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Flow characterization of a microfluidic device to selectively and reliably apply reagents to a cellular network^{†,‡}

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Abstract

A three-dimensional microfluidic device has been successfully fabricated and the flow streams characterized for eventual use in studying communication in an *in vitro* network of nerve cells. The microfluidic system is composed of two layers of channels: a lower layer for the delivery of pharmacological solutions and an upper layer of channels used to direct the flow of the pharmacological solution streams and perfuse the cells with media and nutrients. Flow profiles have been characterized with computational fluid dynamics simulations, confocal fluorescence microscopy, and carbon-fiber amperometry, which have been used to map changes in flow profiles at different bulk flow rates. Ultimately, the microfluidic system and incorporated cell network will show how networked neurons adapt, compensate, and recover after being exposed to different chemical compounds.

Introduction

The use of microfluidics as analytical tools to investigate cellular systems has become widespread in recent years.^{1–5} The field of neuroscience, however, has yet to take full advantage of the technique's utility for studying brain function, particularly communication and behavior in neural networks. Currently, most analytical techniques for studying changes in network communication in the presence of a treatment involve bathing the entire culture in the chemicals being investigated^{6–8} or using glass micropipettes to expose single neurons in an *in vitro* network to different solutions.^{9–11} Unfortunately, neither of these methods allow individual or small patches of cells to be exposed selectively since all cells are exposed to the treatment in the case of a bath or the majority exposed *via* the rapid diffusion of solutions after being “puffed” out of pipettes. While several researchers have begun to integrate fluidic systems for perfusion and treatment with network cultures,^{12–18} these systems feature complex designs that require advanced, multiple-step microfabrication techniques or expensive equipment, such as lasers, and to date do not allow multiple cells to be addressed in culture with different solutions at different locations.

In this paper, a two-layer microfluidic device capable of selective exposure of specific cells and subsequent monitoring of the network response has been created using soft lithography and its flow patterns have been characterized. The microfluidic platform consists of a bottom layer of channels used for the delivery of pharmacological solutions to specific cells in the

[†]The HTML version of this article has been enhanced with colour images.

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network (pharmacological channels) and a top layer of channels for gently directing the flow stream of the pharmacological solutions with low linear flow rates (bulk flow channels). By varying the bulk flow rates, the direction and height of the pharmacological fluid streams can be altered, allowing application of reagents to specific locations. Uniquely, this device is able to deliver different solutions to multiple sites in a network of cells with controllable and well-defined laminar flow for both transient and long-term stimulations. Computational fluid dynamics (CFD) simulations, as well as confocal fluorescence microscopy and carbon-fiber amperometry experiments have been used to determine the direction and height of the flow streams at various bulk flow rates. In the future, this platform will be used to selectively and precisely expose nerve cells to different drugs, toxins, and other pharmacological solutions while monitoring neural network activity by fluorescence microscopy.

Materials and methods

CFD simulations

CFD simulations were performed with Comsol Multiphysics 3.3 (Comsol, Inc., Burlington, MA, USA), a finite element analysis solver. A two-dimensional geometry was used to model the microfluidic system in order to yield a cross-sectional view of the fluid flow. The following constants were used: diffusion coefficient, $D = 4.0 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$; density, $\rho = 1.0 \times 10^3 \text{ kg m}^{-3}$; dynamic viscosity, $\mu = 1.0 \times 10^{-3} \text{ Pa s}$; initial concentration, $c_0 = 3.6 \times 10^{-5} \text{ mol L}^{-1}$. Incompressible Navier–Stokes equations were initially solved, given by:

$$\frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u}$$

$$\nabla \cdot \mathbf{u} = 0$$

where $\mathbf{u}$ is velocity, t is time, ρ is density, p is pressure, and μ is dynamic (absolute) viscosity. Subsequently, the convection/ diffusion equation,

$$\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c = D \nabla^2 c$$

was solved, where c is concentration, t is time, $\mathbf{u}$ is velocity, and D is the diffusion coefficient.

Confocal fluorescence microscopy

Syringe pumps (Harvard Apparatus, Holliston, MA, USA and KD Scientific, Holliston, MA, USA) were used to control the volumetric flow rates of 36 μM fluorescein isothiocyanate (FITC, Sigma, St. Louis, MO, USA) and water in the microfluidic system. The pumps interfaced with the NanoPorts™ and inlet holes of the PDMS microchannels *via* plastic syringes connected to polyethylene tubing. Fluorescence images were acquired with a laser scanning confocal microscope (Carl Zeiss LSM 5 Pascal, Thornwood, NY, USA).

