DESIGN AND FABRICATION OF A CD-LIKE DISPOSABLE MICROFLUIDIC PLATFORM FOR SERIAL DILUTION

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Interest in Lab-on-CD technology has been escalated by application to real world problems, with advantages including low-volume consumption of reagents, miniaturization, improve portability, multiplexing the assays and potential for automation, while one of the major technical challenges is the manufacturability in a cost-effective manner. In this work, we used polyester transparency (overhead transparency film) as substrate and printer toner as adhesive material to fabricate lab-on-CD device rapidly and with a low cost. By simply controlling the rotation speed, the microchip can meter from 200 nL to 2 μL liquid and serially dilute a sample in parallel with a coefficient of variation (CV) less than 5%.

KEYWORD:

Lab on CD, polyester-toner microchip, centrifugal, hydrophobic valving, overflow metering, dilution

INTRODUCTION

Serial dilution involves the stepwise dilute of a solution with a constant dilution factor in each step, resulting in a geometric progression of the concentration.[1] It has been an indispensable procedure intensively used for quantitative analysis in biochemistry, pharmacology and homeopathy.[2,3] The conventional method for serial dilution requires many repeating pippeting steps, rendering the process time-consuming (half hour to several hours) and labor-intensive, and for each dilution step, it consumes hundreds of microlitter reagent, adding up the cost per test.

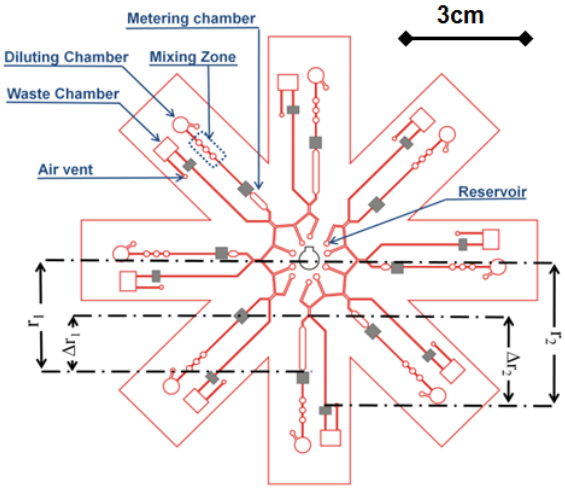

Figure 1: Schematic of the microfluidic network on a centrifugal CD-like PeT metering microchip. Gray bars represent hydrophobic patches on the channel surface.

Lab-on-a-Chip (LOC) technology has been bolstered in the last decade by the ability to control flow through active or passive valving. The advantages of LOCs include low fluidic volume consumption, improved portability, and potential for automation. Previous work on serial dilution in microdevices was focused on preparation of buffer environment where laminar flow was involved; as a result, the dilution process was limited by the diffusion rate of the analytes (e.g., DNA and protein have slow diffusion rates).[4] In this work, we designed and functionalized a CD-like polyester-toner (PeT) microchip where flow is controlled by centrifugal forces to achieve an automated, parallel serial dilution with accurate volume control (Figure 1).

EXPERIMENTS

The PeT microchip can be easily fabricated with simple instrumentation and costs less than 1 USD per microchip (Figure 2), thus it can be used as disposable cartridge. Various centrifuge fluidic functions have been introduced on this CD-like PeT microchip: (1) hydrophobic valving: while the native surface overhead transparency film is hydrophilic, modification of the channel surface can be conveniently achieved by mass-printing hydrophobic printer toner patches on the PeT sheet. (2) Metering by overflow method and (3) chaotic mixing.[5] Control of the fluidic delivering was achieved by simply manipulating the rotation speed of the CD-like polyester-toner microchip. There are two steps involved in the process: metering and mixing. When the microchip is spun at 400 rpm, it generates enough pressure to

burst only the hydrophobic valves that are connected to the waste chambers which retain the liquid in the metering chambers and remove the excessive volume. The retained volumes are geometrically defined by the dimension of the metering chambers. In the mixing step, the spin speed increases to 800 rpm, the burst valves connected to the metering chambers are open, allowing the metered liquids in sample layer and buffer layer passing through the chaotic mixing zone and flowing to the diluting chamber. (Figure 4)

