MODELING AND EXPERIMENTAL VERIFICATION OF CHANNEL GEOMETRY FOR DELIVERY OF STIMULANT WAVEFORMS TO LARGE VOLUME CHAMBERS FOR CELLULAR SYNCHRONIZATION

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ABSTRACT

The *in vivo* environment is dynamic with fluctuations in nutrient, waste, and hormonal levels. An understanding of cellular response can only be achieved by devices that recreate this environment. Microfluidic systems that produce temporal gradients have been used to evaluate dynamic cellular processes. In this report, finite element analysis was used to investigate various microfluidic architectures designed to maintain waveforms in large volume cell chambers. Multiple inputs to the chamber were found to be necessary to reduce attenuation of waveforms and maintain homogeneous concentrations across the chamber. These results were confirmed by synchronizing islets of Langerhans to a glucose wave.

KEYWORDS: Finite Element Analysis, Temporal, Gradient, Stimulant

INTRODUCTION

Microfluidic devices are well suited to mimic the dynamic nature of *in vivo* environments due to the ease in which complex gradients of stimulants can be produced. Although most reports have focused on production of spatial gradients, methods to produce and deliver temporal gradients to cells are gaining in popularity. Temporal gradients, or waveforms of stimulants, provide a means to evaluate the dynamic nature of, for example, genetic networks [1], transcriptional responses [2], or entrainment to external forcing signals [3].

Stimulant waveforms are flowed through a mixing channel to a downstream cell chamber. While passing through the mixing channel, the waveforms broaden, resulting in amplitude attenuation. This effect has been quantified [4] and shown to increase with the amount of time the waveform spends in the channel. Therefore, to reduce attenuation, the length of the channel should be decreased, or the linear velocity of the waveform increased.

While the amount of attenuation in the channel has been quantified, the effect of the cell chamber has not been taken into account. Generally, the cell chamber is larger than the mixing channel, lowering the linear velocity of the fluid and increasing the attenuation of the waveform. Depending on the size of the chamber, the waveforms may have pronounced amplitude attenuation and inhomogeneous concentration across the chamber making the results of the experiments difficult to interpret.

In this work, we investigated the attenuation and heterogeneity of stimulant waveforms in a series of cylindrical cell chambers with a volume of 0.8 μL by finite element analysis (FEA). It was found that if the mixing channel split to produce 4- or 8-inputs into the cell chamber, the amount of attenuation and heterogeneity was lowered as compared to 1- or 2-inputs. The 1- and 4-input channel designs were fabricated and used to produce waveforms of glucose to synchronize intracellular Ca^{2+} oscillations from groups of 20 islets of Langerhans. With the 4-input design, synchronization of islets was observed whereas with the 1-input design, no synchronization was observed indicating that the optimized microfluidic geometry was required to obtain an accurate view of the biological phenomenon.

EXPERIMENTAL

Simulation. COMSOL Multiphysics was used to build the finite element models. 3D cell chambers were drawn and meshed using tetrahedral finite elements. In all simulations, Navier-Stokes equations were first solved for a stationary velocity profile of creeping flows. In the creeping flow module, an input velocity was imposed on the chamber inlet according to a constant total influx rate of 1.5 μL/min provided by the pneumatic pumps. The walls in the model were set with no slip, the outlet was set with no viscous stress, and all liquids were defined with a density of 1000 kg/m^3 and a viscosity of 0.001 Pa·s. After the velocity profile was solved, parabolic partial differential equations for convection and diffusion were then solved for time-dependent profiles of transport of diluted species with a time step of 2 s. All input waveforms simulated in this work were sinusoidal with a period of 5 min, amplitude of 1 mM, and median value of 11 mM. In the transport of diluted species module, all solutes the diffusion coefficient was assumed as 10^{-5} cm^2/s.

Chemicals and Reagents. HNO_3, $CaCl_2$, NaOH, and NaCl were purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Polydimethylsiloxane (PDMS) was from Rogers Corporation (Carol Stream, IL). Glucose (dextrose), $MgCl_2$, HF and Cosmic Calf Serum were from Fisher Scientific (Pittsburgh, PA). KCl, fluorescein, tricine, dimethyl sulfoxide (DMSO) and Type XI collagenase were from Sigma (St. Louis, MO). Fura-2 acetoxymethyl ester (Fura-2 AM), Pluronic F-127, RPMI 1640 with 11 mM glucose, and penicillin-streptomycin were from Invitrogen (Carlsbad, CA). All solutions were made with Milli-Q (Millipore, Bedford, MA) 18 MΩ·cm deionized water. The buffer solution was composed of 2.4 mM $CaCl_2$, 125 mM NaCl, 1.2 mM $MgCl_2$, 5.9 mM KCl and 25 mM tricine.

