

Multiple and High-Throughput Droplet Reactions via Combination of Microsampling Technique and Microfluidic Chip

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Web-Enhanced Feature S Supporting Information

ABSTRACT: Microdroplets offer unique compartments for accommodating a large number of chemical and biological reactions in tiny volume with precise control. A major concern in droplet-based microfluidics is the difficulty to address droplets individually and achieve high throughput at the same time. Here, we have combined an improved cartridge sampling technique with a microfluidic chip to perform droplet screenings and aggressive reaction with minimal (nanoliter-scale) reagent consumption. The droplet composition, distance, volume (nanoliter to subnanoliter scale), number, and sequence could be precisely and digitally programmed through the improved sampling technique, while sample evaporation and crosscontamination are effectively eliminated. Our combined device provides a simple model to utilize multiple droplets for various reactions with low reagent consumption and high throughput.

ver the past few years, droplet-based microfluidics has attracted increasing attention, because a large number of reactions can be independently miniaturized into tiny droplets ranging from nanoliter to picoliter sizes without having to increase the chip size and complexity.¹⁻³ Microfluidic chips have provided a powerful platform for accomplishing droplet formation and manipulation within one chip of only a few square centimeters in size. Passive droplet formation methods based on T-junction or flow-focusing geometries in microfluidic chips are able to generate monodisperse droplets (water in oil) with frequencies from 2 Hz to 5300 Hz.² A variety of droplet manipulations, such as sorting,^{4,5} merging,^{6–8} and even logic functions,^{9–11} have been carried out in microfluidic chips. However, droplet generation and manipulation functions within one chip can easily interfere with each other and are difficult to balance, despite being separated by a long, winding channel. Moreover, in these passive methods, droplet generation for multiple samples is limited by the "world-to-chip" interface problem and cannot be addressed individually.^{12,1}

To overcome the aforementioned limitation, the cartridge technique¹⁴ offers an effective method for introducing different samples to a droplet-based system without interference.¹⁵ Arbitrary one-dimensional (1D) droplet arrays can be generated in a cartridge by sequentially immersing the cartridge or capillary tip into appropriate samples to aspirate the desired volume. Droplets can be mixed with other samples or reagents during or after aspiration in processes that are called the premixing and post-mixing modes.¹⁶

A report showed that by using the pre-mixing mode with a multiwell plate or slotted-vial array (SVA) to develop an automated microfluidics screening assay platform (called



Droplab) for precise liquid metering and mixing, droplet assembly, and droplet array storage.¹⁶ The post-mixing mode has been applied extensively with different techniques (such as passive droplet generation) in microfluidic chips to obtain a much higher throughput. Ismagilov and co-workers have used the preloaded cartridges in microfluidic chips to test or evolve catalysts,¹⁷ and to deliver a sequence of stimuli to a stationary substrate for multiple analyses in parallel.¹⁸ This post-mixing mode was also adopted by Griffiths et al. to generate a series of smaller daughter droplets for the screening of compound libraries.¹⁹

In this context, we show a hybrid device: the cartridge droplet generation technique combined with a microfluidic chip. We improved the cartridge technique with a novel movement scheme: the capillary, instead of the multiwell plate, was moved by a digital and automatic manipulator to aspirate different samples or the covering oil. The experimental results show that samples can be miniaturized into droplets of nanoliter to subnanoliter size, and the droplet composition, distance, volume, number, and sequence can be precisely and digitally programmed via a personal computer (PC). Moreover, sample evaporation and cross-contamination were eliminated by the covering oil. This improved droplet generation technique was directly combined with microfluidic chips for further droplet merging and other manipulations, which is

Received: August 6, 2012 Accepted: October 17, 2012 feasible for large-scale to single-droplet generation and manipulation.

EXPERIMENTAL SECTION

The hybrid droplet device for generation and manipulation consists of four major components (see Figure 1): the



Figure 1. Droplet sampling device setup: (a) manipulator, (b) multiwell plate, (c) capillary, and (d) PDMS chip.

