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Droplet Screens in Nanovolumes Using Static Conditions

Liang L. Wu, Wei Xu, Mark Bachman and G.P. Li

Abstract— **Microfluidic droplet systems have shown great promise in high throughput chemical assays to minimize chemical consumption and increase process efficiency. We report a droplet system that forms nanovolume drops under static conditions. The programmability of drop sizes is determined by geometric configurations and surface tension, and not particularly sensitive to flow rates. The geometry of the device predetermines locations of drops, and thus it is easy to identify the locations of drops and the volumes of the drops within them. Further integration can be made to generate screening assays and utilized in various applications such as crystallization screening and solubility studies. This technology makes hand-operated systems a possibility, since precision control of flow rates is not necessary.**

I. Introduction

Miniaturization of chemical assays to nanovolumes is an emerging paradigm that results in a reduction of the consumption of reagent, and in increase in process efficiency. Typically, robotic systems are used to meter nanoliters and picoliters of reagent in array plates [1]. However, robotic systems are large, costly and not feasible for academic research purpose. In recent years, there has been great interest in developing small volume droplet systems that use immiscible fluids in microchannels. These have the potential to enable large numbers of chemical assays on small footprint, while requiring only small quantities of chemicals [2]. Moreover, water-in-oil systems do not suffer appreciably from reagent contact with channel walls, a serious problem in microchannel systems. High-throughput droplet systems can be used to study protein crystallization [3], perform solubility screens [4], and generate combinatorial conditions for chemical assays. Different methods are used to generate small droplets such as electrowetting [5], dielectrophoresis [6], and voltage pulses [7] to form nanoliter or smaller droplets. A common method uses shear forces produced by flowing oil and water in a microchannel, generating smaller droplets in a T-shaped channel [8], or in micro nozzles [9]. In these systems, fluid flow initiated breakup of the aqueous solution into small droplets. With precise control of flow rate, different chemical concentrations can be varied. Recently, Shim, et al further improved the system to provide small chambers at fixed locations to catch the droplets after formation [10]. Though uniform droplets are produced, these microfluidic devices require sophisticated external flow control, often by a computer controlled syringe pump that connects to the chip through relatively long tubes that waste reagent. We report a microfluidic cassette that may be used to quickly and easily form large numbers of small drops of liquid of predetermined volumes at predetermined locations without the need for external flow control.

The phenomenon of deformation and breakup of drops in oil [11] have been studied by different research groups in various geometry configurations [12-14]. The technology discussed here utilizes variations in the shape of a microfluidic channel to induce droplet breakup at specific sites along the channel. By carefully controlling the shape of a microfluidic device, we can preprogram where each drop will form, and the volume it will have. These droplets may be made to collect in cavities that combine two or more drops together for the purpose of performing multiple chemical assays.

II. System and Methods

A. Microfluidic Device Fabrication and Configuration

Microfluidic devices were made from poly-dimethylsiloxane (PDMS) through cast molding. The fabrication is shown in Fig. 1. Silicon wafers were prepared as micro-molds using standard photolithography and deep reactive ion etch to 180 μm. Resist was then stripped and the surface cleaned, then coated with a mold release (Sigmacote™, Sigma-Aldrich, St. Louis, MO). PDMS (Sylgard 184, Dow Chemical, Midland, MI) was prepared according to manufacturers instructions, and then poured over the mold. After curing, the PDMS was removed then exposed to oxygen plasma to activate the surface. The material was sealed to a second piece of PDMS to form a permanently sealed microchannel device.

As seen in Fig. 2, two reservoirs located on both ends of the flow channel provide fluid loading and waste collection. The fluidic sections, "cavities" of 180 μm in depth consist of a main flow channel of 4 cm in length and 150 μm in width connecting to a series of specially designed diamond-shaped

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L. L. Wu is with the Interdisciplinary Material Science and Engineering Department, University of California, Irvine, CA 92697 USA (phone: 949-824-5096; fax: 949-824-3732; e-mail: lwu@uci.edu).

W. Xu is with the Electrical Engineering and Computer Science Department, University of California, Irvine, CA 92697 USA (e-mail: wxu@uci.edu).

M. Bachman is with the Electrical Engineering and Computer Science Department, University of California, Irvine, CA 92697 USA (e-mail: mbachman@uci.edu).

G. P. Li is with the Electrical Engineering and Computer Science Department, University of California, Irvine, CA 92697 USA (e-mail: gpli@uci.edu).

cavities, "chambers" designed to create and store droplets. Each droplet chamber consisted of a dead volume to collect droplets and two fluid bypass slots. The inclusion of the bypass slots is critical for the successful operation of the device since it provides a pathway for the displacement of oil during the movement of aqueous solution as it forms a droplet in the chamber.

