Supplementary information to accompany

Dynamic formation of a microchannel array enables kinesin-driven microtubule transport between separate compartments on a chip

Kazuya Fujimoto, Moeto Nagai, Hirofumi Shintaku, Hidetoshi Kotera and Ryuji Yokokawa*

*Ryuji Yokokawa

Department of Micro Engineering

Kyoto University

Kyoto daigaku-Katsura, Nishikyo-ku, Kyoto

615-8540 JAPAN

Tel/FAX: +81-75-383-3682

Email: ryuji@me.kyoto-u.ac.jp

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1. Fabrication process.

Top PDMS layer with control channels: A silicon wafer was coated with hexamethyldisilazane to increase adhesion with an SU-8 photoresist followed by baking at 200ºC for 5 min. The SU-8 3050 resist was spin-coated at 3000 rpm for 60 s (1H-D7, Mikasa) and baked consecutively as follows: 65ºC for 2 min, 80ºC for 5 min, and 95ºC for 25 min. The channel pattern on a Cr mask (CS Hardmask Blanks, Clean Surface Technology Co.) was transferred by a mask aligner (PEM 800, Union) and developed in a SU-8 developer (Microchem) for 10 min. After rinsing with isopropyl alcohol for 1 min, the mould was baked for 30 min at 200ºC to reinforce SU-8. Finally the SU-8 mould was silanized for 2 hr with 100 μL trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane in a vacuum chamber for easy removal of the cured PDMS.

Middle PDMS layer with fluidic channels sealed by a thin PDMS membrane: A silicon wafer was coated with hexamethyldisilazane and baked at 200ºC for 5 min. An AZP 4903 photoresist was spin-coated at 2000 rpm for 60 s (1H-D7, Mikasa), yielding ~20 μm in thickness. To reduce residual stress by rapid heating or cooling, the wafer was baked at 60ºC for 2 min, 110ºC for 30 min, and 85ºC for 2 min, and thereafter kept at room temperature for >1 hr. Following UV exposure with a dose of ~600 mJ cm^{-2}, the photoresist was developed in AZ 400k developer solution (AZ Electronic) mixed 1:2 with deionized water for 10 min and rinsed with deionized water for 1 min. The mould was reflowed in an oven at 150ºC for 1 hr to form the rounded shape shown in Fig. S1 and also silanized as described above.

2. Mean square displacement calculation

The time course of mean square displacements (MSDs) along the *x* and *y* axes (see Fig. 3a) was plotted by analyzing sequential images obtained experimentally. MSDs were defined by

$$MSD_x(t) = \sum_{i=1}^{n} \frac{1}{n} \left(x_i(t) - x_i(0)\right)^2, \quad \text{(S1)}$$

$$MSD_y(t) = \sum_{i=1}^{n} \frac{1}{n} \left(y_i(t) - y_i(0)\right)^2, \quad \text{(S2)}$$

where n is a number of trajectories continuously tracked for longer than time t, and x_i and y_i are the coordinate values at i-th step. In Fig. 3c, MSDs were plotted for $n > 5$. Then theoretical MSDs in Fig 3c were calculated by

$$MSD(t) = 2Dt, \quad \text{(S3)}$$

where D is the diffusion coefficient. Assuming the average diameter of a Q-dot is 20 nm, we substituted $D = 2.5 \times 10^{-11}$ ($m^2\ s^{-1}$) calculated by the Stokes-Einstein equation.

3. Flow velocity with or without forming the microchannel array

The relationship between pressure difference, Δp, and average flow velocity, u, in a microfluidic channel was calculated by the Darcy-Weisbach equation expressed as

$$\Delta p = Ref \frac{\eta L}{2{D_\mathrm{h}}^2} u, \tag{S4}$$

where Re is the Reynolds number, f is the Fanning friction factor, D_h is the hydraulic diameter, η is the kinematic viscosity, and L is the channel length.[1] Because the flow in a microchannel is laminar, the Fanning friction factor is expressed as

$$f = \frac{64}{Re}. \tag{S5}$$

Substituting Eq. S4 into Eq. S3, u is expressed as

$$u = \frac{{D_\mathrm{h}}^2}{32\eta L} \Delta p. \tag{S6}$$

By substituting the hydraulic diameter $D_{\mathrm{h}1}$ or $D_{\mathrm{h}2}$ listed in Table S1, flow velocity in the microchannel and fluidic channel were calculated as 6.2 μm s^{-1} and 3.8 mm s^{-1}, respectively.

