Fabrication and Characterization of a Microfluidic Module for Chemical Gradient Generation Utilizing Passive Pumping

Jonathan T.W. Kuo*, Connie Li*-*EMBS Student Member*, and Ellis Meng, *IEEE Senior Member*

***Abstract*— We introduce a micro-biochemical administration module (μBAM) for generating chemical gradients for use in axonal guidance studies. The device is designed to be simple to use, require minimal packaging, and be operated using only a pipette. A passive pumping mechanism is utilized to pump liquid through a SU-8 microchannel and then the micropore on the Parylene cap of the microchannel. The achievable flow rate delivery through the micropore was characterized and manipulated by varying the drop volumes used to passively drive fluid flow into the device. Biochemicals controllably delivered using this module can be combined with neuronal cell cultures to form chemical gradients for axonal guidance studies.**

I. Introduction

Biomolecular gradients play a pivotal role within biological systems guiding the growth, migration, differentiation, apoptosis as well as many other functions of cells through chemical signaling. Chemical signaling is complex with gradient composition, concentrations, and spatiotemporal characteristics all dynamically influencing cellular microenvironments and thus cellular behavior.

However, studying cellular behavior with respect to changes within the cellular microenvironment remains difficult. Conventional tools such as puffer micropipettes, the Transwell assay, or the Zigmond chamber for gradient generation possess limited capabilities for mimicking *in vivo* conditions with relatively static chemical gradient generation potential and high operational difficulty [1]. Oftentimes, whole cell populations are exposed to a chemical gradient whereas it is often desirable to precisely target specific subpopulations or even a subcellular region.

Micromachining technologies have been exploited to construct microfluidic devices capable of presenting chemical gradients in a highly controllable manner, thereby overcoming limitations associated with conventional tools. Agrin was focally delivered to myotubes cultured on top of a microfluidic pore simulating *in vitro* synaptogenesis of a neuromuscular junction [2]. A microfluidic device capable of focally targeting a subpopulation of cells with full user control over the chemical concentration presented was reported [3], but was limited to the same chemical concentration over the entire device. Another microfluidic device consisting of multiple channels each possessing an individual pore enabled multiple focal chemical gradient generations within the same device [4]. Microfluidic devices consisting of multiple input channels have also been constructed for delivering chemical gradients to cells seeded and cultured within the channels [5]. These devices were able to present chemical gradients to subcellular regions by generating subcellular fluid streams through the exploitation of laminar microchannel flow and diffusion-limited mixing of multiple microfluidic inputs [6].

All of the aforementioned microfluidic devices require pressure-driven flow in order to maintain steady-state gradient delivery. Pressure-driven flow is typically achieved using a syringe pump or other off-chip pneumatic sources. The requirement for large external equipment, electrical power, and complicated microfluidic connections places restrictions on microdevice portability and hampers adoption by end-users not accustomed to such infrastructure.

Microdevices for studying axonal growth have been constructed by Taylor *et al.* such that hydrostatic pressure within the device alone was utilized to present a steady-state gradient between two compartmentalized chambers separated by an array of microchannels [7]. Filling one chamber with more fluid than the other and maintaining the fluid height differential generated a constant hydrostatic pressure that drove fluid through the array of microchannels. This technique enabled axonal growth studies by separation of the soma from the axon and presenting a different chemical environment between the two [8]. Moreover, this device was not reliant on external sources of pressure-driven flow; greatly simplifying device operation and significantly decreasing the experimental system footprint.

For this work, the goal was to achieve a microdevice capable of targeted focal delivery of chemicals for axonal growth studies; this is not possible with Taylor *et al.*'s microdevice paradigm which exposes the entire cell population to a uniform chemical gradient. Our approach specifically avoids off-chip pneumatic flow sources and all the complex requisite packaging. Most importantly, our approach was selected to enable an inexpensive, simple-to-use device for neuroscientists and other biologists.

II. Design

A. A Modular Approach

The task of separating neuronal soma and their respective axons is delegated to a micro-compartmented cell culture module (μCCM), a polydimethylsiloxane (PDMS) device similar in design to Taylor *et al.*'s device except with accessible compartments. This separation allows for targeted

*Both authors contributed equally to this work.

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J.T.W. Kuo, C. Li, and E. Meng are with the University of Southern California, Los Angeles, CA 90089 USA (phone: 213-821-3949; e-mail: ellis.meng@usc.edu).

chemical delivery to either the soma or axons and subpopulations with the use of another device, a micro-biochemical administration module (μBAM). μBAM placement controls the spatial characteristics of delivered chemical gradients. Multiple μBAMs can be used in conjunction, enabling experimental flexibility with user-controlled gradient generation (Figure 1). For this work we focus on μBAM.

