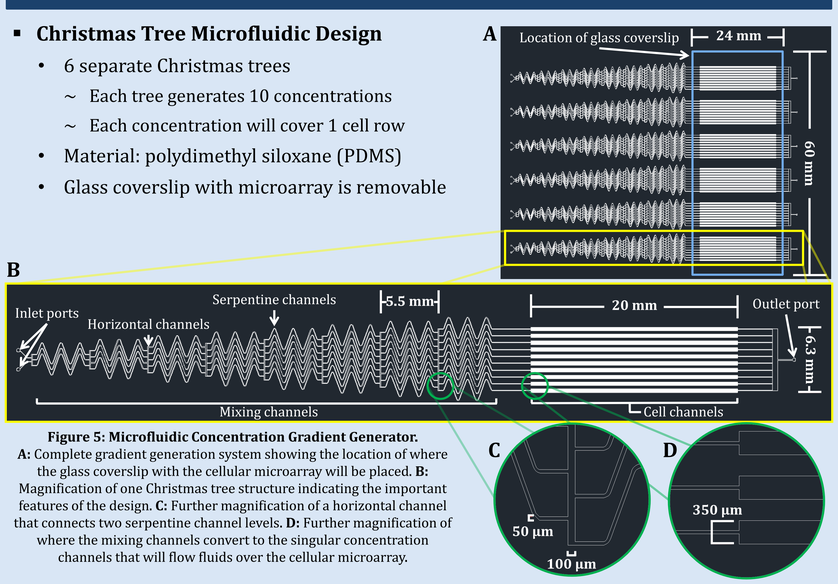

Figure 5: Microfluidic Concentration Gradient Generator.
A: Complete gradient generation system showing the location of where the glass coverslip with the cellular microarray will be placed. **B:** Magnification of one Christmas tree structure indicating the important features of the design. **C:** Further magnification of a horizontal channel that connects two serpentine channel levels. **D:** Further magnification of where the mixing channels convert to the singular concentration channels that will flow fluids over the cellular microarray.

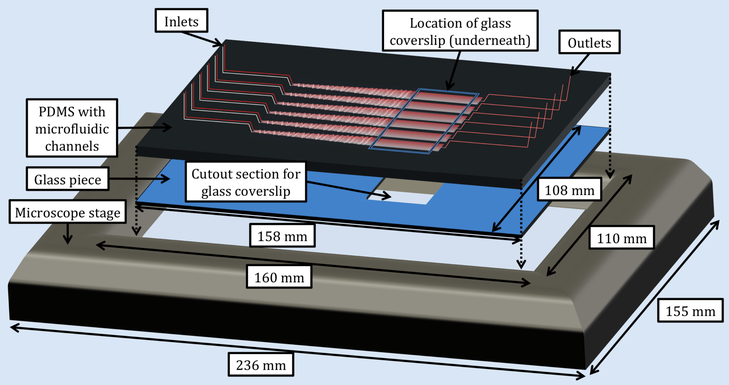

Figure 6: Microfluidic Platform Integration.
Imaging of the device is possible due to an opening in the microscope stage where it can placed and viewed with an objective from below. The PDMS component is oxidized to a glass bottom piece, but they are separated in the figure for ease of viewing. This glass has a section removed to allow for integration of the glass coverslip that contains the cellular microarray. The glass coverslip will be adhered 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

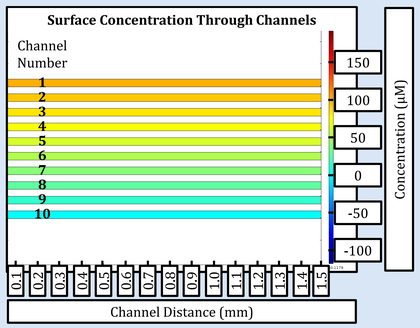

Figure 7: COMSOL Analysis.
Concentrations of 0 and 100 µM were input into the two inlets of one Christmas tree structure. Creep flow was used to simulate the flow of fluids through the channels. The resulting concentrations in the cell channels are shown above.