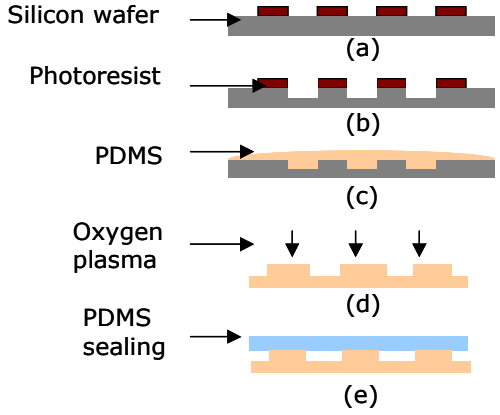

Fig. 1. Fabrication methods of the PMDS microfluidic device: (a) prepare the silicon mold by standard lithography process, (b) deep reactive ion etch of the silicon wafer, (c) cast mold PDMS, (d) Oxygen plasma etch on PDMS surface for sealing purpose; (e) bond the chip to another PDMS substrate. Not shown: treat surfaces to make them hydrophobic.

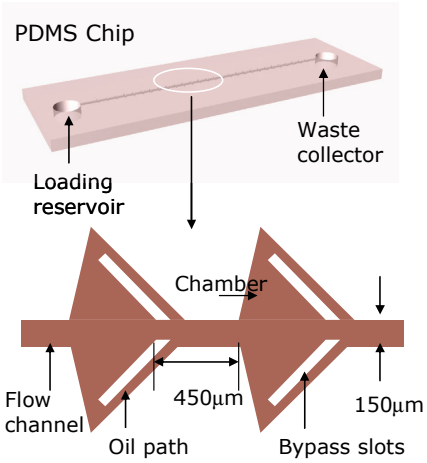

Fig. 2. A top view of the microfluidic channel with a 150 µm wide main flow channel connecting two-sided diamond-shaped chambers with embedded slots. The bridge gap between the two chambers is 450 µm in length. The depth of the entire configuration is 180 µm. Droplets form in the diamond shaped cavities.

B. *Experimental Set-up*

The goal of this work is to produce technology that allows a researcher to readily form nanovolume drops of preprogrammed size using only hand control of the flow and to use those drops in assays. We built many variations of the design shown in Fig. 2. To use the devices, we first filled the channels with vegetable oil, filling them slowly to remove all air bubbles. Then we injected filtered deionized water into the channels and observed droplet formation under a microscope with a video camera attached. Injection of the water was performed by hand using a disposable syringe pipette or by machine using a syringe pump.

In addition to observing the dynamics of droplet formation, we studied the effects of surface hydrophobicity and polymer hydration to determine their importance on the performance of this device. We also demonstrated the ability to first form one drop in the chambers, allow it to sit in a dead region, and then form a second drop from a second fluid in the same chamber.

For the surface hydrophobicity study, we used oxygen plasma to treat the PDMS before sealing. Following this, oil was placed in the device and dyed water was injected while imaging with a video microscope. A second set of devices was allowed to sit for one day in open air after oxygen plasma treatment (which was used to bond the PDMS halves together). Then the treated PDMS surface is known to revert back to its hydrophobic state. To be certain, we loaded the microfluidic devices with SigmaCote™ to make the walls hydrophobic. After draining the SigmaCote™, these channels were filled with oil and the same injection experiments performed.

For hydration experiments, we prepared two devices. We prepared one device to be hydrophobic as discussed above, and then filled with oil. A second device was prepared identically, but then the entire chip (with oil filled channels) was soaked in water for 6 hours. This hydrated the PDMS with channel walls still remained hydrophobic. Following this, lysozyme solution was injected into the devices (by hand pipette) to form nanovolume droplets of protein solution in the chambers. Images and videos of the entire process were acquired by a Nikon Coolpix 990 digital camera through a microscope.

For two-drop experiments, we first filled the channels with a first fluid by manual injection. Oil was then injected to create small droplets with the rest of solutions exited through the main channel and the bypass slots to the waste collector. The small droplets moved to the dead regions of the chambers, and fresh oil was introduced. The new flow of oil did not wash out the droplets in the dead zones of the chambers. Following this, a second fluid was injected into the channels under normal flow, producing droplets in the chambers as usual.