4. Diffusion-driven transport between two compartments

Diffusion-driven transport between compartments separated by the microchannel array was calculated by assuming two conditions: Concentration of sample molecules was uniform in a chamber, and the concentration gradient inside a microchannel was linear. When concentrations in chamber 1 and 2 are given as C_1 and C_2, respectively, the concentration in a microchannel at the distance x from chamber 1, $C(x)$, is described by

$$C(x) = \frac{C_1 - C_2}{L}x + C_2, \tag{S7}$$

where L is the length of the channel. Fick's first law provides the diffusive flux j (molecules transported per unit area per time) as

$$j = D\frac{C_1 - C_2}{L}, \tag{S8}$$

where D is the diffusion coefficient. The change in concentration as a function of time is described as

$$\frac{dC_1}{dt} = -DS\frac{C_1 - C_2}{LV}, \tag{S9}$$

$$\frac{dC_2}{dt} = DS\frac{C_1 - C_2}{LV}, \tag{S10}$$

where S is the total cross-sectional area of the microchannel array and V is the chamber volume. Defining the difference of two concentrations, $C_d = C_1 - C_2$, the change in C_d is constituted from Eq. S9 and S10 as

$$\frac{dC_d}{dt} = -2DS\frac{C_d}{LV}. \tag{S11}$$

Assuming the initial condition $C_1(0) = C$ and $C_2(0) = 0$, a solution to Eq. S11 is given as

$$C_d(t) = Cexp\left(-2\frac{DS}{LV}t\right). \tag{S12}$$

Therefore, C_1 and C_2 are derived as

$$C_1(t) = \frac{C}{2}\left(exp\left(-2\frac{DS}{LV}t\right) + 1\right), \tag{S13}$$

$$C_2(t) = \frac{C}{2}\left(-exp\left(-2\frac{DS}{LV}t\right) + 1\right). \tag{S14}$$

By substituting parameters in Table S1 and t = 10 min into Eq. S13, $C_1(t) = 0.975C$. This implies that 2.5% of molecules in chamber 1 were transferred to chamber 2 by diffusion. When the initial concentration was C = 1 nM, the number of molecules transferred was calculated as 2983 (~3000).

5. Active “molecular shuttle” transport between two compartments

To estimate the number of molecules that can be carried by MTs, we assumed biotin-avidin binding between MTs and molecules.[2–4] Assuming that the molecular ratio between non-labeled tubulin and biotinylated tubulin was 8:2, that 10% of biotin molecules were bound to avidin molecules, and that the average MT length was 10 μm, each MT should transport 325 molecules. By multiplying this number by the increase in the rate of MTs described in the main text (9.3×10^{-2} s^{-1}), the number of molecules transported by MTs in the microchannel array was estimated as ~30 molecules s^{-1}.

6. Supplementary table

Table S1 Parameter nomenclature

Definition	Symbol	Value
Hydraulic diameter (microchannel)	D_{h1}	1.3 µm
Hydraulic diameter (fluidic channel)	D_{h2}	33 µm
Kinematic viscosity	η	8.9×10^{-4} m^2 s^{-1}
Channel length	L	100 µm
Diffusion coefficient	D	2.5×10^{-11} m^2 s^{-1}
Chamber volume	V	2.0×10^{-13} m^3
Channel cross-section	S	3.5×10^{-11} m^2

7. Supplementary figures

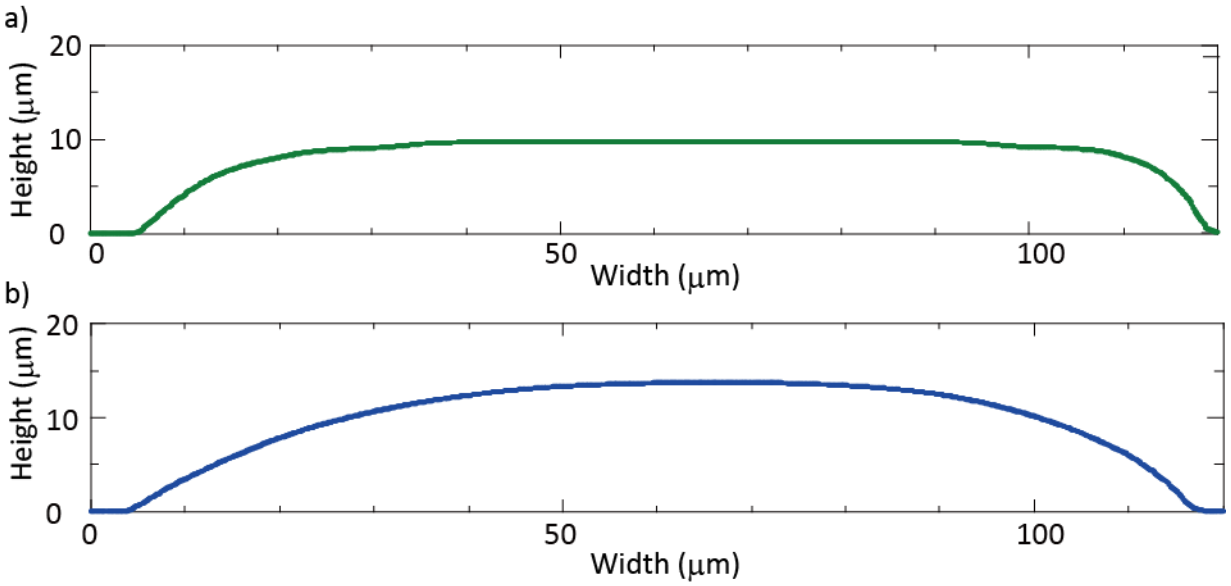

Fig. S1 Measured height of the SU-8 mould for a control channel. a) Before and b) after the reflow process.

