DROPLET MICROFLUIDICS INSIDE PAPER

Hiroki Yasuga¹, Weijin Guo², Jonas Hansson², Tommy Haraldsson²,
Norihisa Miki^{1*} and Wouter van der Wijngaart^{2*}

¹Keio University, Yokohama, JAPAN

²KTH Royal Institute of Technology, Stockholm, SWEDEN

ABSTRACT

Here, we demonstrate, for the first time: the self-digitization, i.e. spontaneous formation, of microdroplets during the imbibition of paper; the on-demand merging of individual microdroplets in paper; and the on-demand ejection of individual microdroplets from the paper. Two technical novelties underlie these novel functions: the formation of free-standing synthetic microfluidic paper, i.e. a porous matrix of slanted and interconnected micropillars without bottom layer; and the hydrophobic surface modification of the paper. The ease of manipulation and the direct access to the microdroplets from the environment makes this an extremely versatile tool, with potential applications in liquid sample digitisation and microparticle generation.

INTRODUCTION

Microdroplet-based systems provide low-cost and high-throughput biological assays or medical diagnosis because the small volume of microdroplets leads to reduction of sample/analyte and acceleration of reactions [1]. EWOD [2], slip-chip [3] and continuous droplet microfluidics [4] are well-established platforms for the generation and manipulation of the discrete microdroplets using electrical, mechanical and pneumatic manipulation, respectively. Self-digitization, i.e. spontaneous droplet generation without the need for complex interfacing has also been demonstrated in spatially arranged mechanical structures; e.g., closed microfluidic system including consecutive bypassed channels [5, 6] and open microfluidic systems of a well array [7-10] or surface energy patterned flat surfaces [11]. These self-digitization methods drastically facilitated the preparation of two-dimensional (2D) arrays of microdroplets, which can be used for digital biological assays [12]. Droplet microfluidics have been recently demonstrated on top of paper-based surfaces, which includes EWOD on hydrophobic paper [13, 14], and droplet generation in channels engraved inside omniphobic paper [15]. Although these previous studies showed the applicability of paper substrate to droplet microfluidics as proof-of-concept, they only replace conventional EWOD and microfluidic device materials on paper and droplet formation and handling inside a porous medium of paper has not yet been shown to the best of our knowledge.

In this study, we demonstrate, for the first time, droplet generation, transport and merging inside a paper substrate consisting of a three-dimensional (3D) porous matrix. We recently introduced synthetic microfluidic paper (SMP), as a novel paper substrate that consists of slanted and interconnected micropillars with well-defined geometry [16]. In the SMP, there are regularly distributed hollows in 3D space, that are surrounded by 8 pillars. We

conceived that these hollows are possible to work for self-digitization of sample solution. To achieve the self-digitization in the SMP, we designed SMP as free-standing, i.e. without a bottom layer, which allows fluid transport not only laterally but also vertically through the paper.

FABRICATION

The free-standing SMP was fabricated in an Off-Stoichiometry-Thiol-Ene (OSTE) polymer (OSTEMER[®] 220 Litho, Mercene Labs, Sweden). Photolithographic definition of the slanted pillars is performed using a collimated, near-UV mercury lamp (OAI, Milpitas) (12 mW/cm² @ 365 nm) and the development process was performed in acetone (VWR, USA) under ultrasonication (EMAG Technology, Germany).

The formation of the free-standing SMP was performed in the following subsequent steps: a 1 mm thick square-shaped OSTE frame was placed on top of a 100 μ m thick transparent film (Xerox, USA); OSTE prepolymer was added in the frame; a film photomask (JD Photo-Tools, UK) was placed on top of the frame; slanted and interlocked micropillar structures in SMP were formed in the OSTE by four-directional lithography (Fig. 1a) by reflecting vertically collimated UV light to angles of 60° using four aluminum mirrors for 100 s; the UV-exposed OSTE device was developed in acetone for 5 min under

Figure 1: a-c) Schematic drawings showing the fabrication process of free-standing synthetic microfluidic paper (SMP): a) multi-directional UV exposure of the OSTE precursor; b) development of SMP in acetone; and c) hydrophobic surface functionalization of SMP. d) A whole image of fabricated SMP. The pillar arrays were fabricated in 10 mm x 10 mm square. e) A magnified view of slanted and interlocked pillar structures. The scale bar is 500 μ m.

ultrasonication (Fig. 1b). The interlocked pillar geometry is defined by the photomask pattern containing transparent squares with side length 250 μm and pitch 1 mm. The pillar arrays are fabricated in 10 mm x 10 mm square.