III. RESULTS AND DISCUSSION

A. *General droplet formation*

The device worked in the following manner, as can be seen in Fig. 3. Oil was introduced in the microfluidic device, completely filling it. Following this, an aqueous solution was injected (by hand pipette, negative pressure, or by a delivery device) into the device. During injection, the solution formed a continuous cylindrically shaped column (a "sausage") that *did not* break up into drops. After injection, the water-oil system began to equilibrate and water gently broke into droplets. The shape of the microchambers created

instabilities at precise locations along the sausage, resulting in breakup into preprogrammed droplet volumes at preprogrammed locations. After breakup, the water formed small spheres of known volume and sat in the center of the droplet chambers. The droplet size and position was determined primarily by the shape and size of the chamber volumes and not by shear forces during flow through the oil. Furthermore, the average droplet volume changed from 32.7 nl to 22.0 nl as flow rates varied from 0.45 ml/min to 4.45 ml/min, which a weak dependence of droplet size on flow rates was shown. This means that this type of device can be used to generate conditions for nanovolume assays.

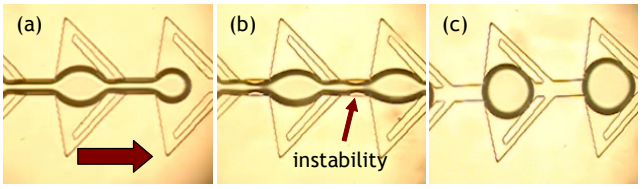

Fig. 3. Video frames of droplet formation in microchambers. (a) Aqueous fluid flows in from the left to form a single column of fluid that passes through each chamber. (b) After fluid flow has stopped, the aqueous fluid column forms instabilities in the neck regions between chambers. (c) Aqueous fluid pulls back into the chamber to form droplets. Oil flows from the chamber into the neck region through the bypass slots on either side of the chambers.

B. *Hydrophobicity of surfaces*

We observed that the success of droplet formation is highly dependent on the hydrophobicity of the walls of the micro device. Devices that were treated to be hydrophobic operated consistently and flawlessly according to the description above and in Fig. 3, typically forming drops in over 94% of the chambers. Hydrophilic devices produced irregular drops or did not form droplets at all. Typical success rates were less than 40% droplets per chamber.

Fig. 4 illustrates the breakup of a droplet in a hydrophilic channel. The hydrophilic neck regions between chambers do not allow the drop to readily separate. Consequently, most regions do not form instability points and remain connected together. Furthermore, if the water does break in the neck, the capillary forces on the neck walls work in opposition to the pull-back forces caused by the droplet's desire to form a more circular shape, resulting in a slow and unsteady droplet formation.

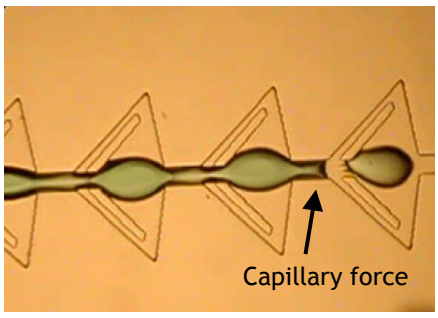

Fig. 4. Droplet formation in a hydrophilic device. Attractive forces on the walls prevent most regions from breaking into drops. Capillary action in the necks resists the pull back of water into droplets.

C. *Hydration of PDMS*

Many assays, such as solubility studies and protein crystallization, take days or weeks to perform and conditions within the droplets must be controlled during that time. PDMS is known to be porous, allowing gas and fluids to diffuse slowly through its bulk. We performed a protein solubility experiment using Lysozyme solution to demonstrate the importance of using a non-porous polymer for fabricating the devices (or alternatively, hydrating the PDMS prior to use). The results are shown in Fig. 5.

In the case of dry PDMS (the usual case in most microfluidic devices), we observed that the droplets soon shrank in size resulting in a precipitate mass at the center of each chamber. By 24 hours, all water had left the chambers. We interpret this to be evaporation of the water into the dry porous spaces of the PDMS at the points where the water contacts the walls of the PDMS.

On the other hand, by first soaking the PDMS in water for 6 hours, we observed that droplets remained unchanged for several days. We interpret this to be a result of the PDMS absorbing water vapor, producing a humid condition in the PDMS that does not favor absorbing water vapor from the droplets. We note that this is a less than ideal situation, however. Apart from the inconvenience of soaking the PDMS in water, the humid PDMS could in principle produce an opposite effect of increasing the water content of the drops, particularly for drops with high salt content. Instead, we recommend manufacturing such devices from a non-porous, hydrophobic material.