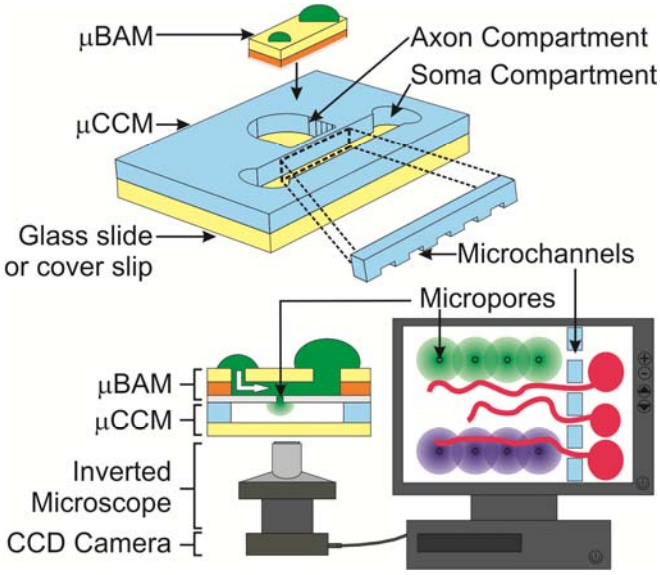

Figure 1. Illustration of modular microfluidic system that enables flexible user-defined chemical gradient presentation for axonal guidance studies. Multiple μBAMs can be placed with a single μCCM (bottom).

B. *Focal Delivery*

Focal delivery was achieved with fluid flow through a micromachined Parylene C pore addressed by a SU-8 microchannel. Parylene C, a United States Pharmacopeial (USP) Class VI material is widely used within biomedical microsystems [9]. Moreover, Parylene can be micromachined through plasma etching [10] with submicron pore diameters [11].

C. *Passive Pumping*

Passive pumping is exploited to drive fluid flow within the microchannel. This approach takes advantage of the surface tension differential present within two liquid bubbles of different radii. When fluidically connected, e.g. through a channel, the internal pressure within the smaller bubble exceeds that of the larger and a pressure gradient is generated that drives fluid flow from the smaller bubble to the larger [12]. Microdevices have been constructed from PDMS for passive pumping since a hydrophobic surface is required to maintain flow [13]. Passive pumping enables fluid flow through a microchannel simply by operating a pipette obviating the need for external fluid pumps. This also makes μBAM an extremely simple-to-use device since the end-user only needs pipetting knowledge for operation.

III. Fabrication

A bare 3" Si wafer was used as a carrier substrate during the fabrication process (Figure 2). A 5 μm thick Parylene layer was first deposited. SU-8 2035 was then spun on (~55 μm). The negative photoresist was relaxed for 10 minutes prior to softbaking on a leveled hotplate. Next, a controlled softbake was performed (65 °C for 10 minutes followed by a temperature ramp to 95 °C and 30 minute bake). The hotplate was turned off resulting in slow, convective cooling to room temperature. Microchannels were patterned into the SU-8 coated wafer (UV exposure of 500 mJ). A post-exposure bake was performed in the same manner as with softbaking. Finally, the SU-8 was developed.

A micropore for chemical gradient delivery was formed through the base Parylene. First, thick AZ 4620 (~9.6 μm) was spun on and patterned to serve as a Parylene etch mask. The Parylene was then selectively removed in an oxygen plasma environment using a reactive ion etcher (100 W: 100 mT) until etched through to the silicon support wafer. The device outline was also patterned and etched during this step.

Finally, the devices were released from the carrier wafer by soaking in DI water and gentle separation from the supporting substrate with tweezers. A standard clean was then used to remove the photoresist etch mask.

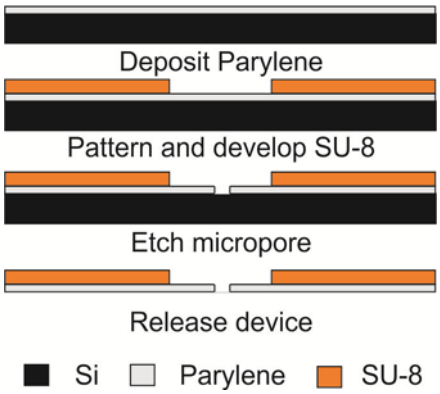

Figure 2. Simplified fabrication process for μBAM.