manipulator, the capillary, the multiwell plate, and the PDMS microfluidic chip. The implementation of our system relies on three general steps: (1) load the multiwell plate with different samples, cover with a layer of oil, and input the parameters of the desired droplet array into the controller via a PC; (2) start the controller to aspirate certain volumes of samples and oil into the capillary sequentially; and (3) introduce the generated droplets into the PDMS chip for merging, mixing, detection, or other manipulation. Detailed description of the manipulator could be found in the Supporting Information. The manipulator can be programed by a controller (see Figure S1 in the Supporting Information), which is connected to a PC. Teflon tubing (PTFE microbore tubing, outer diameter of OD = 350 μ m, inner diameter of ID = 50 μ m) was used as the capillary. To guide the droplets smoothly into the PDMS chip from the Teflon tubing, the adjoining entrance of the microchannel was made to be ~50 μ m (Figure 1), matching the ID of the tubing. The 50-µm-high microchannels and micropillars inside the PDMS chip were fabricated by soft lithography.²⁰ A constant vacuum was maintained at the outlet of the PDMS chip via a solenoid valve. The on/off state of the valve can be triggered by the controller. Droplets can be stopped in the microchannel by turning off the valve. An inverted microscope (Model IX71, Olympus) coupled with a CCD camera (DP70, Olympus) was used to observe the coalescence and measure the fluorescent intensities of droplets within the PDMS chip. The CCD camera and controller were connected to one PC, so that all operations could be controlled via the PC.

The carried fluid was silicone oil with a viscosity of 10 cSt. The starting materials for the precipitation of iron oxide nanoparticles were $FeCl_2 \cdot 4H_2O$ (Sigma–Aldrich), $FeCl_3 \cdot 6H_2O$ (Acros Organics), ammonium hydroxide solution (28% NH₃, Fluka), and hydrochloric acid (37% HCl, Acros Organics). To avoid oxidation of Fe^{II} during the reaction process, all prepared solutions were degassed in a vacuum chamber for 1 h before use. Fluo-4 (pentapotassium salt, Life Technologies), Rhod-2 (tripotassium salt, Life Technologies), La^{III} (lanthanum nitrate

hydrate, Sigma–Aldrich), Yb^{III} (ytterbium acetate tetrahydrate, Sigma–Aldrich), and Y^{III} (yttrium chloride hexahydrate, Sigma–Aldrich) solutions were prepared in 40 mM 3-(*N*-morpholino)propanesulfonic acid buffer (MOPS, Sigma–Aldrich).

RESULTS

Improved Sampling Technique. The capillary aspirates the oil when the cantilever remains in the "up" state. When the cantilever is triggered to stay in the "down" state, the capillary aspirates the sample. The residence time in the oil (T_0) and sample (T_s) could be adjusted respectively to generate droplets with desired distance and volume, although the droplet distance and volume are still determined by the viscosity of the carrier fluid and sample and the total droplet number. Figure 2



Figure 2. Droplet volume versus sample aspiration time (T_s) under 41 and 58 kPa. The droplet sizes were measured and averaged over 20 droplets for each aspiration time. The upper inset shows two 3-nL droplets, two 2.3-nL droplets, three 1.8-nL droplets, three 1.3-nL droplets, and three 0.8-nL droplets. All are the same type of sample: red-dyed deionized (DI) water. The lower inset shows three 470-pL blue droplets, three 560-pL red droplets, three 480-pL blue droplets, three 610-pL red droplets, two 580-pL blue droplets, and two 660-pL red droplets.

provides droplet volume calibration as a function of $T_{\rm S}$ under 41 and 58 kPa, which shows that the sample can be precisely digitalized into nanoliter to subnanoliter droplets via the manipulator. The spatial position, $T_{\rm O}$, and $T_{\rm S}$ of the capillary can be directly input into the manipulator through the PC. Therefore, droplet composition, distance, volume, number and sequence can be precisely and digitally programmed as indicated by the two insets in Figure 2. The switch between the "up" and "down" state of the cantilever requires <0.1 s, and the accuracy of $T_{\rm O}$ and $T_{\rm S}$ is 0.1 s. Such ultrafast and accurate switching makes it possible to produce a minimum droplet volume of 200 pL, using the 50- μ m-ID Teflon tubing. This value could be further decreased using smaller tubing. The droplet generation throughputs in this device are $\sim 1-5$ s per droplet under a vacuum level of 41 kPa, depending on droplet distance, volume, and composition.

Cross-contamination in this device was examined by sequentially aspirating 50 μ M fluorescein and DI water. Data are presented in the form of a figure in Figure S1 in the

Supporting Information. No measurable change was observed in the intensity of the fluorescein and DI water droplet, which indicates that no cross-contamination between these two samples.

