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Cite this: DOI: 10.1039/c2lc00000x

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PAPER

Microfluidic platform for culture and live cell imaging of cellular microarrays

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Received 14th March 2012
DOI: 10.1039/c2lc000000x

Neurodegenerative diseases, such as Parkinson's disease (PD), result from the loss of neuronal structure or function. Due to the limited proliferation capacity of dopaminergic neurons, PD usually results in bad patient prognoses, leaving patients the only option of current treatments which only alleviate the disease's symptoms, not the underlying neuronal loss. Fortunately, a regenerative therapy approach may be possible since neural stem cells have been found to differentiate into all neuron types, including dopaminergic neurons. However, the exact combination of environmental cues necessary for this specific differentiation has yet to be identified. Here, a high-throughput device containing a microfluidic gradient generator and a cellular microarray is described. With this approach, gradients of soluble factors can be generated which will then flow over a cellular microarray, allowing for high-throughput analysis of cell response. This will help elucidate necessary temporal and spatial concentrations needed to induce specific lineage differentiation of neural stem cells.

Introduction

The limited amount of cell proliferation in the mature nervous system is devastating for patients with neurodegenerative diseases resulting from the loss of neuron structures and functions.[1] This is especially true with Parkinson's disease (PD), which is caused by the loss of dopaminergic neurons in the midbrain.[2] Patients with PD experience tremors, compromised coordination, and other diminished motor functions that decrease their quality of life.[2] The National Institute of Health estimates that approximately 500,000 Americans currently suffer from PD, with another 50,000 new incidences occurring annually.[3] Presently, there is no cure for PD; current therapies only treat the symptoms without correcting the underlying dopaminergic neuron loss.[1] Fortunately, promising new research suggests a regenerative therapy approach may be appropriate, as neural stem cells (NSCs) have been shown to differentiate into all the neuron types present in the nervous system.[1] One of the factors that dictates NSC fate *in vivo* is the exposure to concentration gradients of soluble factors.[4] Based on the position of a cell within the gradient, gene expression, and subsequently cell fate, can be tightly controlled.[4] This approach is promising, but many of the factors that induce specific NSC lineage differentiation need to be identified and thoroughly investigated before it becomes a viable treatment option. In addition to this, the differentiation of NSCs into dopaminergic neurons must be highly efficient, because a heterogeneous mixture of cells may lead to an immune response or tumor formation.

Using standard cell culture techniques to test for optimal soluble factor concentrations would be extremely expensive and time consuming. A more high-throughput method for accomplishing this involves the implementation of microfluidics. Previous studies have demonstrated that various microfluidic devices can generate accurate, reproducible concentration gradients of soluble molecules.[5-11] Microfluidics have the additional benefit of being able to be integrated with clonal microarrays, which consist of hundreds to thousands of separate cell colonies seeded onto a patterned surface.[12] Microarrays allow for the high-throughput, simultaneous analysis of many conditions when incorporated into a gradient-generating microfluidic device. Following testing, individual cell colonies can be recovered, expanded with standard cell culture techniques, and examined using biological assays to analyze cell fates.[12]

Following the 'Christmas tree' design first established by Jeon *et al.*[11] to create a gradient generator, we fabricated a platform that integrates this microfluidic device with a cellular microarray for examining NSC responses to soluble factors. This system allows for high-throughput analysis with reduced costs and reagent quantities, efficient manipulation of fluids, and beneficial fluid characteristics, such as laminar flow. With this platform, we hope to be able to determine the specific soluble factor concentrations necessary to elicit NSC differentiation down specific neuronal lineages. This knowledge can then be applied to direct controlled differentiation of NSCs *in vitro* for use in regenerative therapies.

Materials and methods

Microfluidic device description

Our microfluidic device contains seven gradient generators with a 'Christmas tree' design.[11] Jeon *et al.* developed the conventional

