PARALLEL MULTI-TIME POINT CELL STIMULUS AND LYSIS IN A MICROFLUIDIC DEVICE USING CHAOTIC MIXING AND PRESSURE RESISTANCE
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ABSTRACT
A high-throughput two-module device was designed to simultaneously obtain 8 time points of cell activation through stimulation with 30s resolution rapidly and accurately. Rapid mixing and even splitting of reagents into each time-point channel are necessary features of this device. The microfluidic platform allows uniform sample handling with superior temporal resolution compared to conventional methods.

KEYWORDS: Cell stimulation, rapid mixing, parallel processing

INTRODUCTION
Understanding the dynamics of cell signaling networks is important for many biological applications. To build a complete computational model of the dynamic pathway a large data set is necessary. Gene expression and protein activity at various time points during stimulation with an external signal must be known. Many important protein activation events occur within minutes after stimulation [1, 2]. However, with conventional multi-well plate assays, it is difficult to achieve adequate resolution in the appropriate time scales. Microfluidics is a capable alternative, providing uniformity in sample handling to reduce error between experiments [3].

We present here a device for multi time-point lymphocyte stimulation and lysis for downstream analysis of protein activation. Previously El-Ali et al. developed a device achieving cell stimulus and lysis on a microfluidic chip using segmented gas-liquid flow for rapid mixing [4]. This device was the first to demonstrate on-chip multiple step manipulation of cells with fast mixing although with only one time point per experiment and large shear at the gas-liquid interface. In comparison, our device is capable of 8 time points with controlled rapid mixing, precise timed stimulation, and rapid lysis.

EXPERIMENTAL
This two-module device is molded in PDMS from a two-layer SU-8 master. During operation (Fig. 1), a syringe pump drives the flow to only 3 inlets, cells and stimulus are mixed and split into 8 equal streams in Module 1. The majority of the incubation time occurs in the tubing leading to Module 2. There the reaction is quenched and cells are burst with lysis buffer to extract intracellular components.
Rapid mixing is essential to our design for precise definition of stimulation time. A staggered herringbone mixer (HBM), modified from Stroock et al.[5], was used to achieve full mixing of reagents with minimal shear. Because our reagents have different fluidic properties, a COMSOL model was created to probe the mixing effects of viscosity and density. Figure 2 demonstrates the model agreement with data. Our model suggests that slower mixing of fluids with mismatched properties is mainly due to viscosity, not in density. Further confocal microscopy experiments, also shown in Figure 2, were used to visualize the mixing of solutions with mismatched viscosity.

Figure 1. A schematic of the device with inlets, tubing, pressure drop channels and cell lysate outlets for sample collection. The respective residence times (t) in each unit are noted, where the total time is essentially the time in the tubings, varying with length (L) and inner diameter (ID) and volumetric flowrate (Q). Insets (A) shows a close-up of Module 1 and (B) the whole device setup.

Figure 2. Mixing data from COMSOL and experiments. (A) shows the mixing for 1 cycle of herringbone mixers. (B) is the concentration profile color map from the model after 1. (C) is a confocal image of mixing after 1 cycle with one inlet containing rhodamine dye. (D) shows the mixing of two fluids with the same viscosity. (E) shows the mixing of two fluids with mismatched viscosity; the fluid with no fluorescent dye has a viscosity 20 times the other. (B-E) are all in the y-z plane.
Equal flow rates in each stream were achieved by balancing the channel resistance. The resistance increases with increasing length and decreasing cross-sectional area. All channels have the same small dimensions with pressure drops orders of magnitude greater than that of the tubing.

RESULTS AND DISCUSSION

With the current herringbone design, mixing was achieved in less than 0.2 sec on the stimulation chip (and <0.9 sec on the lysis chip), an extremely small percent of stimulation (or lysis) time. Our data suggest the viscosity effect occurs only at viscosity ratio of 6:1 and above for the current HBM design. Our operation condition are below this ratio. Therefore the HBM is adequate for fast mixing of cells and stimulus in Module 1, and of cells and lysis buffer in Module 2.

Although to set up multiple stimulation times we use different lengths of tubings (with varying inner diameters), the evenness of flow rates is not influenced. Figure 3 shows the distribution of flow rates across different timepoints with varying tubing dimensions. This configuration gives us large flexibility of the specific time points for cell stimulation. With commercially available tubings, we can achieve stimulation times ranging from 20 seconds to 1 hour very easily.

CONCLUSIONS

Our device successfully accomplishes a multi-time point stimulation experiment with on-chip cell lysis. Rapid mixing, achieved with the staggered herringbone array, allows precise time point resolution down to ~20 seconds. The large flexibility of this design allows a wide range of time points with simple modification.

REFERENCES