MICROFLUIDIC TEMPORAL CELL STIMULATION

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ABSTRACT

We present a novel microfluidic platform for probing cellular signaling networks, combining a programmable system for generating arbitrarily complex temporal input stimulant concentration profiles with real-time fluorogenic monitoring of cell physiological response. Applying microfluidics to multi-parametric systems biology problems, like the analysis of signal transduction pathways, requires a high level of automation to rigorously control cellular environments and detect output signals. Addressing this challenge, we have developed a Java-based programmable microvalve system for automated control of reagent delivery, enabling the end user to generate complex chemical waveforms and perform multiple assays on individually addressable cell chambers on chip.

KEYWORDS: microfluidics, signal transduction, complex systems, cell

INTRODUCTION

The behaviour and connectivity of cellular signalling networks has typically been inferred from responses to step inputs. More recently, rectangular pulses or slow pulse trains have been introduced [1-3]. Generation of waveforms in these kinds of experiments has involved either complex programmable syringe pump systems or moving a sample through a spatial gradient to create temporal changes [4,5]. This type of stimulation can be insufficient to elucidate behaviours such as high- or low-pass filtering of oscillating inputs [3]. Our device uses computer-controlled elastomer valves to dynamically generate arbitrary concentration profiles (Fig. 1) with waveform periodicities as short as 10 seconds. This kind of complex signal input to cellular networks has not previously been reported.

THEORY

An electrical engineering approach to cell signaling networks is to treat them like a black box [1,7]. The relationship between known inputs and measured outputs can be used to calculate a transfer function that, in turn, elucidates properties of the intervening pathways. We have implemented a simple model of receptor stimulated calcium oscillation adapted from [6], which we will use to infer the connectivity of the internal signaling network from real experimental input/output response data.

EXPERIMENTAL

In our two-layer PDMS device, initial experiments have focused on change of intracellular free calcium in response to histamine. Cells are treated with a dye (fluo4AM, Sigma), which fluoresces only when bound to calcium within the cytoso-

lic compartment. Live cell imaging is used to observe both spontaneous oscillations and response peaks driven by applied waveforms of histamine concentration generated on-chip (Figs. 1D, 2B).

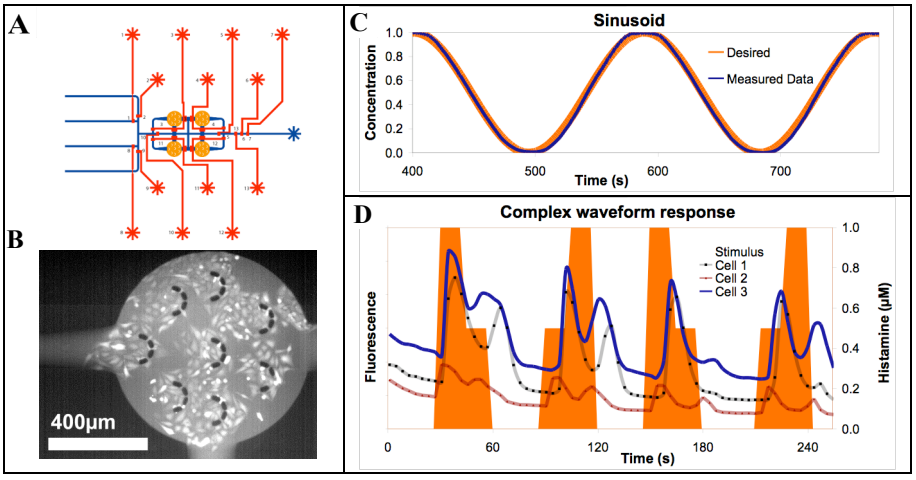

Figure 1. A; two-layer chip design. Control lines in red, cell chambers in orange, flow lines in blue. B; fluorescent image of HeLa cells in a chamber stimulated with 1µM histamine. C; Waveform generation: demonstration of system accuracy reproducing a desired output. D; three individual cell responses to a faster, multi-stepped waveform (blocks in orange).

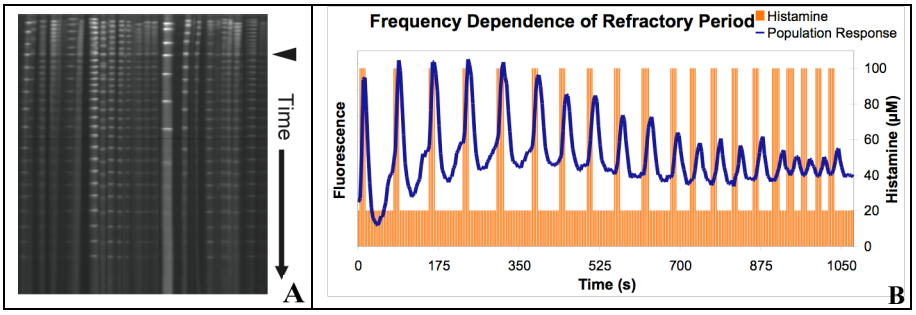

Figure 2. A; composite kymograph of calcium response to histamine. Each striped vertical band indicates an individual oscillating HeLa cell. A lower level (20µM) is applied for 3 minutes and then (▲) 15 second pulses of 100µM histamine are applied with decreasing frequency (1Hz, 0.75Hz, 0.5Hz, 0.25Hz). B; cell response averaged over a population.

With our chip design, we are able to compare responses of separate subpopulations identically cultured on a single chip to different concentrations using isolatable cell chambers. The use of multiple, individually addressable cell chambers is a unique scalable design feature that permits meaningful, simultaneous analysis of multiple input waveforms.

RESULTS AND DISCUSSION

Comparison of early experimental data obtained in our microfluidic platform with the simple oscillatory calcium model provides insight into adaptive and refractory behavior in cell signaling pathways and hints at the importance of cell to cell variability (Figs. 1D, 2A). Each cell responds with a unique natural oscillation frequency to a given steady concentration of histamine (Fig. 2A) and our system allows us to follow the response of individual cells to histamine concentration changes. Cells exhibit an increasing lag in response to a histamine pulse train of decreasing frequency (Fig. 2B). This lag is not seen in simulations using the current model and implies slower calcium release over time or a stable refractory period, a longer-term behavior not normally observable using existing plate-based assay techniques. Using iterative feedback between real, complex temporal input/ output experimental data and model simulations, we will be able to infer and then test previously hidden signal transduction connectivity and behaviour of this system.

CONCLUSIONS

The remarkable complexity of cellular signalling pathways reflects the complex external signals that such pathways were evolved to detect [7]. We have developed a novel platform that allows such complex signals to be reproduced and cellular responses measured, under programmable control. Such novel capability will revolutionize the study of mammalian signal transduction.

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