RAPID AND ACCURATE IC$_{50}$ DETERMINATION
USING LOGARITHMIC CONCENTRATION GENERATOR

Yuta Abe[1,3], Hirotaka Sasaki[1], Toshihisa Osaki[1], Koki Kamiya[1], Ryuji Kawano[1],
Norihisa Miki[1,3] and Shoji Takeuchi[1,2]

[1]*Kanagawa Academy of Science and Technology, *[2]*Institute of Industrial Science, The University of Tokyo,*
[3]*Keio University, Japan*

ABSTRACT

This paper proposes an effective methodology for the determination of the half maximal (50%) inhibitory concentration (IC$_{50}$) by using microfluidic device that generates logarithmic concentration gradients. Dose-response curve is obtained with respect to the concentrations in a logarithmic scale. Although we have presented a high-throughput analysis of ATP binding cassette transporter (ABC-transporter) by using linear concentration generator [1] and fluorescent substrates [2], logarithmic concentration generator is demanded for rapid and accurate IC$_{50}$ determination as shown in Fig. 1. We designed a microfluidic device composed of branching micro-channels where samples are divided and mixed to generate designated concentrations. Finally inhibition analysis of substrate transport was conducted by linear and logarithmic concentration generators. We found that logarithmic concentration generators are useful for the accurate determination of IC$_{50}$ by a fewer number of tests than linear concentration generators.

KEYWORDS

IC$_{50}$, Microfluidics, Drug screening.

INTRODUCTION

In drug development process, the phenomena that depend on the logarithmic concentration are of great importance for safe and effective use of drugs, such as IC$_{50}$ that determines the concentration at which the transport of a drug is inhibited by the other one by 50% [3,4,5]. To determine IC$_{50}$, substrate transport at different concentrations must be evaluated. For the high-throughput IC$_{50}$ determination, microfluidic concentration gradient generator can be a smart approach [6,7]. Although IC$_{50}$ can be estimated by using linear gradient generators [1,8], the uneven concentration distribution in the logarithmic scale (Fig. 1(c,d)) causes less accuracy. To accomplish a rapid and accurate determination of IC$_{50}$, the logarithmic sampling device, which produces an even concentration distribution in the logarithmic scale (Fig. 1(c,d)), is useful and demanded.

In generally, the generation of logarithmic concentrations in the microfluidic devices was completed by a pyramidal network (T-shaped microchannel) [9], serial network [10,11], and combination of serial and volumetric
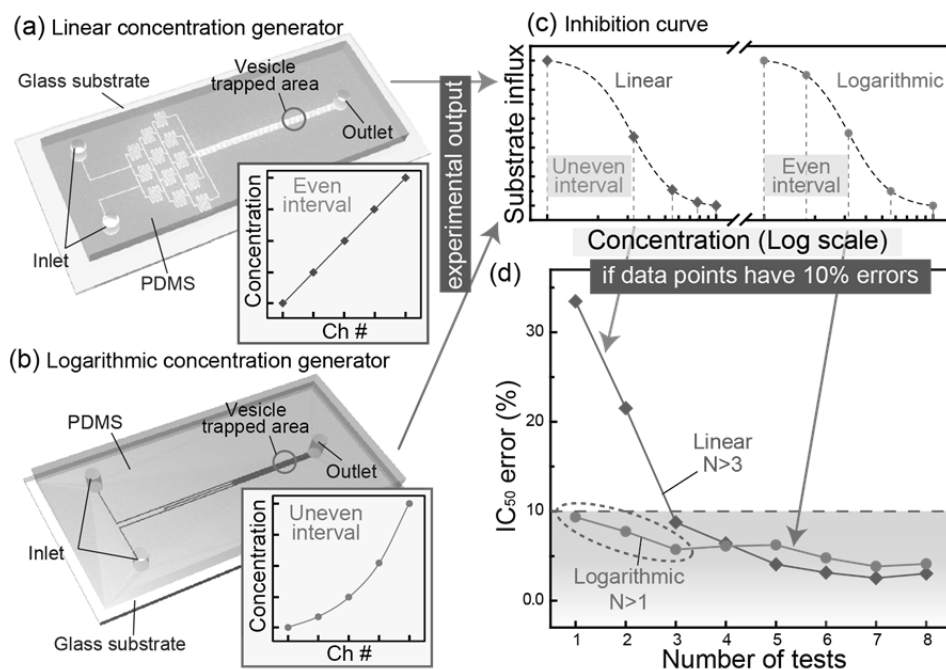

