MICROFLUIDIC NETWORK-BASED COMBINATORIAL DILUTION DEVICE WITH AN INITIAL CONCENTRATION CONTROLLER

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ABSTRACT

In this paper, we present a flow-specific initial concentration controller connected to a combinatorial dilution device, which could generate arbitrary, predictable, and systematic combinatorial dilutions of all the solutions in response to variable input flows. We investigate the performance of the initial controller by computational simulation (CFD-ACE$^+$). The simulated output concentrations had very low tolerance compared to theoretical value with error < 1%. Linearity was tested with fluorescent dye (sodium salt) for 3 samples and DI water for buffer by employing a separate initial concentration module. Then, a combinatorial dilution device integrated with the initial concentration module was tested. The device showed CV error less than 10%. As proof-of-concept, we performed a simple combinatorial cytotoxicity screening with 3 drugs (Mitomycin-C, Doxorubicin, and 5-FU) for MCF-7 cancer cell.

KEYWORDS: Combinatorial device, 3D simplex centroids, microfluidic network, cytotoxicity test

INTRODUCTION

We propose a simple strategy to control initial concentration for combinatorial mixing. Some research groups showed combinatorial microfluidic devices to generate simple combinations of samples [1, 2]. The reported platforms are very powerful to search new combinations of drugs or materials for primary screening. However, the investigation of combinatorial effects in complex biological or chemical experiments needs more specific and various combinatorial dilutions of the samples [3]. In this study, we present a flow-specific initial concentration controller connected to a combinatorial dilution device is shown to generate arbitrary, predictable, and systematic combinatorial dilutions of all the solutions in response to variable input flows. We analyzed the flow-specific combinatorial dilution generator and demonstrated its proof-of-concept by combinatorial cytotoxicity experiment.

WORKING PRINCIPLE AND DESIGN RULE

A concept of using a single common channel was incorporated in an initial concentration controller to generate controllable initial concentrations of samples. Independent flow sources of each sample (Q_A, Q_B, and Q_C) and buffer (Q_D) are merged into the common channel (Fig. 1a). And the merged flow ($Q_T = Q_A + Q_B + Q_C + Q_D$) is divided into three sub-channels in response to channel resistances ($Q_{A*} = Q_{B*} = Q_{C*} = Q_T/3$). As long as Q_T is fixed to a specific value, any sample concentration over the buffer can be generated within allowed diffusion boundary conditions.

For instance, when $\{Q_A:Q_B:Q_C:Q_D\} = \{1:1:1:6\}$, the initial concentration of three samples is $\{C_{A*}:C_{B*}:C_{C*}\} = \{1/3:1/3:1/3\}$. If Q_A decreases into 0.5 and Q_D increases into 6.5 ($\{Q_A:Q_B:Q_C:Q_D\} = \{0.5:1:1:6.5\}$), the concentration will be $\{C_{A*}:C_{B*}:C_{C*}\} = \{1/6:1/3:1/3\}$. In this manner, any initial concentration can be achieved. The integrated flow-

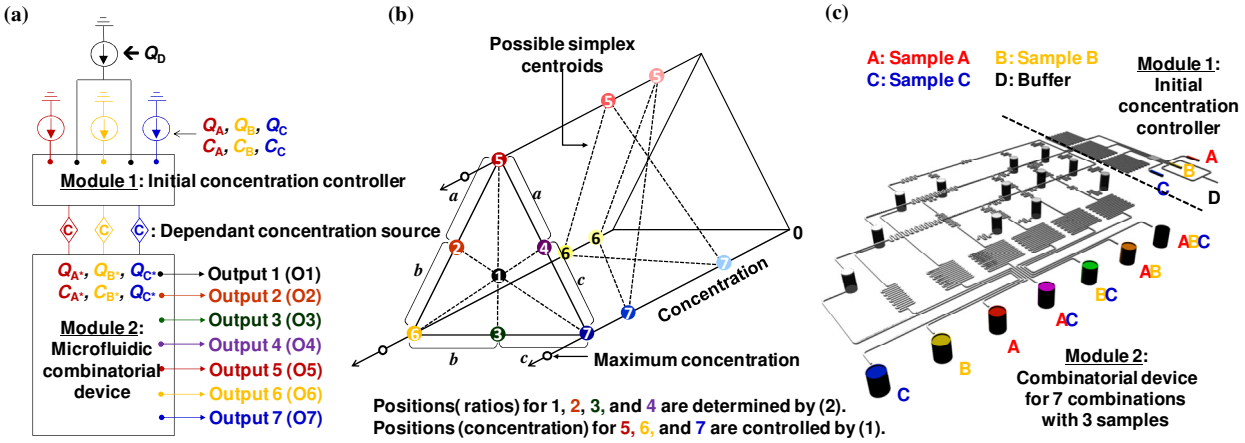

Fig. 1 *(a) The diagram of the proposed combinatorial device with the initial concentration controller. (b) The integrated flow-specific combinatorial dilution generator can produce arbitrary, predictable, and systematic combinatorial dilutions of all three samples (ABC, AB, BC, AC, A, B, and C) in response to the variable input flows. (c) The schematic of the proposed device including an initial concentration generator (module 1) and a combinatorial device (module 2).*