Chip Design and Pumping Program. The device was fabricated by conventional photolithography and chemical etching. The microfluidic chip had two pneumatic pumps that delivered 13 and 3 mM glucose solutions into a mixing channel of 220

μm x 90 μm x 3.3 cm (width x depth x length). The pneumatic pumps were controlled by a program written in LabView (National Instruments, Austin, TX) via a data acquisition card (NI PCI-6221) and solenoid valves (model A00SC232P, Parker Hannifin Corp., Cleveland, OH). The perfusion chamber was drilled using a 0.035" diamond-tipped drill bit (Norton Abrasives, North Tonawanda, NY). In all experiments, a controller (CNi 3233, Omega Engineering, Inc., Stamford, Connecticut), thermofoil (KHLV-0502/5, Omega Engineering), and a thermocouple sensor (SA1-J, Omega Engineering) were attached to the microfluidic chip to maintain 37 °C at the center of the islet chamber bottom.

Isolation of islets of Langerhans. Islets of Langerhans were collected as previously described [3]. For monitoring intracellular [Ca^{2+}], Fura-2 was used as the Ca^{2+} indicator. Briefly, 1.0 μL of 5.0 mM Fura-2 AM, in DMSO and 1.0 μL Pluronic F-127 in DMSO were mixed and transferred into 2 mL RPMI to form a final Fura-2 AM concentration of 2.5 μM. Islets were incubated in this solution at 37 °C and 5% CO_2 for 45 min prior to transferring to the islet chamber in the microfluidic device and washed by perfusing with 3 mM glucose prior to recording Fura-2 fluorescence.

Detection. The microfluidic device was placed on the stage of a Nikon Eclipse Ti microscope for all experiments. A broadband lamp (Lambda XL, Sutter Instrument, Novato, CA) integrated with a filter wheel containing two filters (XF1093 and XF1094, Omega Optical, Brattleboro, VT) at 340 nm and 380 nm was used for excitation of Fura-2. A shutter was used to reduce photobleaching of Fura-2 by opening 150 ms for each filter every 10 s. Passing through neutral density filters (XB17, Omega Optical), the excitation light was focused onto the islet chamber through a 10X, 0.5 NA UV-compatible objective after reflection by a dichroic mirror (XF2002). The Fura-2 emission, collected by the same objective, passed through the dichroic mirror and an emission filter (XF3043) and then reached a CCD camera (Cascade, Photometrics, Tucson, AZ) for imaging. NIS-Elements (Nikon, Melville, NY) was used to control the Sutter filter wheel, shutter, and CCD camera. All measurements of Fura-2 fluorescence are reported as the ratio of Fura-2 emission at 520 nm upon excitation by 340 nm and 380 nm (F_{340}/F_{380}). The F_{340}/F_{380} values are proportional to the intracellular [Ca^{2+}].

RESULTS AND DISCUSSION

To investigate the broadening and delay of waveforms, cell chambers were modeled using FEA. Figure 1 shows a 3D view and a cross-sectional view of a 0.8 μL chamber with a single inlet from the mixing channel. As can be seen, there is significant heterogeneity of the waveform concentration along the bottom of the chamber where cells would be located. This result would lead to each cell sensing a different concentration and make investigations of cell populations difficult.

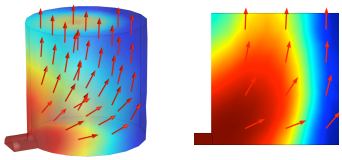

Figure 1: A 3D and cross-sectional view of a concentration waveform as it passes through a chamber with a single inlet. The colors represent the concentration (red is high, blue is low) and the red arrows indicate the direction of the flow.

To reduce the attenuation and heterogeneity of the concentration waveform along the bottom of the chamber, multiple numbers of inlets into the cell chamber were tested. Figure 2 shows a slice of the concentration along the bottom of the 0.8 μL chamber with 1-, 2-, 4-, and 8-inlets.