IV. Packaging

Following device fabrication, a clean 3 mm thick PDMS sheet of identical dimensions was bonded to the anterior side of the device using only surface adhesion to form a watertight seal. This PDMS slab capped the initially open microchannel and provided a hydrophobic surface for the test droplets. By utilizing the transparency of PDMS, the location of the access ports with proper alignment with the ends of the SU-8 microchannel was easily identified. The ports were then cored through the PDMS with a 14 gauge needle to allow for fluid access to the microchannel.

V. Methods

The passive pumping technique was characterized by monitoring the flow rate through the Parylene film micropore. To simulate the wet environment of a neuronal cell culture, the packaged μBAM device rested on two

blocks in a manner that ensured consistent contact with the surface of a distilled water bath. The device was filled with a green dyed delivery fluid to facilitate visualization of the flow into the water bath.

The microchannel and ports were initially filled with dye until the fluids in both ports were level with the PDMS sheet i.e. a primed device. This represents the base level of dye with which each experiment began.

The reservoir drop which consists of a larger volume of dye was first pipetted onto one of the PDMS ports. A smaller volume of dye was then applied to the other port to serve as the pumping drop which is the source of internal pressure for driving passive pumping (Figure 3).

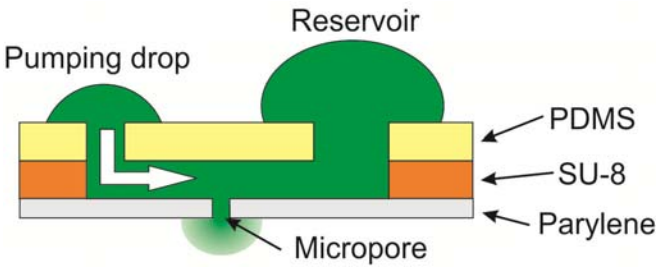

Figure 3. Operating principle for μBAM with the arrow indicating flow direction.

VI. Results

A. Fabrication

While previous passive pumping devices were primarily fabricated with PDMS, we successfully utilized microfabrication techniques to construct a passive pumping device using SU-8 and Parylene. The use of Parylene permitted the creation of a well-defined pore through selective etching (Figure 4). In addition, the use of SU-8 was particularly beneficial for its increased stiffness and chemically inertness compared to PDMS. Operation of the device requires constant chemical exposure; use of PDMS material for construction would result in the risk of chemical absorption, skewing the desired results [14].

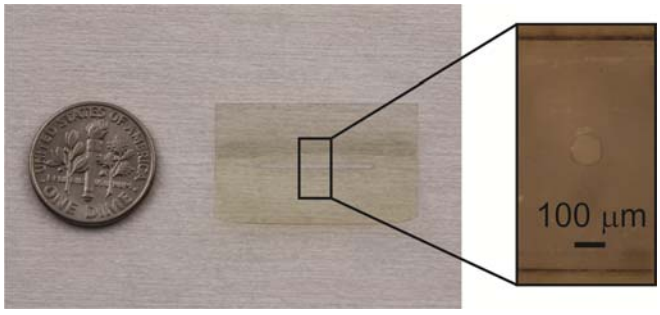

Figure 4. Fabricated μBAM device (left) with etched Parylene pore (right). The slight curvature (left) is due to residual stress within the SU-8 which can be mitigated by bonding with a flat PDMS sheet.

While the PDMS sheet acted as a cover and a hydrophobic surface in which the droplets could be dispensed, the main working components of this device are comprised of Parylene and SU-8. The non-permanent bond between the PDMS and μBAM enables the silicone sheet to be replaced should undesirable absorption be observed. Figure 5 demonstrates the dye flowing across the SU-8 microchannel, through the Parylene film micropore, and into the distilled water bath. It can also be observed that the fluid flows smoothly through the Parylene film micropore to produce an uninterrupted chemical stream into the bath.

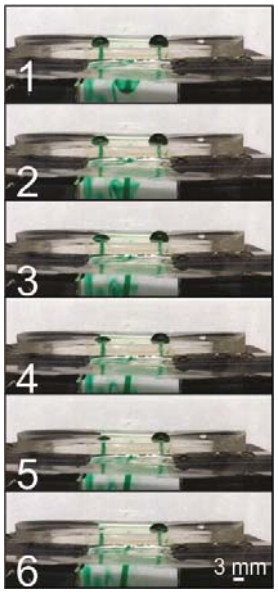

Figure 5. Time-lapse photography (5 s between each image) captures device operation with passive pumping. Drops of green dye are first applied via pipette to the PDMS sheet's access ports. The green dye is successfully delivered into the microchannel and through the micropore to the water bath below without use of an off-chip flow source.