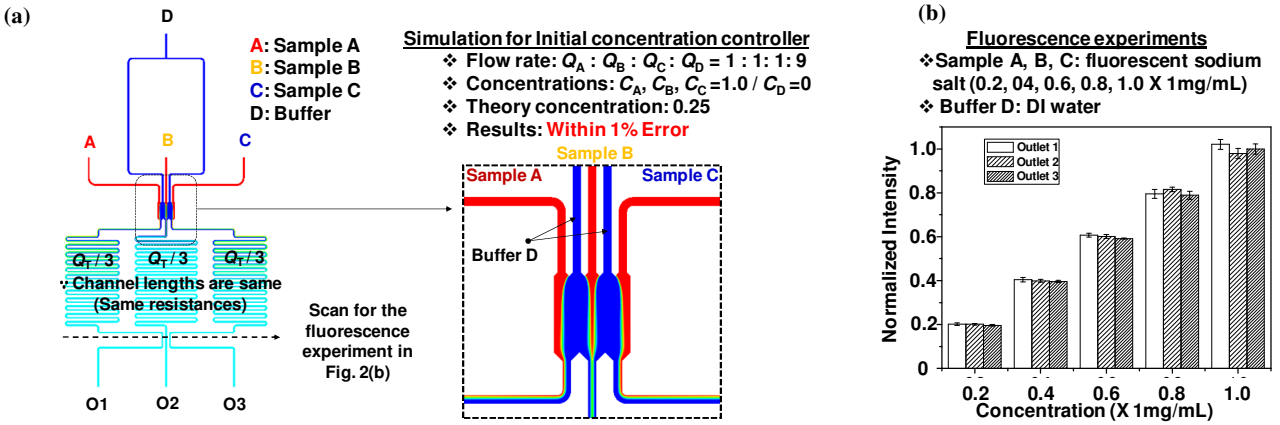

Fig. 2 *The proposed initial concentration controller. (a) Computational fluid dynamic simulation results with CFD-ACE+, (b) the specific conditions for the flow rates and concentrations, and (c) Fluorescence experimental results for linearity test.*

specific combinatorial dilution generator can produce arbitrary, predictable, and systematic combinatorial dilutions of all three samples (ABC, AB, BC, AC, A, B, and C) in response to the variable input flows (Fig. 1b and Fig. 1c).

EXPERIMENTAL

A microfluidic combinatorial device integrated with the initial concentration controller was configured with two channel layers (top and middle) and one substrate layer (bottom). The two channel layers were made by soft-lithography, then punched to make inlets, outlets, and via holes with a diameter of 1 mm for fluidic connections between top and middle layer. After that, the two PDMS layers and the substrate layer (bottom) were aligned and bonded, where all microchannels were 150 μm tall and wide. For a quantitative evaluation, an aqueous fluorescein sodium salt (Sigma Aldrich, 1 mg/mL in water) and distilled water were used as sample liquid and buffer. The solutions were injected by syringe pumps with the calculated input flow rates: $\{Q_A, Q_B, Q_C, Q_D\}$. Fluorescence images were captured with a high resolution monochrome digital camera (Hamamatsu ORCAER) mounted to an Olympus MVX10 epifluorescence microscope, and all quantitative measurements of the fluorescent intensity were obtained using the Olympus Wasabi imaging software package.

RESULT AND DISCUSSION

We investigated the performance of the initial concentration controller by computational simulation (CFD-ACE$^+$). The simulated output concentrations had very low tolerance compared to theoretical values with error < 1% (Fig. 2a). An initial concentration module was fabricated and the linearity of the device was tested with fluorescent dye (sodium salt) for samples A, B, and C, and DI water for buffer (Fig. 2b). The initial concentration controller showed the good linearity with error < 5%. Then, the combinatorial dilution device integrated with the initial concentration controller was designed, simulated, and tested (Fig. 3a). The device showed CV error less than 10% (Fig. 3b).

As proof-of-concept, we performed a simple combinatorial cytotoxicity screening with 3 drugs (Mitomycin-C, Doxorubicin, and 5-FU) for MCF-7 cancer cell. Fig. 4a shows the effect of the drug combinations and the cell inhibition rate had a similar trend compared to the manual evaluation method. In addition, a dose-response curve of Mitomycin-C was fitted with the effect of 4-order logarithmic concentrations to determine the IC_{50} of the drug. As shown in Fig. 4b, the cell inhibition rate had a similar trend compared to the result of the manual method.