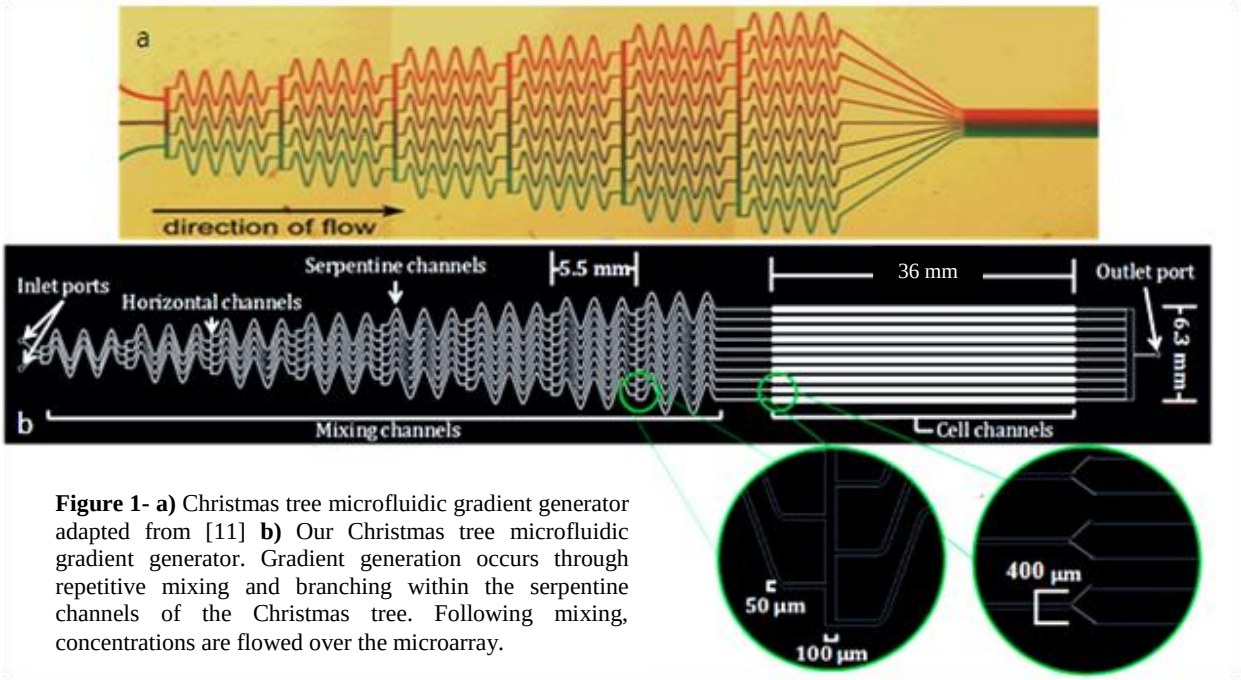

Figure 1- a) Christmas tree microfluidic gradient generator adapted from [11] **b)** Our Christmas tree microfluidic gradient generator. Gradient generation occurs through repetitive mixing and branching within the serpentine channels of the Christmas tree. Following mixing, concentrations are flowed over the microarray.

microfluidic Christmas tree, which generates a concentration gradient in a broad channel with a spatial resolution of 2-20 µm, as seen in Figure 1a.[11] To provide a sustained, specific concentration of soluble molecules in each row of the microarray, one of our seven Christmas trees, shown in Figure 1b, generates a range of discrete concentrations across ten separate cell channels. Since we have seven of these Christmas trees in our device, we can effectively create 70 different concentrations which will then each flow over one row of the microarray. The device was fabricated with polydimethyl siloxane (PDMS) using soft photolithographic techniques and a negative replica silicone master with 200 µm channel heights.[13] The PDMS was developed using 184 Silicone Elastomer Base and Curing Agent (Dow Corning, Midland, MI) in a 10:1 mixture. Elastomer was poured on top of the negative silicone master and a vacuum was pulled for approximately 30 minutes. Next, the PDMS was cured at 100 °C for 1 hour. Plasma oxidization was used to bind the device to a 3.175 mm-thick glass piece for experimentation.

Computational analysis and statistics

A theoretical simulation of the gradient generation was conducted using COMSOL Multiphysics (Comsol Inc., Los Angeles, CA) software. This analysis was performed using the microfluidics module with creeping flow and transport of diluted species modeling. Creeping flow was velocity-driven using an input flow