Amperometry

Syringe pumps were likewise utilized in conjunction with plastic syringes and polyethylene tubing to control the volumetric flow rates of 100 μM catechol (Sigma, St. Louis, MO, USA), 1 M potassium chloride (Sigma, St. Louis, MO, USA), and water in the microfluidic system. A carbon-fiber microelectrode (5 μm diameter) was fabricated as described previously.¹⁹ The electrode was held at a potential of +700 mV *versus* a Ag/AgCl reference electrode for all experiments. The position of the electrode in the fluid was controlled with a micromanipulator under a microscope. The current generated by the oxidation of the catechol was measured and

recorded at different locations within the fluid stream, as the electrode was moved incrementally in a vertical direction.

Results and discussion

The microfluidic platform

The microfluidic platform is comprised of two layers: a bottom layer consisting of nine pharmacological channels, each terminating as $100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$ outlet holes on the surface, and a top layer with channels $200\text{ }\mu\text{m}$ in height and $1.7\text{--}2.0\text{ mm}$ wide at the outlet (Fig. 1). The lower channels are capable of delivering pharmacological agents to cells that will be adhered in between the channel outlet holes on the surface of the device. The top layer consists of bulk flow channels for the flow of water/KCl, or in the future, cell media across the surface. More importantly, the bulk channels flow solutions perpendicular to those exiting the pharmacological channels, thereby controlling the direction and height of the streams of fluid exiting from the pharmacological channels.

Characterization of controlled flow in the device

Microscope images were acquired showing the top view of the pharmacological channel outlet holes (Fig. 2). A solution of FITC dye was flowed from the center pharmacological channel, while water was flowed out of the bulk channel located at the left (Fig. 2a), bottom (Fig. 2b), or the left and bottom bulk channels simultaneously (Fig. 2c). As the dye exited the hole, it flowed upward, out of the plane of the paper. However, the bulk flow of water forced the FITC to flow parallel to the surface and not straight up. It is easy to see that this direction of flow can be used to apply a specific reagent to a group of cells on a surface. Furthermore, the flow is laminar since the fluids to flow in constant, predictable, discrete streams.

In addition to controlling the direction of fluid flow, controlling the height of the flow streams is also important as this allows application of reagents to one set of cells and away from subsequent cells. Initially, two-dimensional CFD simulations were carried out to investigate the interaction between the bulk flow of water and two adjacent pharmacological channels delivering FITC and water. The results of these calculations are shown in Fig. 3a. For these simulations, the flow rates of the solutions exiting the pharmacological channels were held constant at $20\text{ }\mu\text{L min}^{-1}$, while the flow of water from the bulk channel was varied from 1000 to $2000\text{ }\mu\text{L min}^{-1}$ in $250\text{-}\mu\text{L min}^{-1}$ increments (linear velocity at the channel outlet ranged from 4167 to $9804\text{ }\mu\text{m s}^{-1}$). At each flow rate, the height of FITC was measured $100\text{ }\mu\text{m}$ to the right of the water outlet hole. The dye solution flowed upward but quickly turned to the right due to the influence of the bulk flow of water. The FITC stream continued to flow to the right, staying in contact with the surface, until reaching the water stream from the adjacent pharmacological channel. At this point, the flow of water from the adjacent channel then forced the FITC stream upward, driving the FITC away from the surface. This process can clearly be used to apply a reagent to a cell located to the right of the first channel outlet, while avoiding application to cells located beyond the second outlet hole.

Simulations of the FITC flow stream have been compared to flows measured experimentally with confocal fluorescence microscopy under similar conditions. The confocal microscope was used to acquire multiple images, each on a separate focal plane, which were merged together to yield a three-dimensional image viewable from different angles. The flow profile obtained with confocal microscopy (Fig. 3b) shows FITC flowing up after exiting the channel, and then traveling to the right in contact with the surface of the device. As shown with the CFD simulation, once the FITC reached the adjacent water pharmacological channel outlet, the FITC stream flows further upward and the two methods show similar profiles.

Carbon-fiber amperometry was also performed to support data collected with CFD simulations and confocal microscopy. The characterization of flow by amperometry was performed under the same conditions as the confocal experiments, with electroactive catechol replacing FITC and the use of KCl solution in lieu of water for bulk flow to allow current conduction through the solution. A microelectrode was positioned 100 μm to the right of the water outlet hole (Fig. 4a) and the current was measured at various heights. Near the surface, no current was detected, indicating that only water, which is not electroactive at the potential applied to the electrode (+700 mV), was present. However, as the height of the electrode was increased, measurable current was detected, indicating the presence of catechol due to its oxidation to *o*-benzoquinone. Profiles generated from these data correlate well with the confocal images and simulations of the FITC/ water interaction.