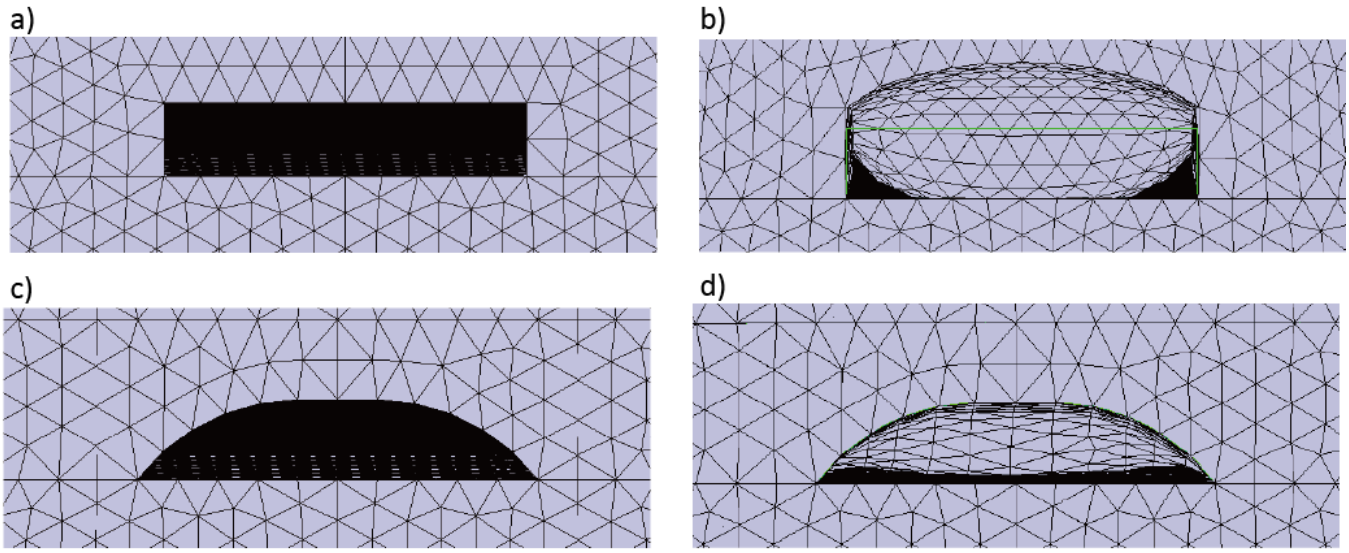

Fig. S2 Finite element model simulation results for the deformation of a flow channel. a) Square-shaped channel not subjected to the photoresist reflow process. b) After applying pressure, the channel collapsed leaving edges open. c) Round cross-shaped channel after reflow, resembling measured shape shown in Fig. S1b. d) After applying pressure, the channel is deformed uniformly compared with the channel shown in panel b. Channel size: width 100 µm, height 20 µm; applied pressure was 80 kPa for the simulation.

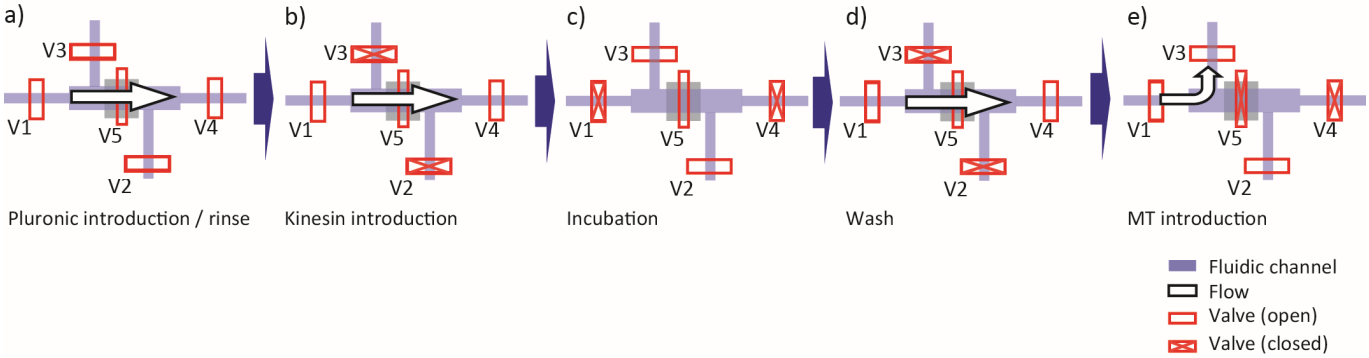

Fig. S3 Schematic sequence of valve operation to introduce solutions into the device. Valves 1–5 (V1–5) are individually actuated by pressure that is controlled by electromagnetic valves. White arrows indicate the direction of solution flow.

8. Supplementary movies

Movie 1. Microtubules gliding in the microchannnel array (30× real time).

Movie 2. Brownian motion of Q-dots in the microchannel array (real time).

References

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