Fig. 1 *(a-b) The conceptual illustrations of the (a) linear and (b) logarithmic concentration generator. Inset graphs show the dispersions of generated concentrations in the devices. (c) Examples of linear (blue) and logarithmic (red) concentration samplings that allow the same inhibition curve (dashed line). (d) Simulation of the percentages of IC$_{50}$ error, which is calculated by giving 10% errors in the sample concentration of each microfluidic channel. In contrast to linear gradient generator (blue), logarithmic gradient device (red) enables low IC$_{50}$ error in a few experimental tests.*
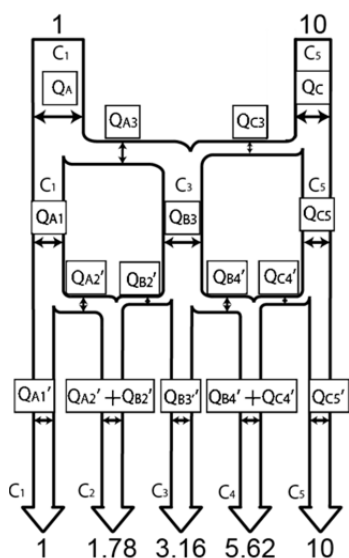
Fig. 2 *Schematics of the flow rate distributions to generate the desired concentrations at the 5 outlets. We assumed that the ratio of branched channel width determines the distribution of the flow rate. Q indicates the flow rate and C is the concentration of compounds. The concentrations were expressed as C (1, 2, 3, 4, 5) =10 0, 0.25, 0.5, 0.75, 1 μM.*

network [12]. The principle of these network designs can be theoretically formulated considering the flow resistance by analogy with electrical circuits [13]. In microfluidic devices, the mixing ratios of two solutions were controlled by adjusting channel length because the flow resistance is purely proportional to the channel length [14]. Therefore, the conventional logarithmic concentration generators have very long channel, multi-inlets, or 3-dimentionals structure to obtain the desired concentrations and become complicated design.

In this report, we developed the simple and useful microfluidics network design to generate logarithmic concentration gradients by unique approach. To control the flow resistance, the channel widths at each branching point are adjusted. Then, the generation of logarithmic concentration gradients was confirmed by COMSOL simulation and fluorescence images in the device. Finally, taking advantage of our previous study [1,2], we performed the IC_{50} assays using ATP-binding cassette transporter (ABC transporter) vesicles. As a result, we found that our logarithmic concentration devices were succeeded in the rapid and accurate IC_{50} determination by a fewer number of tests than linear concentration devices.

DESIGN PRINCIPLE

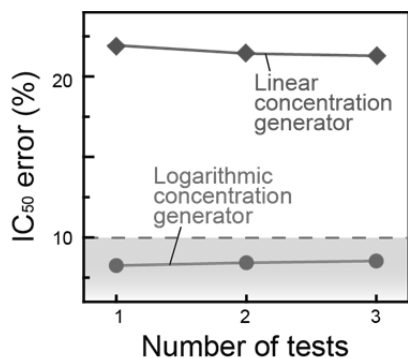We designed a simple logarithmic concentration generator (Fig. 2) that produces concentrations of 10^0, $10^{0.25}$, $10^{0.5}$, $10^{0.75}$, and 10^1. The initial flow rates (Q_A and Q_C), concentrations, and the channel width are the design parameters while the lengths of the channels were set to be long enough to allow complete diffusion-based mixing of the samples. We assumed that the ratio of the branched channel widths determines the distribution of the volumetric flow rate as depicted in Fig. 2. Then, we tried the COMSOL simulation to verify our design principle. As a result, when inlets flow rates (Q_A and Q_C) are 1.0 μL/min and 0.8 μL/min, respectively, the desired concentrations at the outlets can be generated.

