Supplementary Material (ESI) for Lab on a Chip

Rapid generation of spatially and temporally controllable long range concentration gradients in a microfluidic device

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Supplementary

- **Input parameters for the computational simulations.**

The diffusion coefficient is that for FITC-Dextran (MW 10KD) in water [25°C]. The inlet velocity is obtained from the flow rates of the passive pump.

	Inlet Velocity	Outlet Normal Stress	Diffusion Coefficient[1]	Density	Dynamic Viscosity	Simulation Time
Forward flow	0.0008	0	1.70×10^{-10}	1000	1.00×10^{-3}	1
Backward flow	1.01×10^{-5}	0	1.70×10^{-10}	1000	1.00×10^{-3}	50
unit	[m/s]	[Pa]	[m^2/s]	[kg/m^3]	[Pa·s]	[min]

Table 1: Input parameters for computational simulations.

- **Calculate the volumetric flow rate of the forward flow driven by the passive-pump:**

We have developed a Matlab program to calculate the volumetric flow rate of the forward flow in the microchannel based on the analytical equations developed by Walker *et al*[2]:

We found the solution to the following differential equation:

$$\frac{dV}{dt} = \frac{1}{Z}\left(\rho g L - \frac{2\gamma}{R}\right)$$

where the radius of the drop R was determined by

$$R = \left[\frac{3V}{\pi} + h^3\right]\frac{1}{3h^2}$$

and the height h of the drop rising above the port is given by

$$h = \frac{1}{6}\left[108b + 12\left(12a^3 + 81b^2\right)^{\frac{1}{2}}\right]^{\frac{1}{3}} - \frac{2a}{\left[108b + 12\left(12a^3 + 81b^2\right)^{\frac{1}{2}}\right]^{\frac{1}{3}}}$$

where $a = 3r^2$ and $b = 6V/\pi$.

To calculate the average flow rate in the channel, we first calculated the average volumetric flow rate, and then divided by the cross-sectional area of the channel. The average flow rate in channel was calculated as 0.08 cm/s.

Matlab program:

```
function yprime = volume(t,y)
r = .04;
w = .01;
%w is the height of the channel
g = .16;
%g is the width of the channel
u = (8.90*10^(-4));
%u is the viscosity of water
l = 6;
%l is the length of the channel
z = (12*u*10*l)/((w^3)*(g));
%z is the resistance of the channel
a = 3*r^2;
b = 6*y/pi;
h = (1/6)*(108*b+12*(12*a^3+81*b^2)^(.5))^(1/3)-(2*a)/((108*b+12*(12*a^3+81*b^2)^(.5))^(1/3));
R = (3*y/pi+h^3)*(1/3)*(1/h^2);
yprime = 1000*(1/z)*((-1)*98-145.6/R);
end
```

- **Calculate the volumetric flow rate of the backward flow induced by evaporation:**

The evaporation rate of water is calculated as the diffusion rate of water molecules coming out of water through the water/air interface (the boundary layer covering the water surface). The thickness of the boundary layer d is given by the following formula [3]:

$$d = \sqrt{\frac{2vr}{V_{air}}} \tag{1}$$

[4]

The backward flow velocity can be expressed by

$$V = \frac{P_R}{h \cdot w} = \frac{A_E \frac{M}{\rho_1} \frac{D}{R(T+273.15)} \frac{\partial p_V}{\partial x}\Big|_{x=x_0}}{h \cdot w} = \frac{\pi r^2 \frac{M}{\rho_1} \frac{D}{R(T+273.15)} \frac{P_s(1-H)}{d}}{h \cdot w} \tag{2}$$

Where:

d: the thickness of the boundary layer [cm];

v: kinetic viscosity of air [$cm^2\ s^{-1}$];

V_{air} : air flow velocity [$cm\ s^{-1}$];

V: velocity in the microfluidic channel [$cm\ s^{-1}$];

P_R : pump rate [$cm^3\ s^{-1}$];

A_E : area of the water/air interface [cm^2];

M, ρ_1 , D: molecular weight [$g\ mol^{-1}$], density of the water [$g\ cm^{-3}$], diffusion constant of water vapor in air [$cm^2\ s^{-1}$];

p_V , P_s: vapor pressure of water [Pa], saturation vapor pressure of water [Pa];