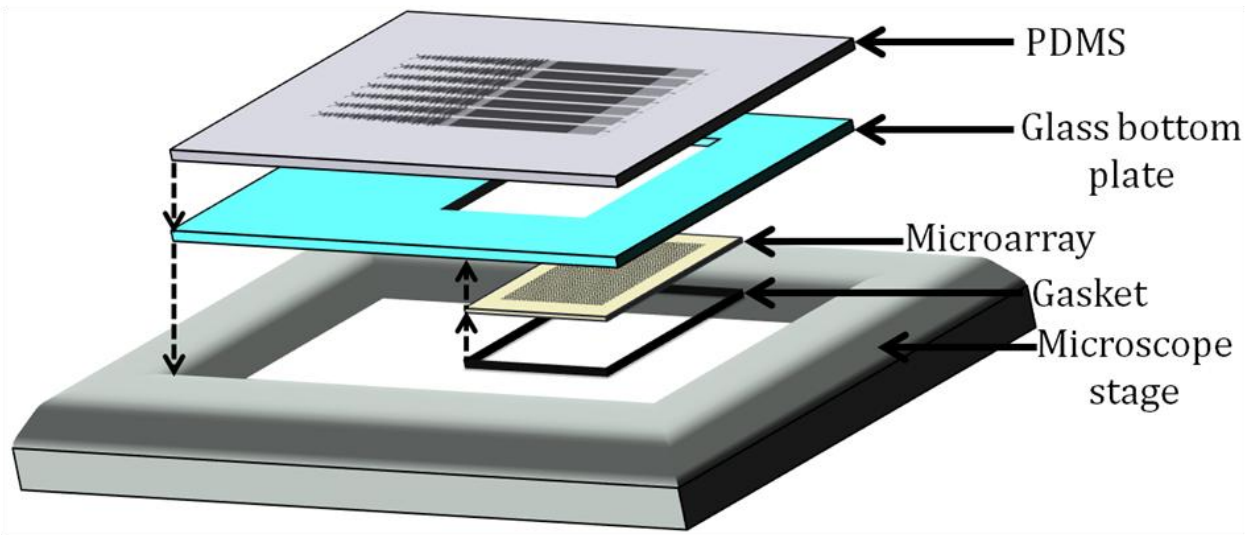

Figure 2: Integration of PDMS mold to glass bottom plate occurs via plasma oxidation. Correct alignment of this allows for the incorporation of the microarray from beneath the device. This entire structure is then incorporated into the microscope stage for imaging of the cell colonies on the microarray.

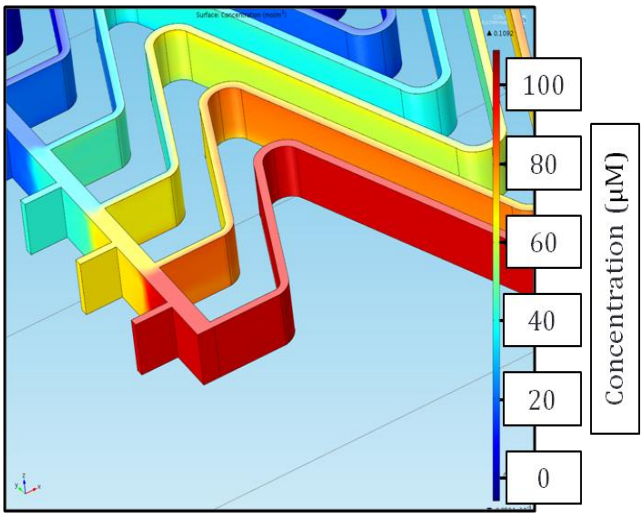

Figure 3: COMSOL 3-dimensional computational model showing diffusive mixing in the serpentine channels.

rate of 1 m/s of incompressible fluid, which corresponded to a volumetric flow rate of approximately 1 μL/min. The transport of diluted species analysis was coupled to the velocity inflow from the creeping flow model. A linear solver was used to calculate the theoretical concentration gradient using an "extremely fine" general physics, tetrahedral mesh. Graphing and statistical analyses were conducted using Sigma Plot (Systat Software Inc., San Jose, CA).

Fluorescence imaging

The microfluidic device was utilized to generate concentration gradients that could then be examined with a fluorescent microscope. Solutions of 0 μM and 25 μM fluorescein isothiocyanate (FITC)-labeled dextran (40 kDa, Sigma Aldrich, St. Louis, MO) were injected through the two inlets of a Christmas tree structure with flow rates of 4.0 μL/min. A gradient was generated and allowed to equilibrate for at least 10 minutes. The device was imaged with an Olympus 1x81 Environmental Microscope (Olympus Valley Inc., Center Valley, PA) using cellSens Dimension software (Olympus Valley Inc., Center Valley, PA). FITC intensity analysis was conducted using ImageJ (free source) software. Three regions of interest were chosen in each cell channel and an average intensity was determined from them. A standard curve was developed to relate intensity to concentration of FITC-dextran. Six samples (0 μM, 5 μM, 10 μM, 15 μM, 20 μM, and 25 μM) were created through serial dilutions and a single standard concentration was pushed through the microfluidic device for imaging and analysis to determine the corresponding intensity. The same exposure rate was used for standards and experimental samples. Ethanol and deionized water were washed through the device between samples.

