Single cell NF-κB dynamics observed under stimulant concentration gradient

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Supporting Information

Experimental Section

Microchip fabrication

The microfluidic chip was fabricated by multilayer soft lithography in polydimethylsiloxane (PDMS; RTV 615 A and B; GE Silicones, Wilton, CT, USA) as described previously [1]. Two photomasks were first generated with the microscale patterns designed by computer-aided design software FreeHand MX (Macromedia, San Francisco, CA, USA) and printed on high-resolution (5,080 dpi) transparencies. The masters for both control layer and fluidic layer were made of negative photoresist SU-8 2025 (Microchem, Newton, MA, USA) spun on 3-inch silicon wafers (University Wafer, South Boston, MA, USA). Afterwards, well-mixed PDMS with a mass ratio of RTV615 A: RTV615 B = 10:1.1 was poured onto the control-layer master to generate a ~ 5 mm thick control layer, and spun onto the fluidic-layer master at 1100 rpm for 35 s, resulting in the thin fluidic layer (~110 μm in the thickness). Both layers of PDMS were partially cured at 80 ºC for 30 min. The control layer was then peeled off from the master after cutting by a razor blade. The control-layer stamp was aligned with and bonded to the fluidic layer after both PDMS surfaces were oxidized in a plasma cleaner (Harrick Plasma, Ithaca, NY, USA). The two-layer PDMS structure was baked at 80 ºC for another 30 min, peeled off from the flow layer master, and punched to produce inlet and outlet reservoirs. Once the two-layer PDMS and a pre-cleaned glass slide were treated with oxygen plasma, they were immediately brought into contact to form closed channels. The assembled chip was baked at 80 ºC for another 1 h to promote the bonding strength between PDMS and glass. Glass slides were cleaned in a basic solution (H_2O: 27% NH_4OH: 30% H_2O_2 = 5: 1: 1, volumetric ratio) at 75 ºC for 3 h and then rinsed with ultrapure water and thoroughly blown dry.

System setup and operation

The microfluidic chip was mounted on an inverted fluorescence microscope (IX-71, Olympus, Melville, NY, USA) equipped with a 10× dry objective. The reagents were introduced into the inlets driven by a syringe pump (Fusion 400; Chemyx, Stafford, TX, USA). The microfluidic valve was actuated by applying pressure in the control channel via a solenoid valve (ASCO Scientific, Florham Park, NJ, USA). The pressure (~20 psi, provided by a nitrogen cylinder) deformed the thin PDMS membrane (~50 μm in the thickness) between the fluidic and control channels and partially closed the fluidic channel. A DAQ card (NI SCB-68; National Instruments, Austin, TX, USA) and a LabVIEW program were employed to control the switching of the solenoid valve. Prior to experiments, the control channels were pre-filled with water to prevent bubble formation in fluidic channels.

Cell culture

Chinese hamster ovary (CHO)/GFP-NFκBp65 cells (Affymetrix, Santa Clara, CA, USA) were grown in Ham's F12K medium (ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 μg/ml hygromycin B (Roche, Indianapolis, IN, USA) and 100 U penicillin-100 μg streptomycin/ml (Invitrogen) at 37 ºC in a humidified incubator containing 5% CO_2. Cells were subcultured every two days to maintain them in exponential growth phase. Once harvested, the cells were centrifuged at 300*g* for 5 min to remove the supernatant, and resuspended in the medium at a concentration of 10^7 cells/ml.

Channel surface preparation

Immediately after the device was made and while the surface was hydrophilic, the microfluidic device was flushed with phosphate buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) to remove the bubbles. 50 μg/ml of fibronectin from bovine plasma (Sigma-Aldrich) in PBS was then pumped through the channels at 2.5 μl/min for 10 min to promote cell adhesion. The device was then incubated at 37 ºC for 1 h to allow adsorption of fibronectin onto the device surfaces. Excess fibronectin was flushed out of the channel with cell culture medium.

Cell seeding, culture and stimulation

CHO/GFP-NFκBp65 cell suspension (~10^7 cells/ml) was loaded from the outlet of the device and allowed to flow under gravity into the microfluidic culture chamber, while the microfluidic valve was partially closed to prevent cell entry into the channel network. Once the appropriate cell density was reached, the device was submerged in culture medium and incubated at 37 ºC to allow the cells to attach to the surface. After incubation for 30 min, a micropipette with the tip cut off was fitted into the punched outlet hole and filled with culture medium to maintain medium perfusion and cell growth. The whole device was then transferred into a 37 ºC, 5% CO_2 humidified incubator. After culturing for 24 h, 100 mg/ml bovine serum albumin (BSA) in PBS was pumped through the network of the channels at 0.05 µl/min for 1 h to suppress nonspecific adsorption. For cell stimulation, a specific concentration of IL-1β (Cell Sciences, Canton, MA, USA) (0.1, 1 or 10 ng/ml) in PBS was flowed through the left inlet at 0.05 µl/min while PBS (without IL-1β) was flowed through the right inlet at the same flow rate to generate the desired gradient in the culture chamber. Cellular activation was measured by capturing images using fluorescence microscopy to track the translocation of GFP-tagged NF-κB from the cytosol into the nucleus. The images were analyzed using ImageJ.