Two parameters in this experiment affected both the heights of the FITC and catechol streams exiting the pharmacological layer, the bulk flow rate and the distance, d , from the bulk flow channel to the FITC/catechol outlet hole (Fig. 3c and 4b). While keeping the pharmacological channel flow rates constant at 20 $\mu\text{L min}^{-1}$, the FITC/catechol streams decreased in height as the bulk flow rate increased. As its rate was increased, bulk flow had more of an influence on the FITC/ catechol height, forcing the solutions to be more parallel with the surface of the device. At low bulk flow rates, the FITC/ catechol flow rate was more dominant, causing it to travel in a more upward direction.

The distance, d , of the bulk flow channel to the FITC/ catechol outlet hole also played a role in the height of the FITC/catechol stream. When the bulk flow channel was closer to the FITC/ catechol outlet hole, the linear flow rate of water was higher *versus* a lower linear flow rate when it is farther away and influences the height of FITC/catechol to a greater degree. Since a typical cell is 10–20 μm in height, the fastest flow rate used in these experiments (2000 $\mu\text{L min}^{-1}$) would cause the FITC or catechol to be approximately 130 μm in height (Fig. 3c and 4b), thereby capable of avoiding application to cells in the region near the water outlet hole in future experiments.

Though the displacement height calculated by CFD simulations follows a similar trend when compared to those measured by confocal microscopy and amperometry (Fig. 3c and 4b), some discrepancies exist between the simulations and experimental results. At higher bulk flow rates, the simulations and experimental data are not significantly different at 95% confidence, while they are significantly different at lower bulk flow rates. These differences occur because the simulations were performed in only two dimensions, the simulations did not take into account the parabolic flow profile in the missing dimension (across the surface of the device). With this flow profile, the highest velocity occurs over the center set of outlet holes while the velocity decreases as the flow spreads outward. Since the confocal and amperometry experiments were completed using the outlets not directly in the center of the flow, the CFD simulations, although similar, do not completely agree with the experimental data.

Conclusions

A PDMS microfluidic device for investigating neural communication has been successfully fabricated by photolithography and oxygen plasma bonding. Computational fluid dynamics simulations, confocal fluorescence microscopy, and carbon-fiber amperometry have been used to characterize the flow profiles by measuring the height and direction of the flow streams. This information will be subsequently used to stimulate and expose cells in different areas of a patterned neural network.

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Fig. 1. Area of interest of the device showing nine pharmacological channel outlet holes and two bulk flow channels. In future studies, cells will be cultured on the surface in between the nine outlet holes. Inlet holes and NanoPorts™ are not shown.

Fig. 2. Fluorescence microscopy images of a stream of FITC ($20 \mu\text{L min}^{-1}$) exiting a pharmacological channel outlet hole and being directed by bulk flow of water ($2000 \mu\text{L min}^{-1}$). Bulk flow channels are not shown in the images. Scale bar = $400 \mu\text{m}$.

Fig. 3.

(a) CFD simulation showing the side-view concentration profile of FITC flow. The height of the FITC stream was measured 100 μm away from the water outlet hole. Scale bar = 100 μm. (b) Confocal fluorescence image of FITC flow viewed from the side, at a 20° angle above the surface. The displaced height of the FITC stream was measured 100 μm away from the water outlet hole. The bulk channel is not shown in the image. Scale bar = 100 μm. (c) Plot of height of FITC flow stream compared to the bulk flow rate of water and distance, d , from the bulk channel to the outlet hole of FITC. Points show theoretical height calculated from simulations ($n = 1$) and average height measured by confocal microscopy ($n = 4$). The standard error of the mean ranges from 3 to 16 for each bulk flow rate for the confocal measurements.

Fig. 4.

(a) Top-view microscope image of the amperometry setup showing two adjacent pharmacological channel outlet holes and carbon fiber electrode. The reference electrode and bulk flow channel are not shown. Scale bar = $100\ \mu\text{m}$. (b) Plot of the displaced height of catechol flow stream compared to the bulk flow rate of aqueous KCl solution and distance, d , from the bulk fluid channel to the catechol channel outlet. Points show theoretical height calculated from simulations ($n = 1$) and average height measured by amperometry ($n = 4$). The standard error of the mean ranges from 15 to 20 for each bulk flow rate for the amperometric measurements.