

Hydrodynamic gating for sample introduction on a microfluidic chip – *Supplementary Information*

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Chip design and fabrication

As illustrated in Fig. 1, a simple microchip with a cross-shaped microchannel was used in the experiments. The length of channel S-O, B-O and O-W were 6.5 mm. The length of channel O-C was 18.5 mm. The channel width and depth were 80 μm and 60 μm respectively. The microchip was fabricated using standard soft lithography techniques with PDMS (Dow Corning, Sylgard 184) as the structural material^{1, 2}. In brief, SU-8TM (GM 1070, Gersteltec Sarl, Switzerland) master was fabricated on a silicon wafer. PDMS structure (base oligomers and curing agent in a ratio of 10:1) was formed by molding the SU-8TM master. After heated at 65 °C for 4 hours, the patterned PDMS structure was peeled off from the master and perforated on each ends of the microchannels by a hole puncher with an inner diameter of 2.5 mm (Rubicon No.18, Japan). The PDMS structure was then bonded to a clean glass slide after oxygen plasma treatment (PDC-GC-M, Weike Spectrum, Chengdu, China). Quartz tubes (inner diameter: 4 mm, height: 12 mm) were attached to the punched holes as inlet and outlet reservoirs.

Chemicals and reagents

NaOH, NaClO, fluorescein, sucrose and D-glucose were purchased from Tianjing Chemical Co. Ltd (Tianjing, China). Penicillin, streptomycin and bovine serum albumin (BSA), Adenosine 5'-triphosphate disodium salt (ATP) were purchased from Sigma-Aldrich (MO, USA). Fluo-3/AM was purchased from Biotium (CA, USA). SGB solution, containing 8.55% sucrose, 1% BSA, 0.1% glucose, 100 units mL^{-1} penicillin, 100 $\mu\text{g mL}^{-1}$ streptomycin and 100 $\mu\text{g mL}^{-1}$ gentamicin, was used as the buffer solution in the injection of nematode eggs. Tyrode's solution, containing NaCl 137 mM, KCl 5.4 mM, CaCl_2 1.3 mM, MgCl_2 1 mM, D-glucose 10 mM, HEPES 10 mM, pH 7.2, was used as the washing buffer in cell stimulation assays. All reagents were of analytical

grade unless specified otherwise. Water was purified by the Millipore-Q system (Millipore, USA). All solutions were filtrated with 0.45 μm microporous membrane filters before use.

Cell culture

HeLa cells were cultured in DMEM (Gibco) supplemented with 10% (v/v) NCS (Gibco), 100 units mL^{-1} penicillin, and 100 $\mu\text{g mL}^{-1}$ streptomycin, at 37°C in a humidified atmosphere with 5% CO_2 . HeLa cells were harvested by treatment of Trypsin-EDTA solution (Gibco), centrifuged at 1000 rpm for 8 minutes, and resuspended in cell culture media. The final cell concentration was adjusted to 10^6 cells mL^{-1} before use.

C. elegans maintenance

GFP transfected *C. elegans* (strain Bristol N2) were constructed by DNA microinjection³. The wild-type and mutant strains were maintained following the standard method⁴. For harvest of the nematode eggs, adult worms were collected from the culture plate and treated with lysis solution (0.5 M NaOH and 1% NaClO)⁵. After sequential wash with water and SGB solution to remove bacteria and debris, the released eggs were collected and resuspended in SGB solution ready for experiments.

Optical setup

Experiments were conducted on an inverted fluorescence microscope (IX 71, Olympus, Japan) (Fig. 1). A mercury lamp (100 W, U-LH100HG, Olympus) was used as the excitation source. The light emitted from the mercury lamp was filtered by a band-pass filter, reflected by a dichroic mirror, and then focused on the microchannel by a 60x objective (NA 0.7). The excited fluorescence was collected through the same objective with a high-pass filter and monitored by an attached photo counter (PMS 400A, B&H GmbH, Berlin, Germany) or a CCD camera (CoolSNAP cf2, Photometrics). Cube U-MWIB2 (460-490 nm band-pass filter, 505 nm dichroic mirror, 510 nm high-pass filter, Olympus, Japan) was used to image fluorescein, Fluo-3 and GFP-transfected nematode eggs.