EXPERIMET

Our device consists of a polydimethylsiloxane (PDMS) microfluidic channel and a glass substrate. The microfluidic devices were fabricated by conventional soft lithography [15]. The PDMS chip was attached to an aldehyde-coated glass with single-strand DNA tethers using oxygen plasma treatment. To prevent DNA removal at the plasma-exposure step, we placed PDMS masks on the glass [1]. After the device preparation, a cholesterol-modified single-strand DNA (ssDNA) was applied to the channels to form duplexes with the ssDNA tethered to the glass surface. ABC-transporter vesicles were then injected into microfluidic channels to be immobilized [2,16], and incubated with quinidine (inhibitor) and 50 nM Rhodamine123 (Rh123) solutions at different concentrations for 1 hour and 37 °C. Here, the inlets concentrations of quinidine, C_1 and C_5, were 0.3 and 3.0 μM, respectively. The fluorescence intensities of ABC transporter vesicles after rinsing with buffer were detected by microscopic images.

RESULT AND DISCUSSION

After device preparation, some experiments were conducted by using Rh123 to verify that our logarithmic device could generate logarithmic concentration gradients. The concentration distributions were estimated by the fluorescence intensity from the channel. The concentration generated in our logarithmic device was well matched with the result of COMSOL simulation (Table 1). Finally, we analyzed the Rh123 transport inhibition with quinidine by linear [1] or logarithmic concentration generator. The IC_{50} was determined by the inhibition curve drawn by the different fluorescence intensity from ABC transporter vesicles. As a result, the IC_{50} values of logarithmic and linear concentration generator were 0.80 ± 0.01 μM and 1.06 ± 0.01 μM, respectively. Fig. 3 represents that IC_{50} can be determined with less than 10% errors by using logarithmic concentration generator. This result indicates that our

Table 1 *Comparison table of the outlet concentrations with desired value, simulation, and experiment. This table shows that simulation and experiment value are agreed well.*

	Ch 1	Ch 2	Ch 3	Ch 4	Ch 5
Desired	1.00	1.78	3.16	5.62	10.00
Simulation	1.00	1.69	3.30	8.12	10.00
Experiment	0.98 ± 0.18	1.79 ± 0.25	3.85 ± 0.70	8.18 ± 0.81	10.36 ± 0.24

Fig. 3 *IC$_{50}$ error of two devises. The percentages were calculated from the each experimental value.*

logarithmic sampling device is readily applicable for the accurate determination of the IC$_{50}$ by ABC transporters.

CONCLUSION

In this study, we developed the platform for the rapid and accurate IC$_{50}$ determination by logarithmic concentration gradient generator. We believe that our approach would accelerate a high-throughput drug screening using MEMS technology.

REFERENCES

[1] Y. Abe, H. Sasaki, T. Osaki, R. Kawano, K. Kamiya, N. Miki, and S. Takeuchi, "A High-throughput Drug-screening System Targeting ABC-transporters: An Application of a Microfluidic Gradient Generator," *Proc. MicroTAS 2011*, 885-887.

[2] H. Sasaki, R. Kawano, T. Osaki, K. Kamiya, and S. Takeuchi, "Single-vesicle Estimation of ATP-binding Cassette Transporters in Microfluidics Channels," *Lab Chip*, 2012, **12**, 702-704.

