ON-CHIP PROCEDURES FOR MAGNETIC PARTICLE-BASED ASSAY IN DROPLETS

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ABSTRACT

We propose on-chip procedures for magnetic particle-based assay in droplets in which excess reagents are removed more than a factor of 1/16 by extracting the magnetic particles into a washing buffer droplet, applying a magnetic field from a permanent magnet. For proof of concept, it is demonstrated to perform immunoassay between streptavidin conjugated on the surface of the magnetic particles and biotin labeled with fluorescence. We expect that by varying the surface functionality of the magnetic particles, the proposed method could be used to perform many useful and analytical immunoassay researches in the continuous flow droplet-based microfluidic platform.

KEYWORDS

Droplet, Magnetic particle-based assay, Continuous flow, Washing

INTRODUCTION

Droplet-based microfluidic platform is a promising technology in biological assays because it serves as a micro-reactor for ultralow volume studies of biological and chemical reactions [1]. However, this technology has a limitation that it is very difficult to wash excess reagents from the micro-reactors. The inability to perform washing steps in droplets limits to multi-step reaction processes and homogeneous assay [2]. In our previous work, we demonstrated a magnetic particle manipulation in a droplet-based platform without a washing step [3]. To overcome this technical challenge, we present a novel method to remove excess reagents in a droplet-based microfluidic device, performing biological reactions on the surface of magnetic particles.

PRINCIPLE

In this device, three major functions (synchronization, electrocoalescence, and magnetic particle manipulation) were integrated **(Fig. 1)**. Two kinds of droplets were generated by injecting (1) washing buffer and (2) reagents co-infused with streptavidin-coated magnetic particles and fluorescently-labeled biotin. The synchronization of droplets was achieved by a cross-flow of carrier oil between a top and a bottom channel by a pressure difference through a ladder network [4]. By incorporating ITO (300 nm) as electrodes on a glass substrate, the synchronized droplets were successfully merged to perform the washing step. Once merged by electrostatic force, the magnetic particles conjugated with the biotin were extracted toward the washing buffer droplet by an externally applied magnetic field, leaving the excess reagents behind. Subsequently, the merged droplets were split into two at a Y-junction: one with the majority of magnetic particles and the other with unbound biotin.

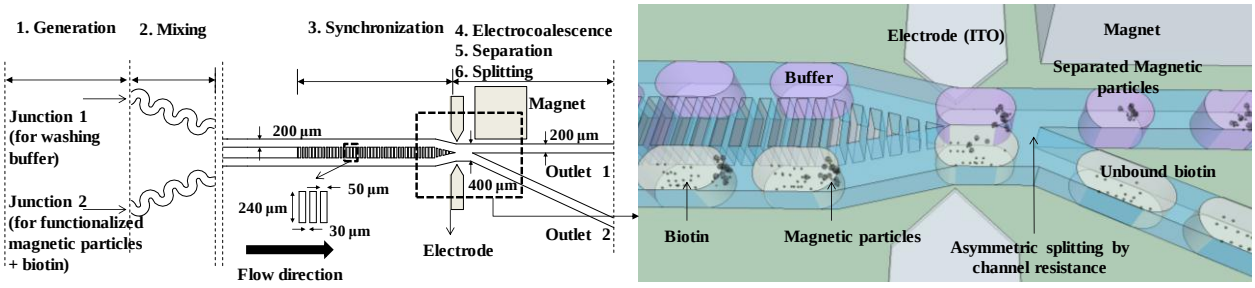

Figure 1: Working principle for on-chip magnetic particle-based assay in droplets. Once two droplets are merged by electrostatic force, the magnetic particles are separated toward the washing buffer droplet.

EXPERIMENT

The mineral oil (M8410, Sigma-Aldrich) was used as the immiscible phase and 2% nonionic surfactant (span-80, S6760, Sigma-Aldrich) was added into the mineral oil to prevent accidental coalescence. The channels were treated with a commercial surface coating agent (Aquapel, 47100, Pittsburgh Glass Works, PA, USA) to enhance hydrophobicity. The concentration of fluorescently labeled biotin (biotin-4-fluorescein, Molecular Probes, Invitrogen) was 2.5 µg/mL. The magnetic beads (2.8 µm diameter, Dynabeads M-270 streptavidin, Invitrogen) were diluted in deionized water. The biotin and magnetic particles were then co-infused into the junction 2 to generate the droplets and they were then completely mixed by a meander-shape channel with short turns to enhance mixing efficiency. The washing buffer droplets were also generated to extract the magnetic particles after the electrocoalescence. The voltage (500 V_{AC}) was supplied to generate the electrical field through ITO electrodes. The magnetic fields were generated by a rectangular neodymium-iron-boron magnet (12.7 × 3.2 × 3.2 mm^3, Emovendo Magnets & Elements, Petersburg, WV, USA). The fabricated microfluidic