Integration

As shown in Figure 2, the surfaces of the PDMS and glass bottom plate were plasma oxidized with a reactive ion etching system and aligned so that the cell channels of the gradient generator were arranged above the opening for the clonal microarray. The microarray was then aligned from underneath and inserted along with a gasket and temporary sealant to secure it in place and prevent the device from leaking at the glass interfaces. After experimentation, the sealant was peeled away and the gasket was removed in order to take out the microarray and enable the colonies to be expanded and analyzed for induced cell fates.

Results and discussion

Computational analysis and statistics

The concentration gradient computed by the coupled COMSOL analysis was consistent with what was expected based on previous descriptions in literature.[11] As depicted in Figure 3, the model accurately shows the three-dimensional mixing in the serpentine channels of the microfluidic device. The final concentrations generated are shown in Figure 4. The concentration values have been normalized to the maximum

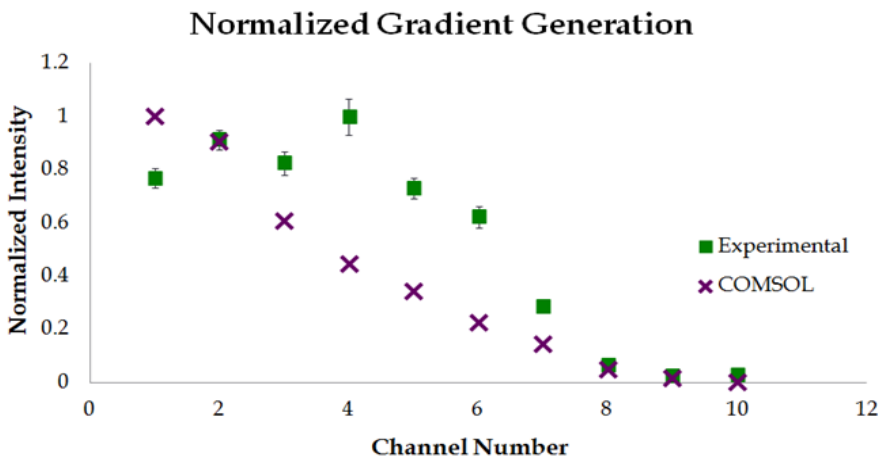

Figure 4: Normalized gradient generation from computational analysis and experimental testing. The computational analysis follows a 3rd degree polynomial as is expected from *Jeon et al.*[11] The experimental testing does not adhere to this polynomial resulting from an error in our master which pushed more fluid to the interior channels due to an increased resistance in the outer channels.

concentration for ease of comparison with experimental results. The gradient generated by the simulation tightly adhered to a 3rd order polynomial with an R^2 value of 0.992. This model accurately predicts the concentration of the constructed Christmas tree gradient for flow rates up to 1 m/s (1 µL/min).

Fluorescence imaging

The generated gradient can be seen in Figure 4. The concentration in each channel was determined from the developed standard curve (not shown); these values were normalized to the maximum concentration for ease of comparison with computational results. Unexpectedly, channel four had the highest intensity and likewise concentration of FITC. This is most likely due to an obstruction in one of the first three channels which increases their resistance, pushing more fluid towards the middle channels. Despite this result, the last three channels follow the computational analysis relatively accurately.

Conclusions

Here, a microfluidic gradient generator was coupled with a cellular microarray. Computational and experimental gradients were developed yet did not overlap due to an error in device fabrication. Our device will be refabricated and tested following the arrival of a new photomask which will hopefully correct the intensity discrepancies between the computer and experimental simulations. However, this microfluidic approach allows for the high-throughput analysis of soluble factor concentrations on inducing dopaminergic neurons differentiation of NSCs. Hopefully, this will help elucidate optimum factor concentrations for specific homogeneous differentiation of NSCs. Such a device can also be used to determine optimal concentration necessary for other stem cell differentiation as well as drug screening to determine harmful drug concentrations.

Notes and references

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