R, T: ideal gas constant [cm^3 Pa $K^{-1}mol^{-1}$] and temperature [^oC] (It is assumed that temperature is constant at the water/air interface);

h, w: height and width of the microfluidic channel [cm];

r: radius of the port in the inlet [cm];

H: Relative humidity of the air

The temperature dependence of D is known to be[3]:

$$D \propto T^{1/2} \quad (3)$$

The temperature dependence of v is known to be[3]:

$$v \propto T^{1.8} \quad (4)$$

Clausius-Clapeyron equation is used to describe dependence of saturated water vapor pressure on temperature[5]. :

$$P_s = 6.112 \exp(\frac{17.67T}{T+243.5}) \quad (5)$$

According to equations (1)-(5), the velocity of backward flow can be expressed as:

$$V = C\frac{T^{-0.4}}{(T+273.5)}\exp(\frac{17.67T}{T+243.5})(1-H) \quad (6)$$

Where C is a constant that combines the diffusion coefficient of water vapor in air, air flow velocity and proportional constants of Equations. (3), (4).

The backward flow rate at current operational condition (T=22^{o}C, H=30%) was 10.1μm s^{-1}. We also estimated the backward flow rate under other operational conditions (at other temperatures and relative humilities) based on equations (6), which were summarized in the table:

Temperature (^{o}C)	Humidity (100%)	Velocity (μm s^{-1})
22	0.3	10.1
27	0.3	12.3
32	0.3	15.1
17	0.3	8.3
12	0.3	7.1
22	0.2	11.5
22	0.1	12.9
22	0.4	8.6
22	0.5	7.2

- **Assess the effects of backward flow on the resulting gradient**

To study the effects of the backward flow on the resulting concentration gradient, we compared the length of the gradient produced by either backward flow or pure diffusion. All simulations started with the same initial concentration profile, produced by the forward flow, and were run with both low and high molecular diffusivities. In the first simulation, we measured the extent of the gradients produced at different times by the backward flow (Table 2), with effects from both convection and diffusion. In the second simulation, the extent of the gradient was measured after the same amounts of time of pure diffusion. The main difference between the two types of simulation is the location of the gradient: the backward flow moves the gradient back toward the inlet, while pure diffusion leaves it in place. This indicates the main role of the backward flow is to place the gradient. In both cases, diffusion acts to smooth the gradient. The results summarized in Table 2 also demonstrate that the backward flow slightly elongates the gradient by 1%-10%, especially for cases with the low molecular diffusivity. This elongation may be due the flow-induced reversal of the parabolic shape of the concentration profiles.

	Low diffusivity (1.7×10^{-11} m^2/s)		High diffusivity (1.7×10^{-10} m^2/s)	
	Backward flow	**Diffusion**	**Backward flow**	**Diffusion**
Time (min)	**Length of the gradient (cm)**			
4	1.01	0.91	1.06	1
8	1.01	0.96	1.06	1.06
16	1.01	0.96	1.11	1.1
32	1.06	0.96	1.16	1.16

Table 2: Comparison of the effects of backward flow and pure diffusion on the length of the gradient for high and low molecular diffusivity

- **Quantify the effect of diffusion on the change of the gradient profile**

Following the slow backward flow stage, the flow is stopped and the concentration obeys the 3D diffusion equation subject to the boundary conditions of no-flux of chemical through the walls and ends of the channel. Exact solutions in terms of Fourier series are well known for any initial concentration. The chemical spreads and its concentration exponentially approaches a constant equilibrium value along the channel over a timescale $T^* = L^2/(\pi^2 D)$, where $L = 1$ cm is the characteristic axial length and D is the molecular diffusivity. For example, for $D = 10^{-7}$ and 10^{-6} cm^2/s, the gradient will decay over $T^* = 281$ and 28 hours, respectively. To be more precise, note that the concentration c approaches its equilibrium constant value as $\exp(-\pi^2 Dt/L^2)$. Thus, to maintain the concentration gradient to within 10% of its initial state, we must have $\exp(-\pi^2 Dt/L^2) > 0.9$. Rearranging yields $t < \ln(1/0.9)T^* \approx 0.1T^*$. So, with $D = 10^{-7}$ and 10^{-6} cm^2/s, the gradients remain within 10% of their initial states over time intervals of approximately 28 and 2.8 hours, respectively, in agreement with the numerical calculations.