Simulation of concentration gradient

The diffusion coefficient of a protein D was calculated using the below Einstein-Stokes equation, assumed to provide isotropic diffusion.

$$D = \frac{k_B T}{6\pi\eta r}$$

Where k_B is Boltzmann's constant, T is absolute temperature, η is the viscosity of the surrounding liquid, and r is the hydrodynamic radius of the protein. The hydrodynamic radii of IL-1β (17 kDa) and β-Casein (24 kDa) were estimated using a curve constructed with known hydrodynamic radii and molecular weights of various proteins. The concentration gradient system was modeled using COMSOL Multiphysics (COMSOL, Burlington, MA, USA). Navier-Stokes equation, continuity equation, and diffusion-convection equation were solved in order to simulate the velocity and the concentration

profile of the system. PBS was simplified in the model as water due to their similarity in viscosity and taken to be at 20 ºC. All the microfluidic channel walls were assumed to have no slip conditions. Pressure was assumed to be atmospheric at the outlet. Inlet velocities were set as 0.3086 mm/s at the inlets, which correspond to 0.1 µl/min for the combined flow. The concentrations in the two inlets were set as 1 and 0 in order to provide simulation results as fractions or percentages of the solute compared to the initial concentration. The model was solved for steady-state and the flow was assumed to be incompressible.

References

(1) Unger, M. A.; Chou, H. P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288*, 113.

Supplemental Figure and Movie

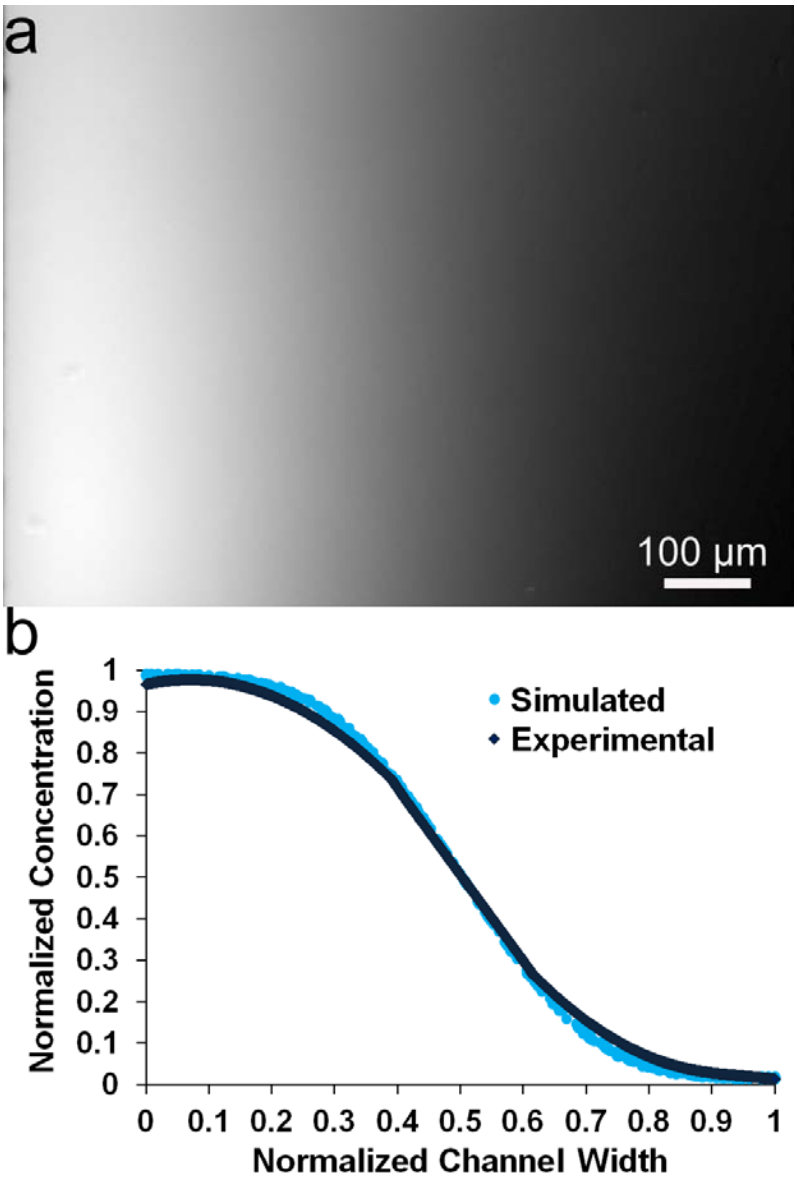

Figure S1. The comparison of concentrations generated by COMSOL modeling and experiments using labeled β-casein (24 kDa) as an example. **(a)** A fluorescent image in the microfluidic chamber (Z=3.65 mm) when FITC-conjugated β-Casein was introduced from one inlet and PBS was flowed from the other inlet, both at 0.05 µl/min. The microfluidic chamber did not contain seeded cells. **(b)** Comparison of simulated (by COMSOL, light blue) and experimental (dark blue) concentration profiles (both at Z=3.65 mm).

Movie S1. A typical video (.avi) showing single cell NF-κB dynamics observed in the concentration gradient device. The video shows CHO/GFP-NFκBp65 cells stimulated with IL-1β at the concentration of 1 ng/ml coming in from the left inlet (and PBS in from the right inlet). The images were captured at Z = 2.95 mm in the microfluidic culture chamber. The interval between images was 5 min, and the total duration was 130 min. The percentage of activated cells decreases and the response time increases from left to right, due to IL-1β concentration decrease in the range of 0.1-1 ng/ml.