[3] K. M. Giacomini, S. –M. Huang, and D. J. Tweedie, "Membrane Transporters in Drug Development," *Nat. Rev. Drug Discov.*, 2010, **9**, 215-236.

[4] J. Rautio, J. E. Humphreys, L. O. Webster, A. Balakrishnan, J. P. Keogh, J. R. Kunta, C. J. Serabjit-Singh, and J. W. Poll, "In vitro P-grycoprotein Inhibition Assays for Assessment of Clinical Drug Interaction Potential of New Drug Candidates: A Recommendation for Probe Substrate," *DMD*, 2006, **34**, 786-792.

[5] K. S. Fenner, M. D. Troutman, S. Kempshall, J. A. Ware, D. A. Smith, and C. A. Lee, "Drug-drug Interactions Mediated Through P-grycoprotein: Clinical Relevance and *In Vitro-In Vivo* Correlation Using Digoxin as a Probe Drug," *Clin. Pharmacol. Ther.*, 2009, **85**, 173-181.

[6] D. Lombardi and P. S. Dittrich, "Advances in Microfluidics for Drug Discovery," *Expert Opin. Drug Discov.*, 2010, **5**, 1081-1094.

[7] G. M. Whitesides, "The Origins and the Future of Microfluidics," *Nature*, 2006, **442**, 368-373.

[8] C. Yang, Y. Wu, Z. Xu, and J. Wang, "A Radial Microfluidic Concentration Gradient Generator with High-density Channels for Cell Apotosis Assay," *Lab Chip*, 2011, **11**, 3305-3312.

[9] S. K. W. Dertinger, D. T. Chiu, N. L. Jeon, and G. M. Whitesides, "Generation of Gradients Having Complex Shapes Using Microfluidic Networks," *Anal. Chem.*, 2001, **73**, 1240-1246.

[10] K. Lee, C. Kim, B. Ahn, R. Panchapakesan, A. R. Full, L. Nordee, J. Y. Kang, and K. W. Oh, "Generalized Serial Dilution Module for Monotonic and Arbitrary Microfluidics Gradient Generator," *Lab Chip*, 2009, **9**, 709-717.

[11] K. Hattori, S. Sugiura, and T. Kanayama, "Generation of Arbitrary Monotonic Concentration Profiles by a Serial Dilution Microfluidic Network Composed of Microchannels with a High Fluidic-resistance Ratio," *Lab Chip*, 2009, **9**, 1763-1772.

[12] K. Lee, C. Kim, Y. Kim, K. Jung, B. Ahn, J. Y. Kang, and K. W. Oh, "2-layer Based Microfluidic Concentration Generator by Hybrid Serial and Volumetric Dilutions," *Biomed. Microdevices*, 2010, **12**, 297-309.

[13] C. Chen, A. M. Wo, and D. Jong, "A Microfluidic Concentration Generator for Dose-response Assays on Ion Channel Pharmacology," *Lab Chip*, 2012, **12**, 794-801.

[14] K. W. Oh, K. Lee, B. Ahn, and E. P. Furlani, "Design of Pressure-driven Microfluidic Networks Using Electric Circuit Analogy," *Lab Chip*, 2012, **12**, 515-545.

[15] J. C. Mcdonald, and G. M. Whitesides, "Poly(dimethylsiloxane) as a Material for Fabricating Microfluidic Devices, *Acc. Chem. Res.*, 2002, **35**, 491-499.

[16] K. Bailey, M. Bally, W. Leifert, J. Vörös, and T. McMurchie, "G-protein Coupled Receptor Array Technologies: Site Directed Immobilisation of Liposomes Containing the H$_1$-histamine or M$_2$-muscarinic Receptors," *Proteomics,* 2009, **9**, 2052-2063.

CONTACT

*Y. Abe, Kanagawa Academy of Science and Technology (KAST), 3-2-1 Sakado, KSP East 303, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan, Tel: +81-44-819-2037, Email: yuta.a@a6.keio.jp