Combined Droplet Device Performance. Because cartridge droplet generation occurs in a droplet-on-demand manner, we can input desired droplets into a microfluidic chip for further manipulation. Here, the merging of droplets was demonstrated in a PDMS chip. The merging chamber design was based on pillar-induced droplet fusion described by Niu et al.⁷ Detailed dimensions of the merging chamber can be found in Figure S2 in the Supporting Information. Figure 3 shows that



Figure 3. (a-d) A sequence of images demonstrating the coalescence of two types of droplets: red- and blue-dyed DI water. The merging chamber size was designed according to the total volume of the two droplets.

two types of droplets, red- and blue-dyed DI water, were successfully merged together. These two types of droplets were sucked from the capillary into the PDMS channel and the merging chamber one by one. First, one red droplet was confined by surface tension in the merging chamber while it waited for the following blue droplet to arrive. Then, the blue droplet went into the merging chamber and merged with the red droplet. The merged large droplet was then pushed out of the merging chamber. After mixing completely, the merged droplet was output to a sinuous channel for display and detection. The coalescence of three types of droplets (red-, blue-, and yellow-dyed DI water) was also achieved with a volume ratio of 1.5:2:1 (see Figure S3 in the Supporting Information). It also should be noted that the capillary/chip interface that we designed was found to be effective in preventing large droplets (up to 10 nL) from fission and small droplets (down to 200 pL) from adhesion in the entrance without leakage. Altogether, multiple droplets were successfully generated and flowed smoothly from capillary to chip for nextstep merging in our combined and hybrid device.

In the following two applications, a series of two types of droplets were generated, merged, and detected in a chip. We denoted these two types of droplets and the output droplet after the merge as A, B, and C.

High-Throughput Droplet Screening. In the first application, a series of A_i and $B_{m,n}$ (i = 1-2, m = 1-3, and n = 1-6) were generated. A_1 and A_2 are Fluo-4 and Rhod-2 solutions with the same concentration (10.8 μ M), which are indicators that exhibit an increase in fluorescence (green/red) upon metal ion bonding. A value of m = 1-3 represent metals ion solutions of La^{III}, Yb^{III}, and Y^{III}, respectively, while n = 1-6 are six different metal ion concentrations (0, 2.5, 5, 10, 20, and 40 μ M) of these solutions. The droplet array contains 72 1-nL droplets in the form of { A_1 , $B_{1,1}$, A_1 , $B_{1,2}$, ..., A_i , $B_{m,n}$, ..., A_2 , $B_{3,6}$ }. If $C_{i,m,n}$ is used to represent the merged droplet, the output droplet array is { $C_{1,1,1}$, $C_{1,1,2}$, ..., $C_{2,3,6}$ } with 36 2-nL droplets.

Figures 4a and 4b are the fluorescent images for Fluo-4 and Rhod-2 droplets merged with six different La^{III} solution droplets, which were captured when these merged droplets stopped in the sinuous display channel. The corresponding



Figure 4. Fluorescent micrographs of a series of 2-nL droplets merged from (a) Fluo-4 and (b) Rhod-2 and six different La^{III} concentrations: 0, 2.5, 5, 10, 20, and 40 μ M. The image in panel (a) was captured under blue excitation, and the image in panel (b) was captured under green excitation. (c) Metal ion response calibration and screening for Fluo-4 and Rhod-2: relative fluorescence intensities of 36 droplets. The results are plotted as fluorescence changes relative to the ion-free (0 μ M) reference droplet expressed as ($F - F_0$)/ F_0 , where F is the fluorescence intensity of ion-containing solutions and F_0 is the fluorescence intensity of the reference solution. All final droplet volumes are 2 nL, merged from two identical volume droplets.

movies, M2 (Fluo-4 & La) and M3 (Rhod-2 & La), are provided as web-enhanced files. By comparing the fluorescence intensity of each merged droplet, complete mixing was found to be achieved when the merged droplet moved just out of the merging chamber. Such rapid mixing can be used to perform chemical kinetics measurements and to study violent reactions while using only nanoliters of the reactants.¹⁹

Figure 4c shows the relative fluorescence intensities of the droplet array {C_{1,1,1}, C_{1,1,2}, ..., C_{2,3,6}}, which were plotted as six response screening curves of the three metal ions for Fluo-4 and Rhod-2. In all curves, the relative fluorescence intensities increase rapidly from 0 μ M to 10 μ M and reach a plateau after 10 μ M. Such a plateau means that 10.8 μ M Fluo-4 and Rhod-2 were both saturated by 10 μ M concentrations of these metal ions. However, their fluorescence enhancements are different: for Fluo, Yb^{III} \approx Y^{III} > La^{III}; for Rhod-2, Yb^{III} > La^{III} > Y^{III}.

The 36 pairs of droplet reactions used ~72 nL of solution and required 1.8 min to complete. Therefore, the reagent consumption for each measurement could be reduced to 1 nL per sample, and the reaction throughput is 1200 h⁻¹, which can be much faster by increasing the flow velocity. The screening droplets could be stored in the PDMS chip after we stopped the vacuum. Since PDMS chip outlet in the present design is connected to big tubing (ID = 900 μ m), the screening droplets would be merged all together in the outlet and could not be collected out of chip. However, if we make the outlet the same design as that of the inlet, we could collect the screening droplets out of chip to the capillary or another chip for successive analysis or subsequent application.

