SINGLE-LAYER MICROFLUIDIC NETWORK-BASED COMBINATORIAL DILUTION FOR A STANDARD SIMPLEX-LATTICE COMBINATORIAL DESIGN

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ABSTRACT

In this paper, we presented a straightforward strategy to generate 15 combinations with 3 samples, using single-layer microfluidic network. First, we investigated the performance of the single-layer based design by computational simulation (CFD-ACE$^+$). The simulated output concentrations were extremely close to the expected values within absolute error < 1%. Based on the simulated design, a PDMS device was fabricated by soft-lithography and tested with fluorescent dye (sodium salt). The combinatorial mixing results for 15 combinations showed good performance with absolute error less than 4%. In addition, we have also conceptually presented two liquid handing methods (bottom-up and top-down) for high-throughput screening and assay.

KEYWORDS: Combinatorial dilution, microfluidic network, simplex lattice design, high-throughput screening (HTS)

INTRODUCTION

Combinatorial chemistry is one of the promising technologies to optimize a specific range of mixture effect for several kinds of samples. Recently, some groups have nicely demonstrated their combinatorial dilution devices using microfluidic device [1, 2]. However, much highly-controlled sample dilution are still required to achieve better optimization of the samples. Previously, we demonstrated a two-layer combinatorial dilution device integrated with a flow-specific initial concentration controller, covering only 7 combinations with 3 samples [3]. In the combinatorial mixture design of experiment (DOE), increased numbers of the mixture combinations are able to make better screening and optimization. Here, we present a very simple method to configure and handle 15 combinations with 3 samples for a combinatorial mixture DOE, using a single-layer microfluidic network-based circuit.

PRINCIPLE/DESIGN

Figure 1a shows the combinatorial configuration of a standard *Simplex-lattice* design with a 3-sample mixture. A straightforward approach was used to generate such combinations using a single-layer (two dimensional microfluidic network) as shown in Figure 1b. Each sample was evenly and symmetrically divided into sub-channels and flowed through mixing channels in the 1st stage. The same procedure was performed in the subsequent 2nd and 3rd stages. The microfluidic circuit was symmetrically designed with appropriate channel lengths that could control flow rates for desired volumetric mixing ratios, which could be easily analyzed based on a simple electrical circuit analysis (Figure 1c) [4]. In addition, the resulted combinatorial mixtures can be collected by two liquid handling methods: a pipette-based system (bottom-up) and a spotting/dropping

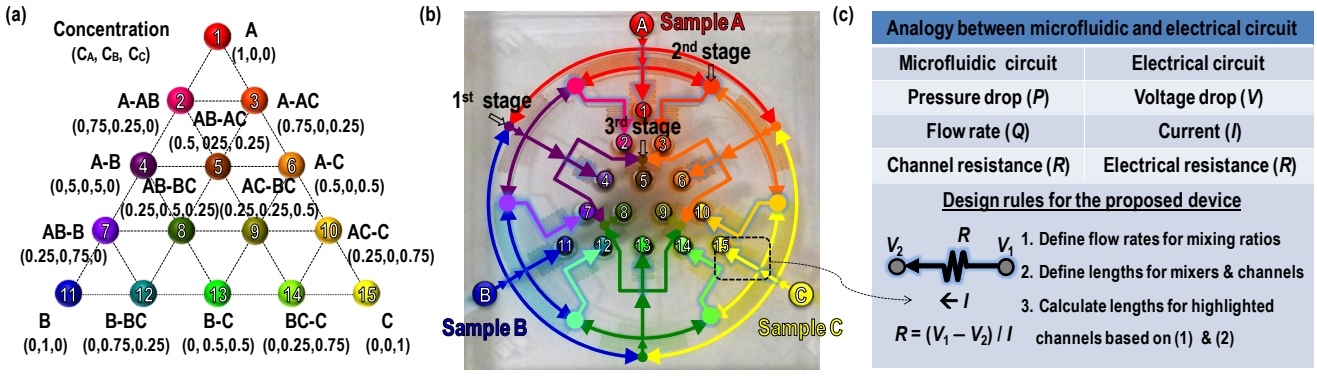

Figure 1: (a) Configuration of a standard Simplex-Lattice combinatorial design containing 15 combinations with a 3-component mixture. (b) A single-layer microfluidic circuit generating the combinatorial mixture with 3 samples (sample A, sample B, and sample C). (c) An analogy between microfluidic circuits and electric circuits to design the proposed microfluidic network based combinatorial device.