Numerical simulation

Calculations indicated that the establishment of a critical gating state requires a pressure ratio of

1.92 between P_c and P_w . To evaluate this theory, three-dimensional numerical simulation was conducted using COMSOL multiphysics (COMSOL AB, Sweden). The flow patterns of sample were characterized by the incompressible Navier-Stokes equation based on finite element method. Given a static negative pressure on C, the flow patterns gradually reached the critical gating state (Fig. S1a) with an increasing negative pressure on W. Five different static pressures were tested on C. The resulting pressures on W at the critical gating state were summarized in Fig. S1c, indicating a close agreement between numerical simulations and theoretical calculations.

Flow visualization

To further validate the theory for hydrodynamic gating, we visualized the laminar flow by supplying 1×10^{-5} M fluorescein and pure water to inlet S and B respectively. A static pressure was applied on C and the pressure on W was adjusted to achieve hydrodynamic gating. The five static pressures used for numerical simulations were tested on C. A typical flow pattern at the critical gating state is shown in Fig. S1b. Results are summarized in Fig. S1c, which is consistent with both numerical simulations and theoretical calculations. This consistency validated the theory for hydrodynamic gating. It also served as guidance for setting up the pressures on W and C in the following experiments. Since the 1.92 ratio between P_c and P_w referred to the critical gating state, we chose a 1.5 ratio in practice to avoid any possible leakage of samples to the outlet channel.

Application to cell analysis

Cell analysis was demonstrated using hydrodynamic gated injection. Repeated injections of 20 μ M histamine were used to stimulate individual HeLa cells that were attached to the substrate in channel O-C within 300 μ m to the intersection O. An injection time of 20 s was used for stimulation every 100 s, and intracellular Ca^{2+} concentrations were monitored as changes in fluorescence intensity (Fig. S3). A decline of peak amplitude was observed with sequential stimulations, indicating a desensitization of H1 receptors over time, consistent with previous findings^{6,7}.

Reference

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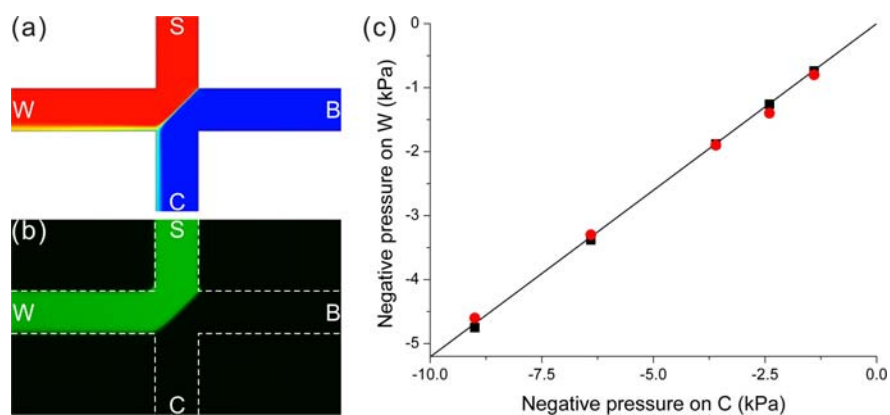


Fig. S1 Validation of the theory for hydrodynamic gating. (a) Numerical simulation of the critical gating state. (b) Flow visualization of the critical gating state with fluorescein. Dotted lines indicate the microchannel outline. (c) A comparison among theoretical calculation (black line), numerical simulation (black square) and flow visualization experiment (red circle) at five different static pressures on C, -1.4, -2.4, -3.6, -6.4, and -9 kPa.