B. Fluid Delivery into Microchannel

In order to characterize how the flow rate might be modified in accordance with user specification, different pumping drop volumes were applied and the time it took to for the pumping drop to completely dispense was observed (Figure 6). The total volume between the pumping and reservoir drops was maintained at ~60 μL for each trial.

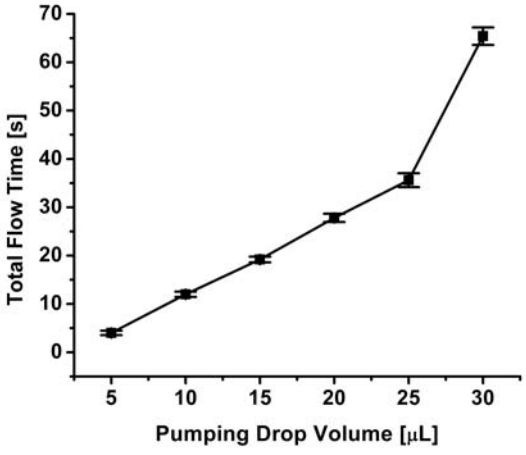

Figure 6. Pumping droplet size and corresponding duration of generated flow (mean ± S.E. with $n = 5$).

For pumping drop volumes between 5-25 μL, a linear increase in total flow time was observed. This corresponds to a consistent microchannel flow rate of ~0.63 μL/s for all pumping volumes within this regime. This result by itself was surprising since it was expected that different pumping drop sizes would possess different internal pressures resulting in varying flow rates. For the pumping drop volume of 30 μL, the flow rate was ~0.46 μL/s, a decrease of ~30%. This decrease was attributed to the pumping drop being close in size to the reservoir drop (35 μL), resulting in a reduction of internal pressure differential that drives passive pumping.

C. Fluid Delivery Through Micropore

To further investigate, different ratios between the pumping and reservoir drop volumes were investigated for their effects on flow rate through the micropore. The full range was explored, from a ratio of 0 in which only one drop was pipetted with no reservoir, to parity between reservoir and pumping drops (Figure 7).

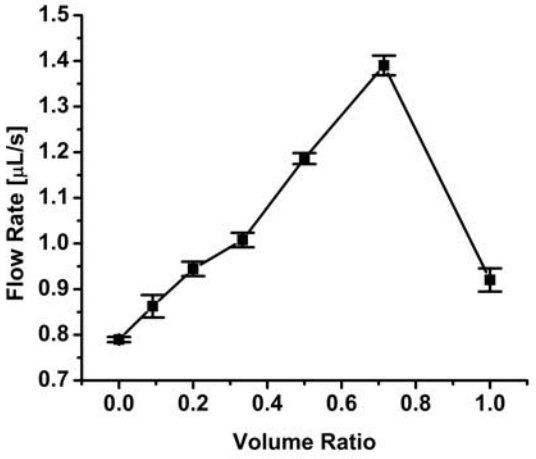

Figure 7. Different volume ratios between pumping and reservoir drops were examined for possible effects on flow rate (mean ± S.E. with $n = 5$).

The flow rate was observed to increase with increasing volume ratio however, at parity the flow rate decreased just as before. This suggests that chemical gradient generation can be manipulated by varying drop volume ratios.

An interesting observation occurred at the extremes with a ratio of 0 and ratio of 1. A ratio of 0 would not be expected to experience flow since there is no reservoir drop. However, flow did occur and the drop volume flowed completely through the micropore into the water bath. This indicates that passive pumping can occur within μBAM without a reservoir drop since the fluidic connection made through the micropore can act as a reservoir with no internal pressure. This hypothesis was further confirmed with the observation that after the complete dispensing of the smaller pumping drop, the larger reservoir drop also dispensed through the micropore until it was completely gone. While gravity and a relatively large pore diameter may be factors influencing this behavior, we suspect that surface tension effects associated with passive pumping play a more dominant role.

With a ratio of 1 in which both drops were of the same volume, all of the dye in both drops eventually flowed through the micropore. The decreased flow rate could be due to opposing fluid flows within the microchannel since both drops would possess the same internal pressures.

VII. Conclusion

With the use of passive pumping, chemical gradient generation can be highly tailored with easy-to-use microfluidic modules. With respect to μBAM, the observed behavior that a reservoir drop is not required points to an even more simplified design. However, other variables such as micropore diameter, microchannel dimensions, and environmental factors must be examined more in depth. The ease of use of this device offers advantages in flexibility and user convenience.

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