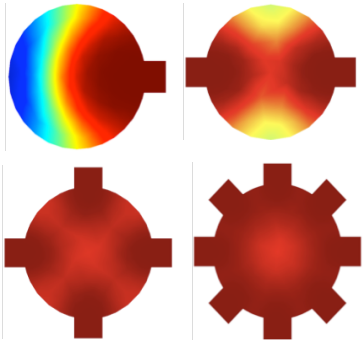

Figure 2: Multiple inlets to bring the waveform into the chamber resulted in homogeneous concentrations across the chamber for the 4- and 8-inlet designs.

The 1-inlet chamber had the widest concentration distribution and largest broadening and delay of waveforms among all four designs. With increased inlet numbers, the concentration distribution became more narrow. However, an 8-inlet chamber was difficult to fabricate because the thin walls between channels were easy to break while drilling the chamber. In contrast, a 4-inlet design was easy to fabricate and provided similar concentration profiles as the 8-input design.

To demonstrate the influence of delay and broadening of waveforms to stimulation of multiple cells and the significance to make a more homogeneous concentration across the chamber, the 0.8 μL chambers with 1- and 4-inlets were fabricated and used to demonstrate synchronization of islets of Langerhans to glucose waveforms. It has been shown that insulin release from multiple islets of Langerhans is synchronized to a periodic input of glucose[5]; and we reproduced this finding using the 4-inlet design as shown in Figure 3 (left panel). The oscillations of intracellular Ca^{2+} from 20 islets synchronized upon initiation of the glucose waveforms (green line) resulting in an average signal that showed strong and robust Ca^{2+} waves (black line). In contrast, using the 1-inlet chamber, glucose waveforms could not synchronize oscillations of intracellular Ca^{2+} from 19 islets (Figure 3 right panel) resulting in an average signal that was flat.

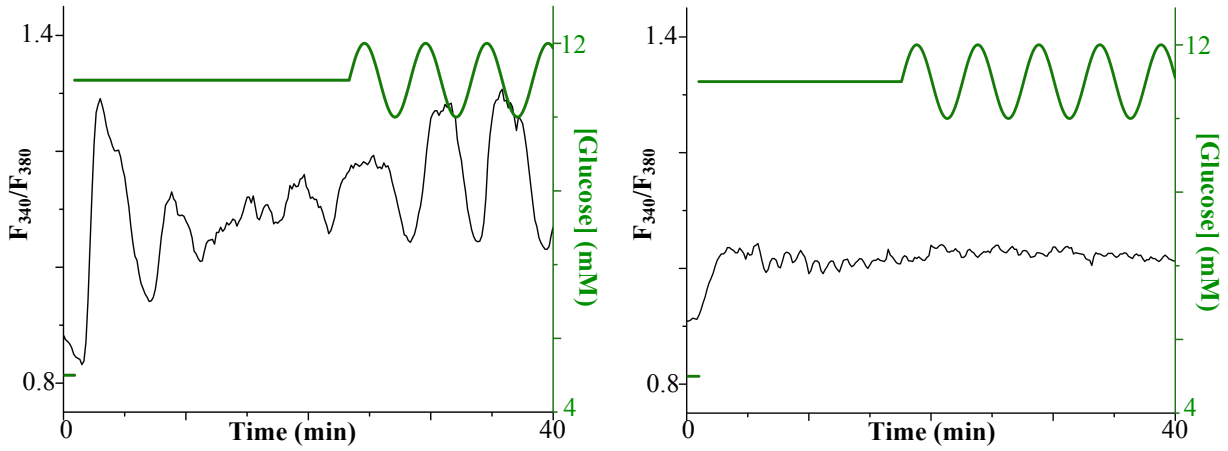

Figure 3: The average Fura-2 fluorescence value is shown from 20 islets (left panel) and 19 islets (right panel). Oscillations of intracellular Ca^{2+} (black lines) synchronized upon initiation of glucose waveforms (green line) when the chamber with 4-inlets was used (left panel) and did not synchronize when the chamber with 1-inlet was used (right panel).

The reason for these different observations were that the 1-inlet chamber had a large delay and attenuation of glucose waveforms resulting in each islet in the chamber sensing a different glucose concentration. Therefore, none of the islets entrained to the same waveform resulting in an unsynchronized response. In contrast, using the 4-inlet design, the glucose concentration was essentially homogeneous across the chamber allowing all islets to entrain to the same waveform. This result then produces a synchronized response from the islet population.

CONCLUSION

A microfluidic device was optimized using FEA to maintain stimulant waveforms in a large volume chamber. Optimized and unoptimized devices produced different biological results highlighting the importance of proper microfluidic design.

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