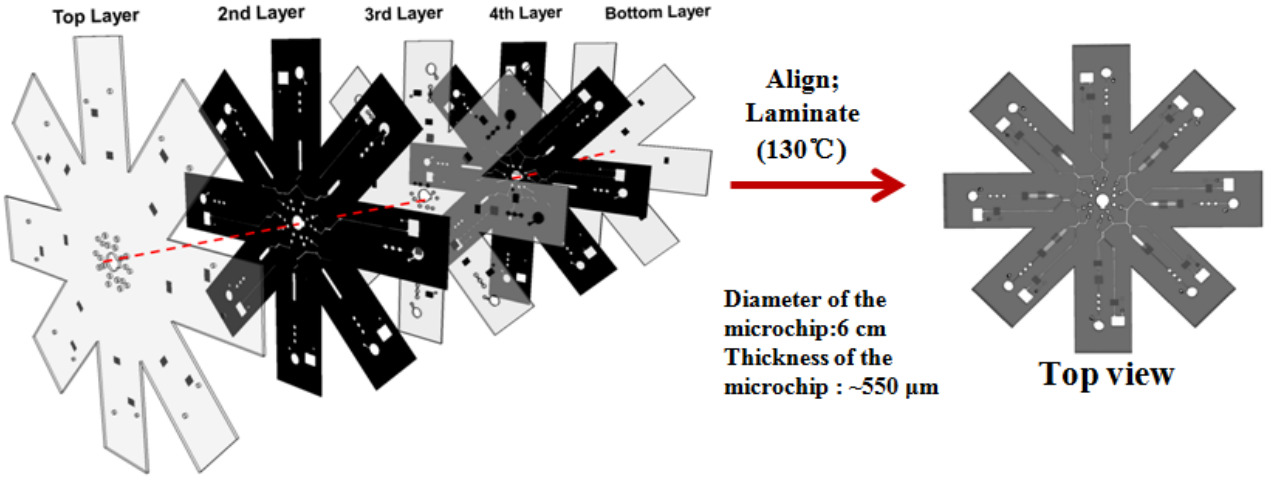

Figure 2: Schematic showing assembly of a dilution microchip, consisting of polyester transparent film and printer toner. The top, 3rd and bottom layers are transparency film, first patterned by printer toner to define the hydrophobic zones. The 2nd and 4th layers are transparency film which is uniformly coated with toner by both sides. Laser-ablation is used to create micro-structures on each layer. Thermal lamination was by a standard laminator.

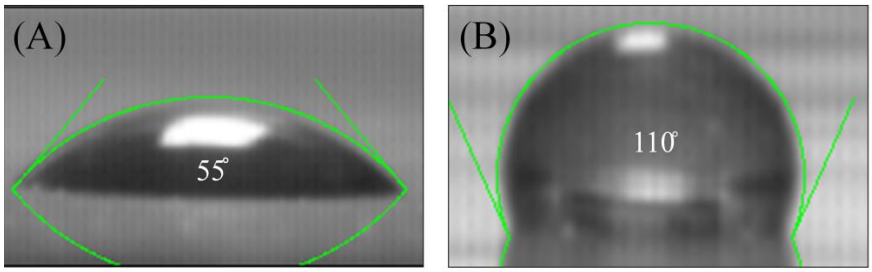

Figure 3: Image of 3 μL water droplet on polyester film (CG5000; 3M) without (A) and with (B) toner-coated surface.

Colorimetric image analysis was used to quantitatively validate the metering and dilution performance of the CD-like microchip. It is confirmed that by changing the geometry of the metering chambers, volume ranges from~200 nL to 2000 nL could be metered.(Figure 5) A two-fold serial dilution of one sample across four to eight different concentrations was generated simultaneously on a disc PeT microchip in 2 min requiring less than 30 uL sample and reagent in total.(Figure 6). The actual dilution ratio correlates with the calculated ones with a CV smaller than 5%. Due to the intrinsic of parallel metering microfluidic network, this method is free from the error propagation which exists in other serial dilution methods.

Current effort includes integrating colorimetric assays (e.g, protein or antibody) will be integrated on this CD-like microchip. By depositing different indicators in the dilution chamber, this CD-like microchip can potentially quantify target analytes in the blood or urine. The low requirement of manual intervention of this assay could greatly lighten the workload and minimize potential human exposure to high-risk biohazardous material. When combined with detection system, this cost-effective assay will provide a simple strategy for a rapid point-of-care diagnosis.

