Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary material

Temporal concentration exchange

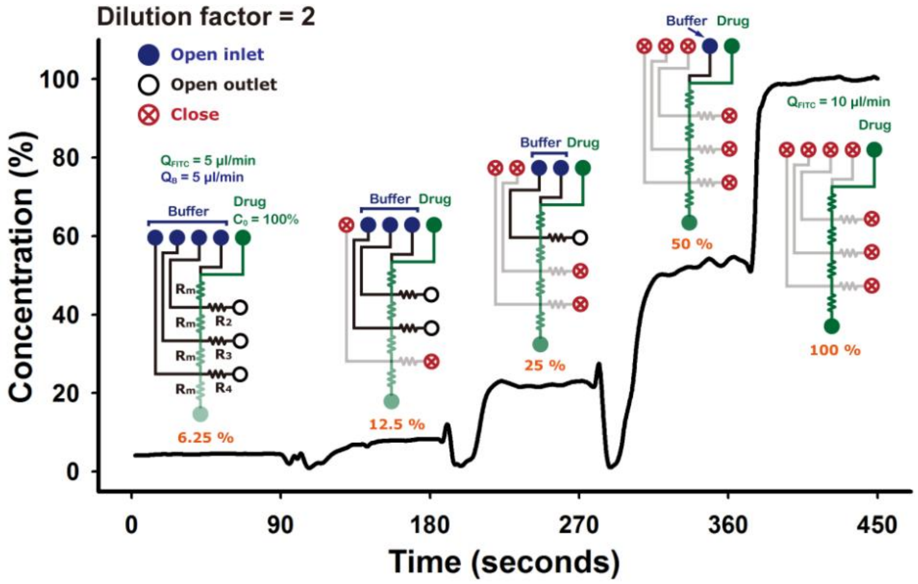

Fig. 1S Generation of dynamically temporal concentration. The concentration was sequentially changed every 90 seconds from 6.25% to 100% with doubling concentration for each switching. The valve actuation was illustrated at each stage. The perturbation during the switch process is due to the non-stabilized residual pressure. The time required for fully concentration exchange in this design is ~45 seconds.

Figure 1S demonstrates dynamic change of the concentration via different combination of the valve actuation, as illustrated. The solutions in Fig. 4C were replaced by FITC-dextran fluorescent dye and PBS buffer for quantifying the concentration temporally. The concentration was sequentially changed every 90 seconds from 6.25% to 100% with doubling concentration for each switching. With all valves open, the initial concentration undergone 4 times dilution and resulted in $1/2^4$ of the initial concentration ($100\%/2^4=6.25\%$). Next, the valves connected to the lowest buffer inlet and R_4 outlet were closed so that the green dye experienced only 3 times dilution from inlet to the temporal concentration outlet, since no split and combination occurred at the last branching point. Hence the concentration was changed to 12.5% ($100\%/2^3$) of the initial concentration. The concentration can be switched to desired logarithmic concentration by the same concept of the valve actuation. At the last stage with all valves closed, the flow rate of fluorescent dye inlet was adjusted to 10 µl/min to maintain constant flow rate at the temporal concentration outlet. During the switching, concentration fluctuation was apparently observed. It is likely caused by non-stabilized residual pressure in the microchannel when the macro-scale valves were performing. Possibly the perturbation could be greatly eliminated by integrating microvalves directly in the microchannels. The time required for fully concentration exchange in this design is ~45 seconds, and that the exchanging time could be reduced by minimized the disturbance during the valve actuation.

In present study, the ion channel recordings do not reveal significant influence due to the perturbation. The ion channels response rapidly to the concentration exchange and tend to fully recover promptly. However, the disturbance will be eliminated in the future study in case of studying cell responses which do not recuperate their cell state immediately (or show some memory effect).

<u>Estimation of percentage of drug usage in temporal generator</u>

Consumption of drug is estimated by liquid utilization times drug concentration, which is referred to mole of drug amount. (**Flow rate *Q*** times **duration *t*** times **concentration *C***)

Temporal outlet concentration	Perfused drug at inlet	Perfused drug at temporal outlet
$\frac{C_0}{k^{n-1}}$	$Q_D \times t \times C_0$	$(Q_D + Q_B) \times t \times \frac{C_0}{k^{n-1}}$
$\frac{C_0}{k^{n-2}}$	$Q_D \times t \times C_0$	$(Q_D + Q_B) \times t \times \frac{C_0}{k^{n-2}}$
$\vdots$	$\vdots$	$\vdots$
$\frac{C_0}{k^{2}}$	$Q_D \times t \times C_0$	$(Q_D + Q_B) \times t \times \frac{C_0}{k^{2}}$
$\frac{C_0}{k^{1}}$	$Q_D \times t \times C_0$	$(Q_D + Q_B) \times t \times \frac{C_0}{k^{1}}$
$\frac{C_0}{k^{0}}$	$k \times Q_D \times t \times C_0$ *	$(Q_D + Q_B) \times t \times \frac{C_0}{k^{0}}$

Table 1S. Consumption of drug at different temporal outlet concentrations.

* At the last stage with all valves closed, the flow rate was adjusted to $k \times Q_D$ to maintain constant flow rate at the temporal concentration outlet.

$Q_{Drug} : Q_{Buffer} = 1 : k-1 \rightarrow Q_{Buffer} = (k-1) \times Q_{Drug}$

$Q_{Drug} + Q_{Buffer} = k \times Q_{Drug}$

$$\text{Percentage of drug usage} = \frac{\text{Perfused drug at temporal outlet}}{\text{Perfused drug at inlet}} \times 100\%$$

$$= \frac{\left(\frac{1}{k^{-1}} + \frac{1}{k^0} + \frac{1}{k^1} + \ldots + \frac{1}{k^{n-2}}\right) \times Q_D \times t \times C_0}{(k+n-1) \times Q_D \times t \times C_0} \times 100\% = \frac{\sum_{m=-1}^{n-2} \frac{1}{k^m}}{k+n-1} \times 100\%$$