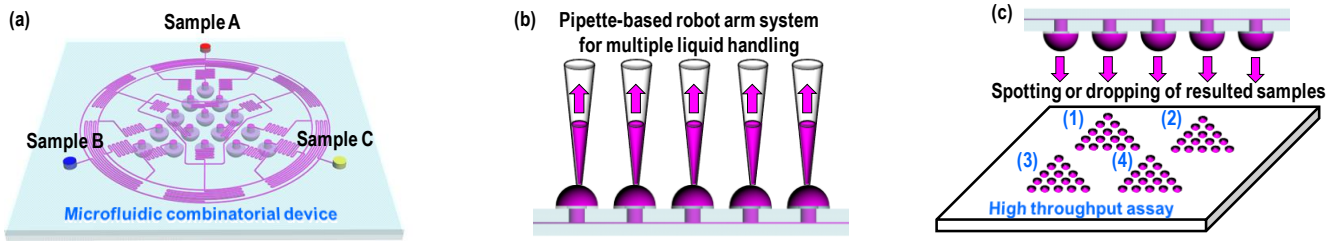

Figure 2: (a) Schematic of the proposed microfluidic network-based combinatorial dilution device. Available methods to collect samples: (b) a pipette-based robot arm system and (c) a spotting (or dropping) system for high-throughput assays.

system (top-down) (Figure 2).

EXPERIMENTAL

The devices consisted of a top PDMS layer with the microfluidic circuits fabricated by soft lithography and a bottom PDMS layer. A master mold with patterned SU-8 2050 photoresist was used to replicate the microchannels (height: 100 μm, width: 200 μm). The top layer was then punched to create inlets and outlets of 2 mm diameter. Finally, an irreversible bond between the two PDMS layers was achieved by a surface treatment with O_2 plasma.

For a quantitative evaluation, an aqueous fluorescein sodium salt (1 μg/ml in water Sigma-Aldrich, Germany) and distilled water were used as samples and buffers. The solutions were injected by syringe pumps with calculated input flow rates. Fluorescence images were captured with a high-resolution monochrome digital camera (Hamamatsu ORCAER, Japan) mounted to an Olympus MVX10 epifluorescence microscope, and all quantitative measurements of the fluorescent intensity were obtained using an Olympus Wasabi imaging software package.

RESULTS AND DISCUSSION

We investigated the performance of the proposed design by computational simulation (CFD-ACE+). The output concentrations from the CFD simulation were highly accurate to the theoretical values within 1% absolute error (Figure 3). Based on the design, a PDMS device was fabricated by soft-lithography and its performance was tested with fluorescent dye. Only one sample was injected with the fluorescent dye (10 μL/min) and the other samples were injected with DI water (10 μL/min). The experiments were alternatively repeated under the same condition for C_A, C_B, and C_C as shown Figure 3. First, for a qualitative evaluation of the device performance, fluidic behaviors in the mixing regions were observed. The results showed that the fluorescent solution and the DI water were evenly merged with one-to-one ratio in the mixing regions (Figure 4a). After collecting the solutions of 15 combinatorial mixtures from the device, their fluorescent intensities were analyzed. The results had

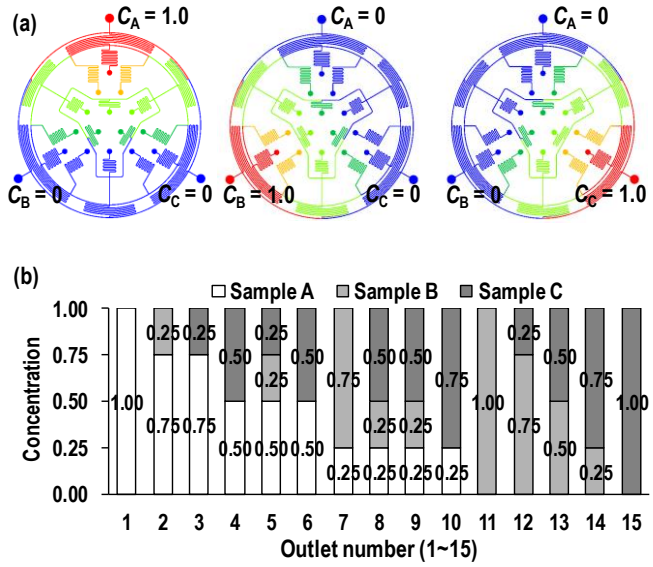

Figure 3 (a) CFD simulation results with the input concentrations of (C_A, C_B, C_C) = (1, 0, 0), (0, 1, 0), and (0, 0, 1), respectively. (b) The resulted output concentrations in each output (absolute error <1%).