The formed free-standing SMPs were made hydrophobic in a surface treatment by UV grafting. SMPs were placed in a solution of 20% w/w 3,3,4,4,5,5,6,7,7,8,8,9,9,10,10,10 heptafluorodecyl methacrylate (FDM, Sigma-Aldrich), 0.1% irgacure 819 (BASF Corp., USA), and 0.1% benzophenone (Sigma-Aldrich, USA) in isopropyl alcohol (IPA, BASF) and illuminated by collimated UV-light for 300 s and thereafter incubated in IPA for 300 s. The contact angle of ultrapure water (prepared using a Milli-Q system, Merck Millipore, USA) to the flat surface of functionalized OSTe was $121.6 \pm 6.9^\circ$. An image of fabricated free-standing SMP and a magnified image of slanted and interlocked pillars are shown in Fig. 1d and c, respectively.

EXPERIMENTS

Droplet Generation

The self-digitization of sample liquid into droplets in the SMP was performed in 4 steps (Fig. 2): i) priming the hydrophobic synthetic paper with acetone; ii) replacing the acetone by ultrapure water with food dye (Fig. 2a); iii) adding mineral oil (Sigma-Aldrich, USA) on top of the paper (Fig. 2b); iv) placing the SMP on top of a paper tissue (Contec, USA) to induce the self-digitization by the vertical oil flow (Fig. 2c).

Droplet Transport

We conducted experiments to transport droplets from within to outside the SMP (Fig. 3a). To transport droplets onto a plastic film lying underneath the SMP, one pillar of the SMP from the top at the position of an individual droplet was mechanically pushed. The transported droplets were inspected under light microscopy using 4–20 $\times$ long-range objective lens (Leica, Germany).

Droplet Merging

We conducted experiments to merge droplets between different pieces of SMPs. Merging of generated droplets were attempted as follows (Fig. 3b): two pieces of SMP with 2D droplet arrays were placed on top of one another. Single droplets from the top layer were mechanically pushed into the bottom layer. During this process, the top droplets were merged with a droplet in the bottom layer.

Figure 2: Schematic drawings of self-digitization of sample liquid droplets. a) Replacement of impregnated liquid from acetone to sample aqueous solution. b) Addition of mineral oil on top of the sample solution and the synthetic microfluidic paper. c) Drainage of aqueous solution to tissue lying under the synthetic microfluidic paper, inducing self-digitization.

Figure 3: Schematic drawings of droplet transport and merging in synthetic microfluidic paper (SMP). a) Pushing one pillar of the SMP from the top at the position of an individual droplet results in the ejection of that droplet on a plastic film below. b) Pushing one pillar of the top SMP layer of a two-layer stack results in the droplet underneath that pillar merging with a droplet in the bottom layer.

RESULTS AND DISCUSSION

Droplet Generation

Fig. 4 shows top and side view photographs of the operation for self-digitization. We confirmed successful generation of droplets inside the SMP. In all steps, liquid was added on top of the paper, liquid flowed vertically through the paper, and excess liquid was drained into the underlying tissue.

Droplet Transport

Fig. 5 a and b show the results of droplet transport. We successfully ejected droplets from an SMP by pushing a pillar top with tweezers on a plastic film below. The diameter of droplets ejected immediately after formation was $651 \pm 34 \mu\text{m}$ ($n = 10$).

Figure 4: Side and top view photographs of each step of droplet generation in synthetic microfluidic paper (SMP). a) ultrapure water with blue food dye imbibed in the SMP, b) mineral oil added on the SMP and the water, and c) generated droplets inside the SMP. All scale bars are 2.5 mm.

Figure 5: Photographs on droplet transport and merging in synthetic microfluidic paper (SMP). a) Pushing one pillar of SMP to eject a droplet with tweezers. b) A droplet transported on a plastic film. The resultant diameter was $651\ \mu\text{m} \pm 34\ \mu\text{m}$ ($n = 10$). c) Two pieces of SMP arrays placed on top of one another. A droplet array in each paper is dyed with blue and red food dye. d) Merged droplets in the bottom SMP. The scale bars are 2.5 mm.

Droplet Merging

Merging of droplets between SMPs was demonstrated. Fig. 5c shows two droplet arrays inside SMPs placed on top of one another and a pillar top pushed by tweezers. As shown in Fig. 5d, we observed droplets with purple colour resulted from pushing different pillars, which confirmed droplets were merged by the process.

CONCLUSION

This work introduces a new platform for the microfluidic generation and manipulation of droplets in paper. The ease of manipulation and the direct access to the liquid from the environment makes this an extremely versatile tool, with potential applications in the point-of-care setting, for digital assays, in microtissue growth, etc.

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CONTACT

*Norihiisa Miki and Wouter van der Wijngaart are shared last authors and corresponding authors.
N. Miki, tel: +8145-563-1141, miki@mech.keio.ac.jp
W. van der Wijngaart, tel: +46-8-7906613, wouter@kth.se