Experimental

- Proved gradient in experimental device
  - Qualitative gradients observed
  - No leakage seen
  - Bubbles at front corners of cell channels

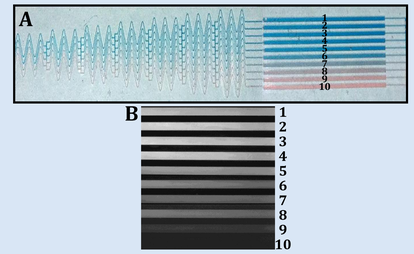

Figure 8: Experimental Device Performance Verification.
A: Red and blue dyed water was input into a Christmas tree at a rate of 3 µL/min. A gradient was observed. **B:** Fluorescence testing with ultra-filtered deionized water and 100 µM dextran-fluorescein isothiocyanate (FITC) input into the inlet ports at a rate of 3 µL/min. A fluorescence microscope was used to image the cell channels, shown in grayscale above.

Comparison

- Quantitatively compared results from COMSOL analysis and experimental device
  - Determined accuracy of experimental device
- COMSOL parameters
  - Flow rate: creep flow
  - Inlet 1 concentration: 100 µM
  - Inlet 2 concentration: 0 µM
- Experimental device parameters
  - Flow rate: 3 µL/min
  - Inlet 1 concentration: 100 µM
  - Inlet 2 concentration: 0 µM
- Compared concentrations in each channel as percentages of maximum concentration

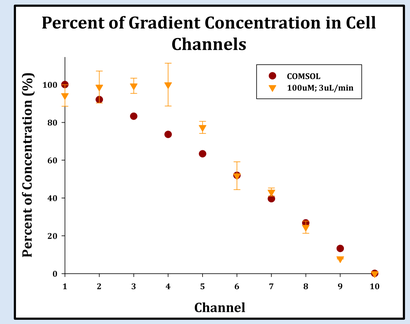

Figure 9: Comparison of COMSOL Analysis and Experimental Device Performance.
A solution of 100 µM dextran-FITC was input into one inlet and ultra-filtered deionized water was input into the other, both at a rate of 3 µL/min. Fluorescence was measured at three locations in each of the cell channels, and averages were compared to the expected results based on COMSOL analysis. Additional concentrations and flow rates yielded similar results.

Future Work

- Continue fluorescence testing
- Modify final design to:
  - Increase inlet and outlet lengths
  - Prevent bubble formation in channels
- Utilize two-layer PDMS microfluidics
  - Allows for different heights of gradient-generating and cell channels
- Integrate microarray
  - Confirm adequate seal is formed
  - Confirm removal without damage
- Incorporate cells
  - Ensure viability
  - Determine response to factors

Acknowledgements

Dr. John Puccinelli, Dr. Randolph Ashton, Greg Czaplewski, Dave Buschke, Brian Freeman, John Guckenberger, Tracy Drier, Chris Pearce

References

1. NINDS. (2011, 12 October). National Institute of Neurological Disorders and Stroke - Parkinson's Disease Backgrounder. Available: http://www.ninds.nih.gov/disorders/parkinsons_disease/parkinsons_disease_backgrounder.htm
2. University of California, San Francisco. (2011, 8 December). Biology of Neurodegeneration. Available: http://ind.ucsf.edu/causetocure/biology
3. NINDS. (2011, 8 December). National Institute of Neurological Disorders and Stroke – The Life and Death of a Neuron. Available: http://www.ninds.nih.gov/disorders/brain_basics/ninds_neuron.htm
4. D. E. Discher, *et al.*, "Growth factors, matrices, and forces combine and control stem cells," Science, vol. 324, p. 1673, 2009.
5. G. M. Whitesides, "The origins and the future of microfluidics," *Nature*, vol. 442, no. 27, pp. 368-373, Jul. 2006.
6. N. L. Jeon, *et al.*, "Generation of solution and surface gradients using microfluidic systems," *Langmuir*, vol. 16, pp. 8311-8316, 2000.
7. A. Naik. (2011, 6 November). Parkinson's Disease. Available: http://drarunlnaik.com/parkinsons_disease
8. T. Petros, *et al.*, "Pluripotent stem cells for the study of CNS development," *Frontiers in Molecular Neuroscience*, vol. 4, p. 1-12.
9. http://neurosciencenews.com/morphogens-brain-development-wnt-vax-tcf712/
10. R. S. Ashton, *et al.*, "High Throughput Screening of Gene Function in Stem Cells Using Clonal Microarrays," *Stem Cells*, vol. 25, pp. 2928-2935, 2007.
11. Ficks: S. Bird, Transport Phenomena Revised Second Edition: John Wiley & Sons, 2007.
12. Shear: L. Kim, et al., "Microfluidic arrays for logarithmically perfused embryonic stem cell culture," Lab Chip, vol. 6, pp. 394-406, 2006.