Aggressive Droplet Reaction. In the second application, coprecipitation of iron oxide in different conditions was demonstrated. In this case, only one droplet reaction was programmed each time. The sequence of images in Figures 5a–

	9 _{VA} : VB	Droplet after fusion
b	2.2 : 1.0	C 19
	1.8 : 1.0	C B
	1.4 : 1.0	
d and	1.0 : 1.0	
e	1.0 : 1.4	2
	1.0 : 1.8	<u> </u>
	1.0 : 2.2	

Figure 5. (a–f) Formation of iron oxide precipitates after fusion of droplets A and B (A = 2 M NH₄OH and B = 480 mM FeCl₂ + 240 mM FeCl₃ + 480 mM HCl). $V_A = V_B = 1$ nL. (g) Fusion of droplets A and B with different volume ratios.

f demonstrates the coalescence of droplets A and B, which both had volumes of 1 nL. (The corresponding movie, M1 is provided as a web-enhanced file.) In Figure 5g, the reaction results of seven different mixing volume ratios are shown. Only ~14 nL of solution was consumed in the seven different volume ratio reactions. From 2.2:1.0 to 1.0:2.2, reagents A and B were precisely metered to produce reaction conditions ranging from excess alkali to excess acid. The precipitation of iron oxide was found to decrease gradually, and the iron oxide even dissolved and disappeared under the final set of conditions. Importantly, from these images, we found that the coprecipitation is so fast that it immediately forms particles when two droplets come into contact with each other. Moreover, in each volume ratio, the precipitation initiated by the fusion of two droplets did not block the microchannel at such high concentrations. Such flexible, controllable and miniaturized reaction conditions are extremely suitable for aggressive, fast, or even explosive reactions that generate precipitation or heat.²¹

DISCUSSION

Multiple and high-throughput microdroplet reactions described above confirm that our combined system worked seamlessly together for droplet generation and manipulation. The cartridge droplet generation method provides an efficient way for introducing different nanoliter droplets into the microfluidic chip. Meanwhile, the microfluidic chip offers a platform for delicate manipulation such as merging. This feature is valuable for reducing the reaction screening and optimization cost and time and is particularly useful for drug screening, which requires the parsimonious use of precious reagents and rapidly automated processing. It also is noted that we digitally programed not only the mixing volume ratio but also the reaction droplet number in coprecipitation of iron oxide. Our device could be adopted for various microdroplet reactions not only general chemcial and biological reations, but also aggressive reaction such as precipitation and exothermic reaction. Therefore, our combined device promises more droplet applications.

For comparison with the other cartridge droplet generation,^{16,22} our design used a digital and automatic manipulator to move the capillary instead of the multiwell plate. The samples in the multiwell plate were prevented from being pitched and rolled. Besides, the capillary can be quickly and precisely located to the desired sample well (position) and moved up and down to aspirate different samples or the covering oil. Using designs of such a type, the samples could be directly digitalized into nanoliter to subnanoliter droplets without chopping them into smaller daughter droplets in chip. More importantly, individual droplet addressability and highthroughput were realized simultaneously through this simple but effective improvement. Subsequently, the covering oil was ingeniously used as a carrier fluid (continuous phase) to avoid evaporation and cross-contamination. This strategy is different from aforementioned cartridge devices, in which oil was loaded in a separate well. In addition, Teflon tubing was used as the capillary here. Because the droplets are completely surrounded by the carrier fluid²³ and Teflon behaves as a nonstick surface for most liquids, cross-contamination is eliminated during the aspiration of different samples.

In summary, droplet generation in our improved cartridge technique is shown to be individually and digitally addressable, which facilitates exquisite maneuverability and control over droplet composition, distance, volume, number, and sequence. The droplet generation throughputs in our sampling device increased to 1-5 s per droplet, which is efficient and versatile for arbitrary, multiple, and high-throughput droplet generation and could be increased by increasing the flow velocity. This improved cartridge technique and microfluidic chip were integrated to make use of their respective advantages for performing response calibrations and aggressive reaction and screening multiple reaction conditions with minimal (nL)

reagent consumption. Finally, it would be interesting to see that other chips designed with different droplet manipulations (such as logic functions) are adopted to perform complex tasks with ease.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Web-Enhanced Features

Movies showing the droplet coalescence of droplets A (composed of NH_4OH) and B (composed of $FeCl_2$, $FeCl_3$, and HCl) (Movie M1), as well as the merger of La^{III} solution droplets with Fluo-4 (Movie M2) and Rhod-2 (Movie M3).

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Notes

The authors declare no competing financial interest.

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