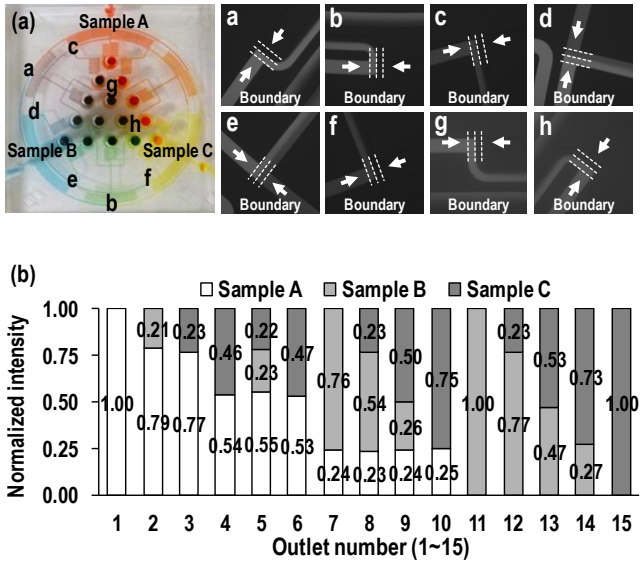

Figure 4 (a) Fluorescent experiments with the fabricated PDMS microfluidic network-based combinatorial device and the images in the mixing regions. (b) The normalized intensities in each output (absolute error < 4%).

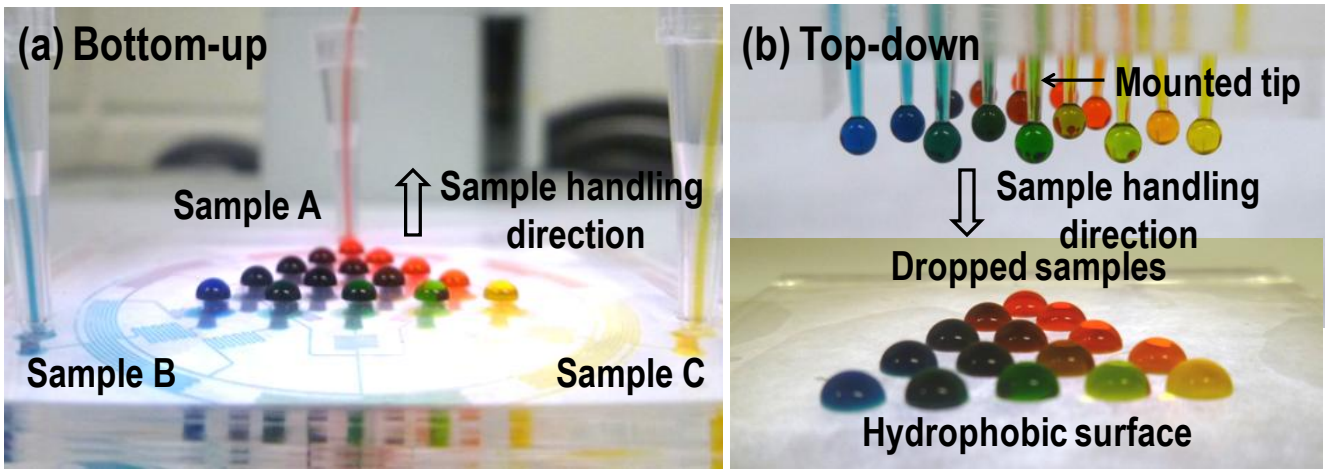

Figure 5: Sample handling strategies for high-throughput systems (HTS): (a) a bottom-up liquid handling method for the pipette-based robot arm system and (b) a top-down liquid handling method for the spotting or dropping of samples. The resulted combinatorial mixtures were dropped on a hydrophobic surface using customized tips for stable drop generations.

good agreement with the theoretical values with maximum of 4% absolute error (Figure 4b). In addition, the two liquid handling strategies (bottom-up for pipetting and top-down for spotting/dropping) were conceptually tried to collect the combinatorial mixtures for potential high-throughput system (HTS) applications (Figure 5).

CONCLUSION

The single-layer microfluidic network-based combinatorial dilution, covering 15 combinations with 3 samples for the combinatorial mixture DOE, has been successfully tested by mathematical modeling, simulation, and fluidic experiments. We have also conceptually presented two liquid handing methods (bottom-up and top-down). In the future, we will study on the geometries of outlets for stable spotting/dropping system (e.g., mounted tips), as well as mixing structures for high-throughput applications (e.g., higher flow rate). Thus, we expect that the proposed device will be valuable in many areas of biological and material researches for high-throughput screening and optimization.

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