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Generating spatially and temporally controllable long-range concentration gradients in a microfluidic device

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Abstract—Concentration gradients have important applications in chemical and biological studies. Here we have achieved rapid generation of spatially and temporally controllable concentration gradients of diffusible molecules (i.e., proteins or toxins) in a portable microfluidic device. The formation of the concentration gradients was initiated by a passive-pump-induced forward flow and further optimized during an evaporation-induced backward flow. We were able to stabilize the gradient for up to 12 hours by stopping the flow. The computational simulations illustrated the combined effects of convection and diffusion on the gradient generation, and fit well with the experimental data. HL-1 cardiac cells were cultured in the device, and were shown to be responsive to the stabilized concentration gradient of a cardiac toxin, alpha-cypermethrin. The approach presented here may be useful for many biological and chemical processes that require rapid generation of long-range gradients in a portable microfluidic device.

I. INTRODUCTION

Several chemical and biological phenomena (e.g., chemotaxis, morphogenesis and wound healing) involve concentration gradients of diffusible molecules (chemical compounds or biomolecules) [1]. While a variety of approaches exist for generating gradients of diffusible molecules, these techniques typically require external pumping equipment and a large quantity of reagents [2]. These factors limit the portability of the device, and also constrain their applications for expensive materials (i.e., growth factors, drugs).

A pump-less and portable microfluidic device that generates controllable gradients is quite beneficial for field testing and high-throughput studies. Long-range gradients can be used to test the effects of molecular dose responses on cell behaviors. For our study, we implemented the passive-pump technology, which was first developed by Walker et al., in order to eliminate the use of external pumps. Passive pumping requires only a pipette to produce small drops of liquid [2,3]. The surface tension difference between the larger drop of solution at the outlet and the smaller drop of solution at the inlet was used to pump the small drop of liquid through the microchannel. Evaporation has also been used as the driving force in ‘pump-less’ microfluidic devices to induce a backward flow.

In this study, we utilized both the forward flow and the evaporation-driven reversed flow to rapidly establish centimeter-long concentration gradients of molecules along the channel of a simple and portable microfluidic device. First, we introduced diffusible molecules into the device and generated a forward flow, by passive-pump technology. Evaporation-induced backward flow from the outlet to the inlet of the channel followed, and resulted in dynamic concentration gradients of the molecule. The spatially and temporally controllable concentration gradients were parallel with the flow along the channel, and by stopping the flow we were able to stabilize a particular gradient. HL-1 cardiac cells were seeded along a channel, and treated with a stabilized gradient of a cardiac toxin to test the response of the cells.

II. RESULTS

A. Generation of the concentration gradient by passive-pump-induced forward flow and evaporation-induced backward flow

We aimed to generate a stable concentration gradient by using the process shown in Fig. 1. The microfluidic channel was initially filled with DPBS, and a 200 µL drop of DPBS was pipetted onto the outlet. A small drop of 2 µL DPBS containing FITC-Dextran was then dropped onto the inlet (Fig. 1) and entered the channel automatically due to the differential surface tensions between the drops. After the small drop entered the channel completely, a second drop was pipetted onto the inlet to continue the forward flow.

Fig. 1. Introduction of molecules into the channel by the passive pump phenomenon.

Evaporation followed the forward flow, resulting in the
formation of the concentration gradient. We were able to stabilize the gradient for up to 4 hours using the oil sealing method, and up to 12 hours under 100% humidity conditions.

In our device, three drops containing the molecule of interest were sufficient to for the fluid to reach the outlet of the 5 cm-long channel. The evolution of the centimeter-long concentration gradient that formed along the channel during the 50 min backward flow was quantified by measuring the fluorescent intensity along the channel, which was assumed to be proportional to the concentration of the fluorescent molecules. During the backward flow, the fluorescent dye and the concentration gradient moved backward toward the inlet, and the gradient steepened. The evolution of the concentration gradient, after the introduction of two drops of fluorescent dye, is shown in Fig. 2. The experimental gradient results agree with the computational simulations.

Fig. 2. Fluorescence images demonstrating the evolution of a concentration gradient due to the evaporation-driven backward flow, after the introduction of two drops of a fluorescent dye into the channel.

B. Applying the stabilized concentration gradient for cytotoxicity testing

We demonstrated the applications of the spatially and temporally controllable concentration gradient by utilizing a cytotoxicity test. We treated the HL-1 cardiac muscle cell line with a cardiac toxin, alpha-cypermethrin [4,5]. As seen in Fig. 3, there is a correlation of cell viability with the toxin concentration gradient along the channel. These results corresponded to the similar experiment performed in a 96-well plate.

Fig. 3. Toxicity of a stabilized concentration gradient of alpha-cypermethrin on the HL-1 cells from outlet (5 cm) to inlet (0 cm) after 4 hours

III. CONCLUSIONS

We achieved rapid generation of centimeter-long concentration gradients of molecules using a reversed flow in a simple and portable microfluidic device. The gradients along the microfluidic channel could be spatially and temporally controlled and stabilized. Our cell viability test results indicated that the cells responded to this concentration gradient of toxins. We believe that this approach for gradient generation is a promising platform for biomedical applications (i.e. drug testing) and studying biological phenomena (i.e. chemotaxis) [6].

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REFERENCES