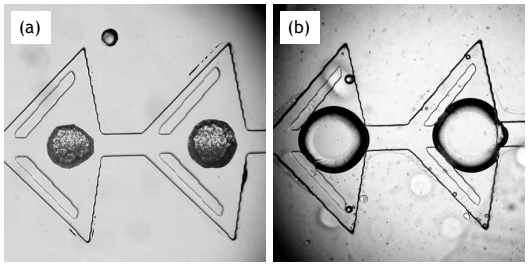

Fig. 5. Pictures of two solubility experiments using Lysozyme solution after 24 hours. (a) The first experiment used non-hydrated walls. The water in the solution diffused into the PDMS polymer leaving a precipitate in the chamber. (b) The same solution in a hydrated chamber remained in liquid form throughout the experiment.

D. *Two drops per chamber*

To further realize the benefit of micro droplet systems, we performed experiments to explore the possibility of generating a combinatorial assay by fusing two different droplets in the same chamber. The first sets of droplets (red) were trapped in the dead volume inside the chamber as shown in Fig. 6. A second set of droplets (blue) then were generated by hand injection of dyed water into the channel. Upon applying gravity or centrifugal forces, two droplets fused together successfully and remained at their fixed location as seen in Fig. 6. A screen assay can be generated using this method by using different reagents to form droplets respectively and fusing the drops for the desired content.

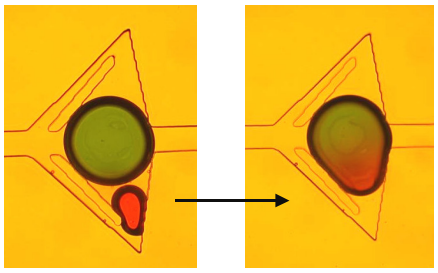

Fig. 6. (a) Two droplets were generated in the same chamber with the smaller droplet generated in the dead volume in prior injection. (b) Two droplets were fused in the same chamber by gravity or centrifugal forces. The dyes in the solution indicated diffusion of the fluids.

E. Droplet screening devices

The previous results demonstrate successful droplet formation by utilizing only geometric design and surface tension to produce preprogrammed droplet volumes at preprogrammed locations. This process may be used to produce screening and combinatorial assays by generating different sizes of drops through various geometric designs such as varying the chamber sizes as envisioned in Fig. 7. This would be valuable as a screening device where a large number of screening drops are prepared in advance on a chip. These pre-metered drops may be stored in the device for use at a later time. When the user injects the liquid sample, the small drops that subsequently form may be moved to the reservoirs containing the pre-metered drops to form a pre-programmed set of chemical mixtures as shown in Fig. 7. This could be used, for example, in a screening experiment for protein crystallization.

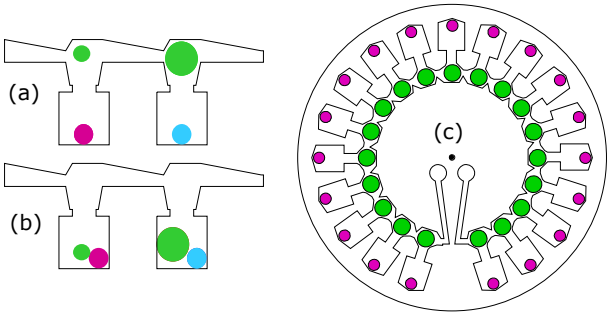

Fig. 7. (a) Droplets generated with different volume and composition by appropriate geometric designs of the device. (b) Fusion of two droplets by cavities path, gravity of centrifugal force to create a chemical assay of different concentration. (c) An envisioned device meters commonly used reagents in advance to be combined with small volumes of a second solution at a convenient later time. A circular platter can house many drops for use in a carousel that automates imaging.

III. CONCLUSION

We report a static droplet system that works by creating the conditions for passive drop breakup without the need for precision control of flow. The shape of the cavities, not flow rates, determines the programmability of drop sizes and locations. This formation mechanism is passive and not particularly sensitive to fluid flow rates enabling a human operator to reliably generate large numbers of nanovolume drops. We studied performance of this method for different geometries, surface properties, and material properties of PDMS. Our results indicate that hydrophobic walls and pre-hydrated (or non-porous) device are required for successful droplet formation and preventing water evaporation. Multiple drops can be fused together in the chambers after formation, enabling one to design devices that can do chemical screens. We believe this approach to nanovolume droplets will allow the creation of inexpensive, hand-operated devices for performing high throughput, low volume chemical assays.

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