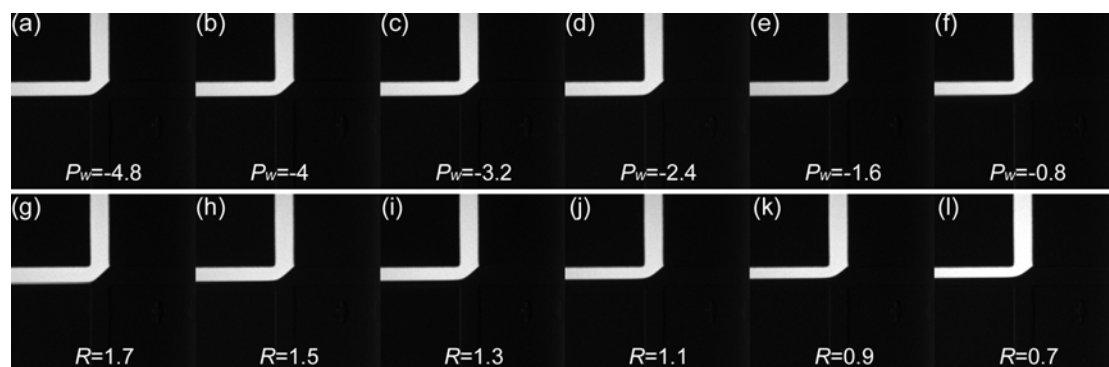


Fig. S2 Fluorescence images of gating state at the intersection of the microchannels. (a-f) The ratio (R) between P_c and P_w was maintained at 1.5 with varying P_w . (g-l) P_c was maintained at -3.6 kPa with varying R . No diffusion of samples (fluorescein, 1×10^{-5} M) to channel O-C was observed.

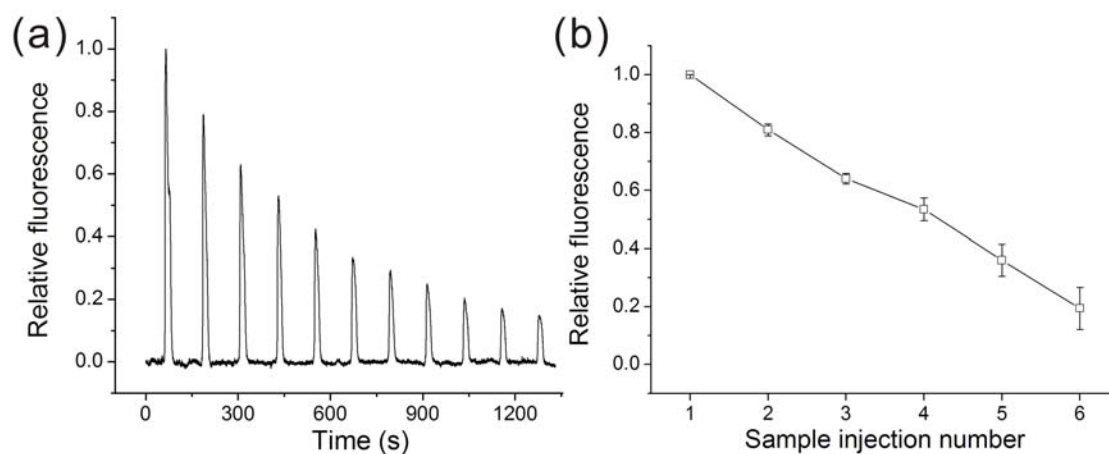


Fig. S3 Stimulation of cells with repetitive ATP stimuli. (a) Eleven consecutive ATP stimuli (injection time, 20 s; interval, 100 s). (b) Statistics for the peak height of the first six consecutive ATP stimuli (n=4).

Table S1

P_c (-kPa)	P_w (-kPa)	Ratio	Peak height* (Normalized)		Peak area* (Normalized)		Rise time [†] (ms)		Decay time [†] (ms)	
			Mean	RSD	Mean	RSD	Mean	SD	Mean	SD
1.2	0.8	1.5	0.98	0.005	1.00	0.017	46.6	1.3	56.8	1.7
2.4	1.6	1.5	1.02	0.009	1.02	0.017	32.2	2.4	30.6	1.3
3.6	2.4	1.5	1.00	0.016	1.00	0.019	23.2	1.4	21.8	1.1
4.8	3.2	1.5	1.12	0.011	1.12	0.014	16.6	1.3	16.4	0.8
6	4	1.5	1.08	0.011	1.08	0.015	15.0	1.4	14.0	1.3
7.2	4.8	1.5	1.16	0.004	1.17	0.008	10.8	1.0	12.4	1.3

Notes: * Peak heights and peak areas were normalized to the condition at ratio 1.5, where P_c and P_w were -3.6 kPa and -2.4 kPa respectively (n=20).

[†] Rise times and decay times correspond to 90% changes in fluorescence (n=10).

Video S1

Video S1 demonstrates 20 repeated injections of 1×10^{-5} M fluorescein. Samples were injected every 500 ms with 100 ms injection time. Pressures on C and W were -3.6 kPa and -2.4 kPa respectively.