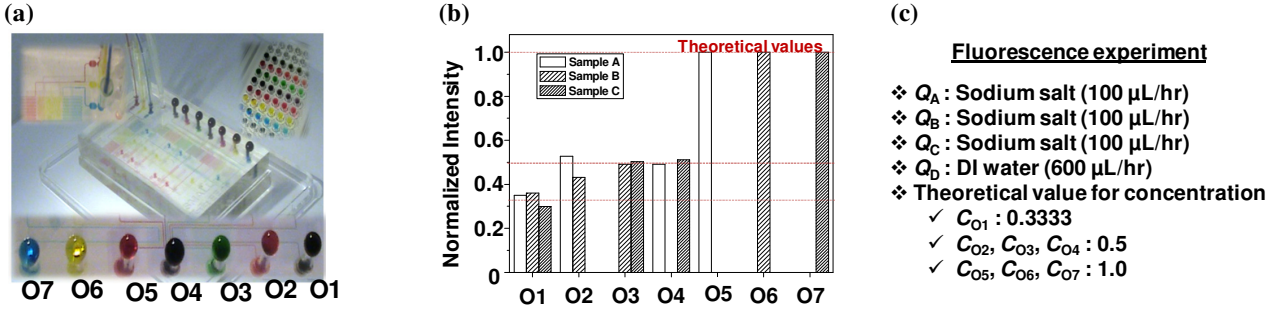

Fig. 3 *(a) The proposed device fabricated by soft-lithography working with color dyes (red: sample A, yellow: sample B, and blue: sample C), (b) The fluorescence experimental results for quantitative analysis with sodium salt and (c) the detail condition including flow rate and concentration for the fluorescence experiments.*

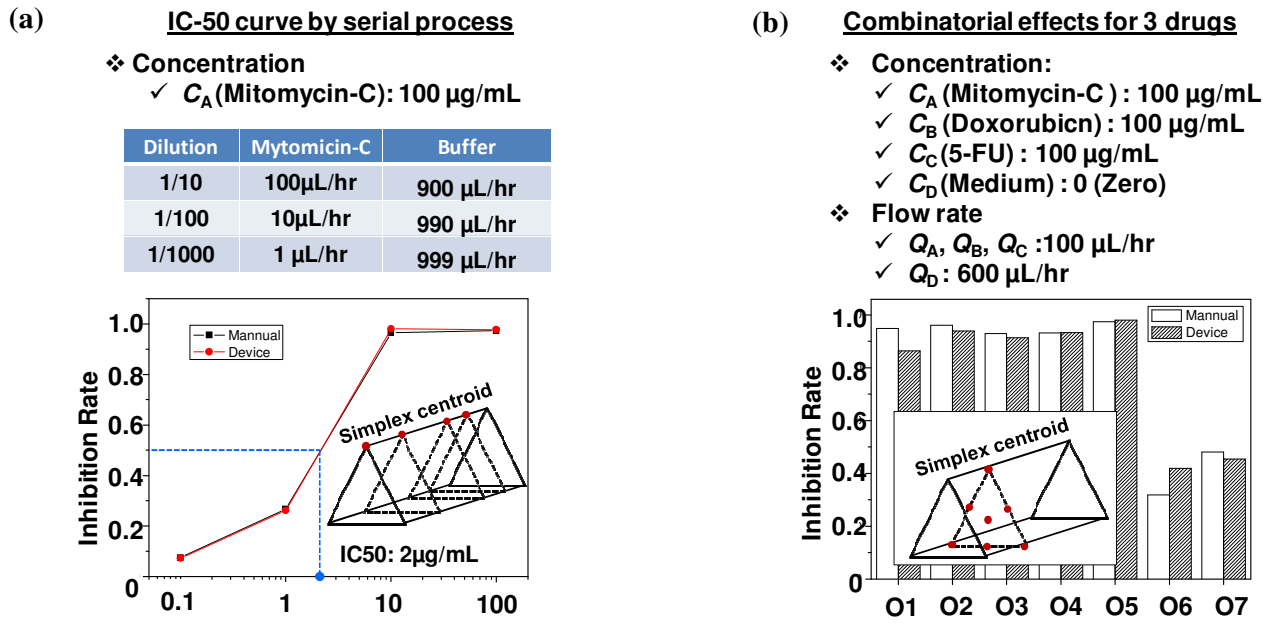

Fig. 4 *The proof-of-concept experiments using the proposed flow-specific combinatorial dilution generator. (a) IC_{50} curve for MCF-7 cell with selected combinatorial dilutions. (b) Combinatorial effects of three drugs (Mitomycin-C, Doxorubicin, and 5-FU) with selected combinatorial dilutions.*

CONCLUSION

The microfluidic network-based combinatorial dilution device integrated with the flow-specific initial concentration controller, capable of covering 3D simplex centroids, was successfully presented. The proposed method for controlling the desirable initial concentrations with one single buffer was verified by mathematical modeling, simulation, and fluorescence experiments. The experimental results showed the good conformance with < 1% error to the theory values. With the integration of the initial concentration controller, the combinatorial device could systemically cover 7 combinations (ABC, AB, BC, AC, A, B, and C) for 3 samples (A, B, and C) within the range of desirable combinatorial mixing ratios and their concentrations using two channel layers and one substrate layer. In addition, to establish the proof-of-concept, combinatorial cytotoxicity screening was performed with three drugs (Mitomycin-C, Doxorubicin, and 5-Fu), and the results had good agreements with those of manual methods. Therefore, we expect that the proposed device will be valuable for high throughput screening and optimization in many biotechnology, chemical, material science, and pharmaceutical fields.

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