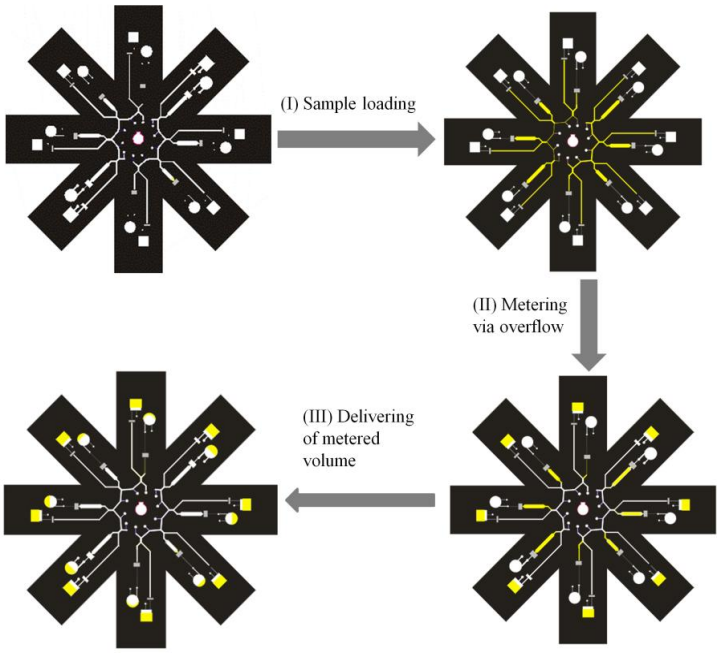

Figure 4: Schematic illustration of liquid metering. (I) Reagent primes the channel by capillary force but stops when it encounters the hydrophobic zone. (II) Depletion of excess liquid via the overflow at low spin rate. (III) Geometrically-defined volume of liquid is delivered by increasing rotating speed (high spin rate) to provide enough pressure to burst the valve. Yellow dye was used to mimic the sample liquid for better visualization

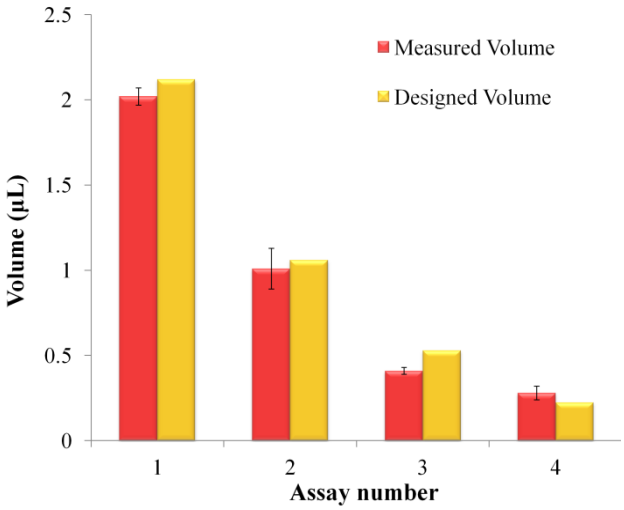

Figure 5: Validation of metering performance. Comparison of empirical volume with calculated metered volume (n=4). The chip was scanned in color and image was imported into Wolfram Mathematic 8. Calibration curve correlating pixel number with metering dye volume was used to quantitatively determine the actual metering volume in the microchip.

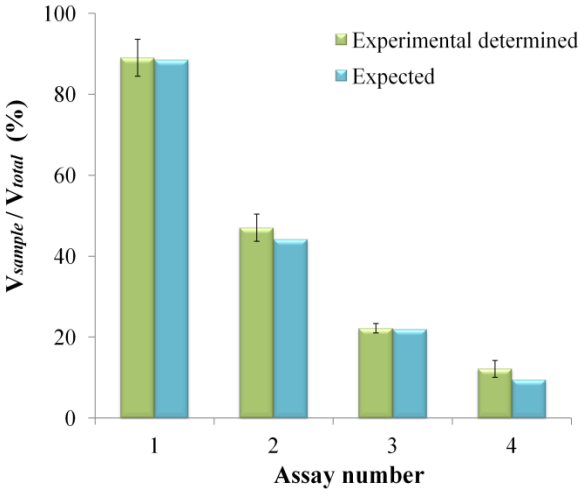

Figure 6: Validation of dilution performance. Comparison of empirical dilution with calculated metering (n=4). Blue and green dyes were loaded to the microchip to mimic the sample reagent and buffer reagent, respectively. Calibration curve correlating the mean intensity of the Hue in the detection zone was used to calculate the actual mixing ratio in the chip.

REFERENCE

[1] Serial Dilution from Wikipedia. http://en.wikipedia.org/wiki/Serial_dilution

[2] Bang,H., Lim,S.H. et al . J. Micromech. Microeng. 2004, 14, 1165

[3] Kim,C., Lee, K., Kim, J.H.,Shin, K.S., Lee, K.,Kim, T.S., Kang,J.Y. Lab on Chip, pp. 473-479, (2008)

[4] Jiang, X., Ng, J. M. K., Stroock, A. D., Dertinger, S. K. W., Whitesides, G.M., J. AM. Chem. SOC., pp. 5293-5295, (2003).

[5] Lago, C. L. do; Silva, H. D. T. da; Neves, C. A.; Brito-Neto, J. G. A.; Silva, J. A. F. da Analytical chemistry.,pp 3853-3858, (2003).

[6] Gyros AB. Appl. Rep. 101, Gyrolab MALDA SP1, Uppsala, Sweden.