- **Cytotoxicity testing of Alpha-cypermethrin on HL-1 cells cultured in 96-well microplate**

HL-1 cells were seeded into the 96-well microplate pre-coated with a mixture of extracellular matrices (0.02% gelatin (w/w) and 5 μg/mL fibronectin) at a density of 6.6×10^4 cell/well and cultured in 100 μL Claycomb medium. The cells were exposed to

medium containing various concentrations of Alpha-cypermethrin (from 12.5 mM to 0 mM) for 5 min and 4 h respectively. For the 5 min cytotoxicity testing, the medium containing toxins was removed from the wells after 5 min and replaced with normal medium. After 4 h, the morphology and cell viability were tested. As shown in Fig. S1, after 4 h of exposure of HL-1 cells to Alpha-cypermethrin, drastic changes of cell morphology were observed corresponding to the toxin concentration. We quantified cell viability after 4 h toxin exposure using image-based live/dead staining. In contrast, short-term exposure of HL-1 cells to Alpha-cypermethrin for 5 min did not induce observable changes of morphology or cell death (data not shown).

Supplementary Videos:

Video 1: Video of experimental results of the time-lapse fluorescence images to show the dynamic concentration gradient generation during the backflow driven by evaporation.

Video 2: Video of simulation results to show the dynamic concentration gradient generation during forward flow when 3 drops of solution containing interested molecules were introduced into the channel.

Video 3: Video of simulation results to show the dynamic concentration gradient generation during the backward flow with a molecular diffusivity of $10^{-7}cm^2s^{-1}$.

Video 4: Video of simulation results to show the dynamic changes of concentration gradient driven by pure diffusion after the forward flow was stopped (with molecular diffusivity of $10^{-7}cm^2s^{-1}$).

Supplementary figures:

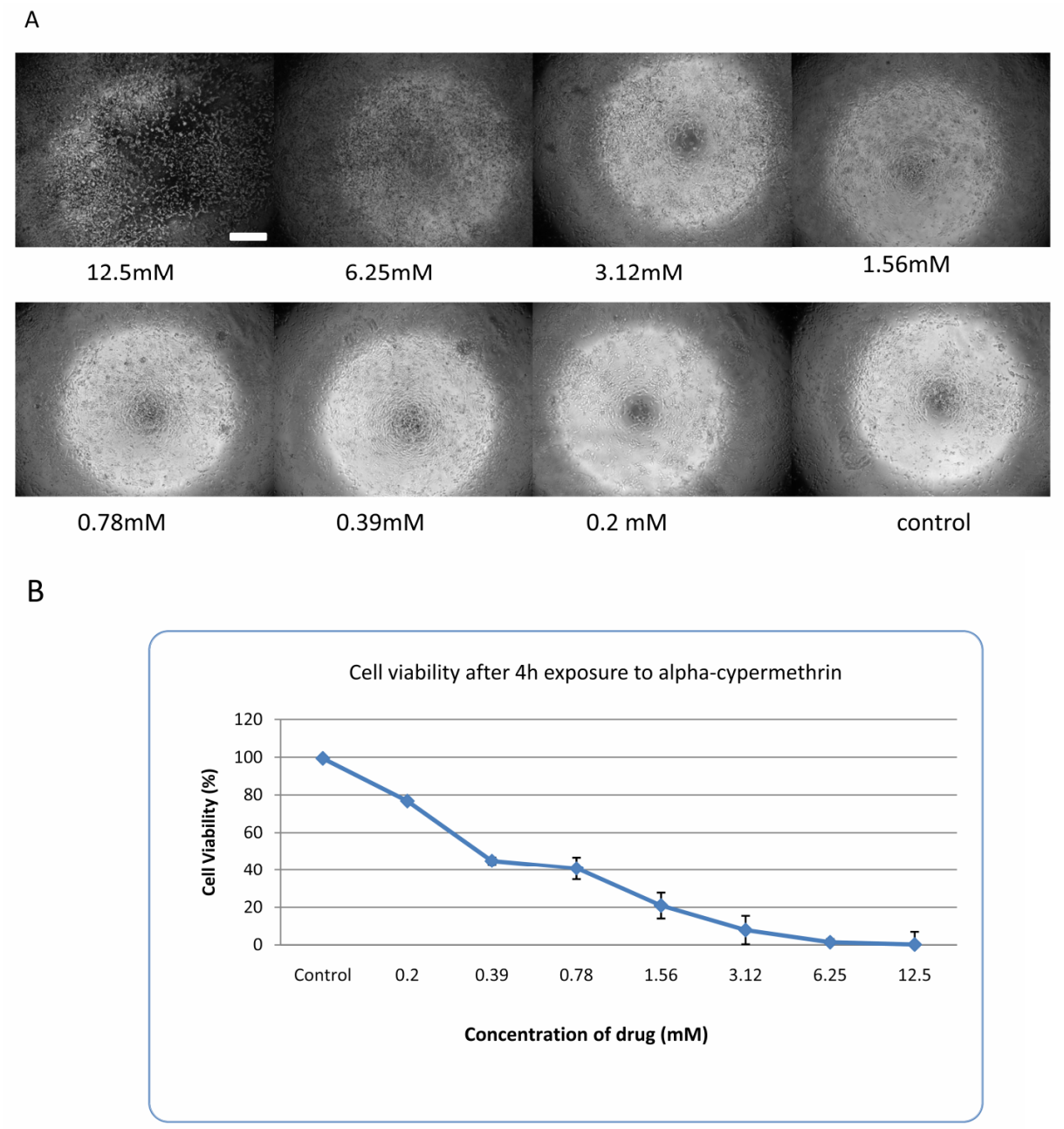

Figure. S1: Cytotoxicity testing of Alpha-cypermethrin on HL-1 cells cultured in 96-well microplate: A) HL-1 cell morphology; B) live/dead assay of HL-1 cell after exposure to various concentrations of Alpha-cypermethrin for 4h (scale bar: 400µm).

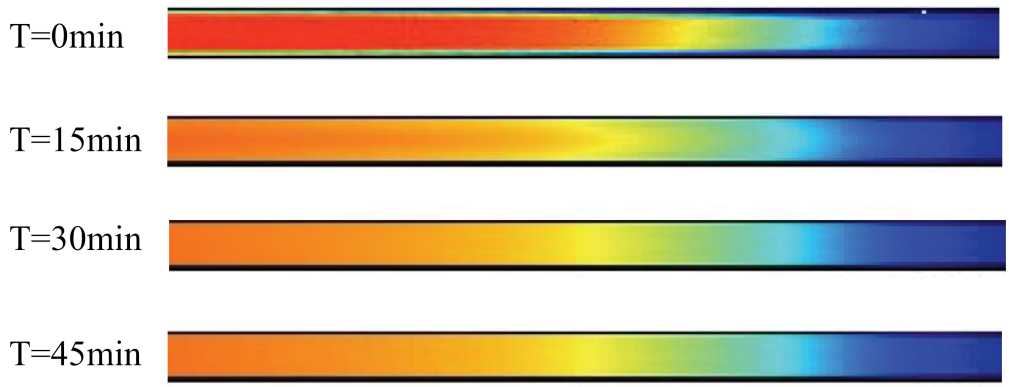

Figure. S2: Top-down view of the simulation results of the dynamic changes of concentration gradient driven by pure diffusion after the forward flow was stopped (with a molecular diffusivity of $1.7\times10^{-6}cm^2/s$).

References:

1. B. G. Chung, F. Lin and N. L. Jeon, *Lab on a chip*, 2006, **6**, 764-768.
2. G. M. Walker and D. J. Beebe, *Lab on a chip*, 2002, **2**, 131-134.
3. H. Kazuo, T. Satoko and A. Youko, *Journal of Applied Physics*, 1993, **73**, 7395-7401.
4. F. Heuck, T. Hug, T. Akiyama, P. L. T. M. Frederix, A. Engel, A. Meister, H. Heinzelmann, N. F. d. Rooij and U. Staufer, *Microelectron. Eng.*, 2008, **85**, 1302-1305.
5. D. Bolton, *Monthly weather review*, 1980, **108**, 1046-1053.