A MICROFLUIDIC PLATFORM FOR FOCAL CHEMICAL STIMULATION OF CELLS

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ABSTRACT

A new microfluidic platform for focal stimulation of cells and tissue has been developed. The device consists of a 100 μm x 4 μm x 6 mm Parylene microchannel with a 5, 10, or 20 μm diameter pore. First results of real time focal chemical stimulation of cell cultures are presented.

KEYWORDS: Microfluidics, Focal Chemical Stimulation, Parylene, PC12

INTRODUCTION

Focal delivery of chemicals at cellular and sub-cellular resolution enables understanding biological responses of cells and tissue, and provides a means of interfacing with the nervous system beyond electrical stimulation [1-4]. A new microfluidic platform has been developed for focal delivery of chemicals to cell cultures and tissue. For the first time, fluid intake as well as ejection and passive diffusion are possible. This platform is easily scaled to an array with multiple individually-addressed stimulation sites. First results of real time focal chemical stimulation of cell cultures are presented.

EXPERIMENTAL

The basic unit of the platform is a surface micromachined Parylene C microchannel (100 μm x 4 μm x 6 mm) perforated with a single central pore (20 μm diameter) (Fig. 1). The device has integrated platinum thermal flow sensors and SU-8 microfluidic interconnects at each end. The Parylene microchannel wall is 2 μm thick, supported by posts, and selectively reinforced with a 75 μm thick layer of SU-8 to prevent buckling and deformation of the channels and interconnects. Custom packaging connects the platform to a gas-tight syringe (Fig. 2). A syringe pump controls fluid flow in the microchannel.

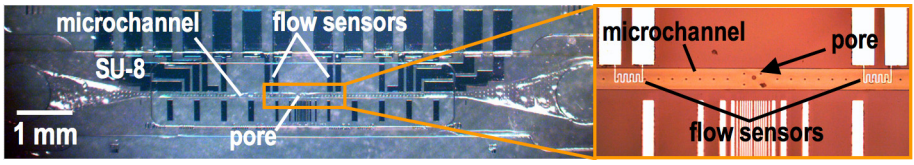

Figure 1. Microfluidic platform photographs showing the microchannel, pore location, thermal flow sensors, SU-8 reinforcement and inlets/outlets (circle and funnel at either end). Right figure is a magnified view of the pore and surrounding thermal flow sensing elements.

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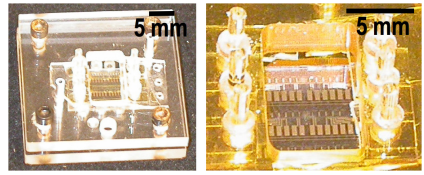

Figure 2. Packaged devices: left panel shows a die with three microchannels in an acrylic jig and the right panel shows a close up of a die in an Ultem jig.

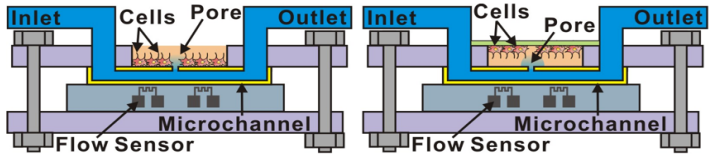

Figure 3. Testing setup: left panel shows a die with cells cultured on top of microchannel and the right panel shows cells attached to an inverted glass cover slip.

Rat pheochromocytoma cells (PC12) [5] were cultured overnight on a device pretreated with polyethyleneimine (PEI) to promote cell adhesion [6]. After loading with fluo-4 dye, a pulse of a 10 mM bradykinin solution was delivered through the pore (Fig. 3). Fluo-4 is a fluorescent Ca^{2+} indicator dye that enables spatiotemporal tracking of intracellular Ca^{2+} [7]. Bradykinin induces a concentration-dependent release of intracellular Ca^{2+} stores [8], which was observed by fluorescence microscopy. The time evolution of average cell brightness as a function of distance from the pore is shown in Figure 4.

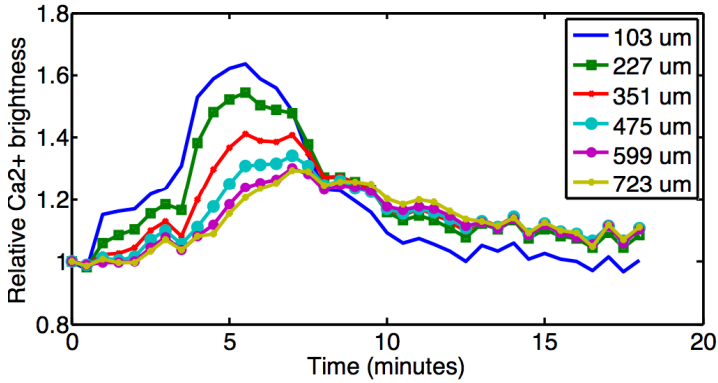

Figure 4. Comparison of mean light curves from 6 concentric 150-pixel thick annuli at different distances from the pore after focal delivery of a burst of bradykinin.

RESULTS AND DISCUSSION

The peak brightness and the timing of bradykinin-induced Ca^{2+} fluorescence changes as a function of distance from the site of delivery, as shown in Figure 4. Cells closest to the pore have the brightest, narrowest peak and show the earliest rise

in brightness. Cells furthest from the pore have the faintest peak brightness, broadest peak, and latest increase in brightness. This spectrum of bradykinin-induced responses demonstrates a decreasing bradykinin concentration with increasing distance from the pore. In a second test used to simulate tissue, PC12 cells were attached to a PEI-coated glass chip which was inverted and suspended 75 μm above the pore (Fig. 3). Continuous delivery of ~30 mM Rhodamine B clearly showed a slow radial progression of Rhodamine uptake by the cells as a function of time for a given flow rate from the pore (15 nL/min) (Fig. 5).

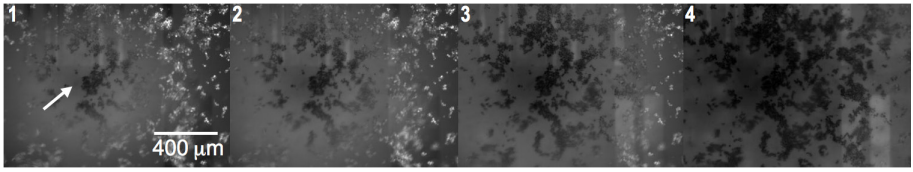

Figure 5. Time sequence of continuous ~30 mM Rhodamine B delivery to a culture of PC12 cells spanning eight minutes. Each frame measures 1 x 1.3 mm, and the location of the pore is indicated by the arrow. White cells are fluorescing after treatment with fluo-4, whereas black cells have taken up the Rhodamine B.

CONCLUSIONS

Focal chemical delivery and stimulation from a microchannel-addressed pore has been demonstrated. This approach is scalable to a high density pore platform in which each pore is individually-addressed such that specific cell populations can be targeted. Integration of electrodes will enable a multi-modal neural interface.

ACKNOWLEDGEMENTS

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