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device was connected through a silicone tube to a syringe pump (KDS100W, Fisher Scientific, IL, USA). All experimental results were captured by a CCD camera mounted on a Nikon stereo-type microscope.

Table 1 Flow rate conditions to generate the washing buffer droplet at the junction 1 and droplet co-infused with magnetic particles and biotin at the junction 2.

Flow rate condition	Flow focusing junction 1		Flow focusing junction 2		
	oil ($\mu l/h^{-1}$)	water ($\mu l/h^{-1}$)	oil ($\mu l/h^{-1}$)	water ($\mu l/h^{-1}$)	water ($\mu l/h^{-1}$)
A	35	10	35	5	5
B	35	20	35	10	10
C	**35**	**30**	**35**	**15**	**15**
D	35	40	35	20	20
E	35	60	35	30	30
F	35	80	35	40	40

RESULT/DISCUSSION

For the stable droplet synchronization and electrocoalescence, we investigated the diameter of droplets by controlling flow rate ratio ($Q_{ratio}=Q_{oil}/Q_{water}$) using conditions listed in **Table 1. Fig. 2(a)** represents the diameter of droplets as a function of flow rate ratio. When the flow rate ratio is low (case F), accidental electrocoalescence was generated as they get close with their vicinity, which led to unstable electrocoalescence. When it is high (case A), they could not get touch each other because of the small size of droplets. For these reason, it is significantly critical to control the size of droplet to perform the synchronization and electrocoalescence. Our optimized experiment condition of flow rates was Q_{ratio} = 0.58 (case C). **Fig. 2(b)** represents the electrocoalescence efficiency according to the flow rate ratio: the efficiency is defined as the number merged droplets over total number of generated droplets. The maximum efficiency was 94.3% (case C). It should be noted that the unmerged droplets were caused only due to the difference of droplet generation frequency at each junction. We used image analysis to determine the intensity of fluorescence emitted by the droplet before and after electrocoalescence. The intensity of droplet initially containing fluorescence of biotin ($C_{initial}$) was compared with that of washing buffer droplet (C_{final}) after splitting of droplets **(Fig. 3)**. As a result, the excess reagents were significantly removed more than a factor of 1/16 while the magnetic particles were extracted into the washing buffer droplet.

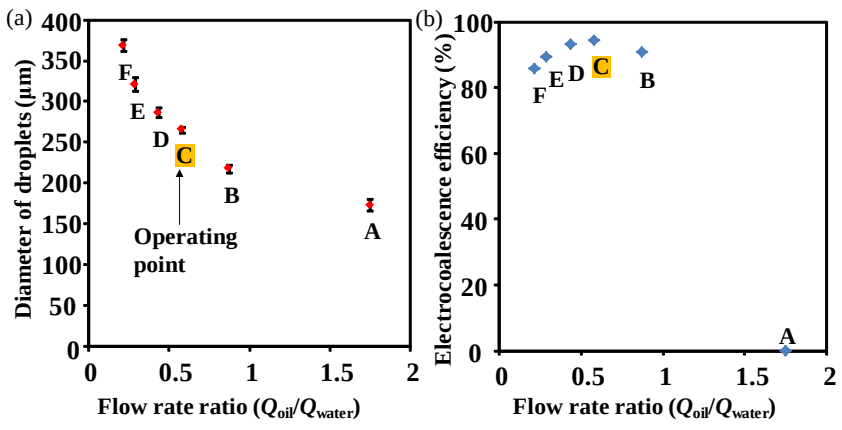

Figure 2: (a) The diameter of droplets as a function of flow rate ratio: the arrow indicates optimal operating condition for experimental. The average diameter of droplets was varied, ranging from 173.1 μm to 368.3 μm. (b) The electrocoalescence efficiency as a function of flow rate ratio: In the case A, the droplets were not merged due to the small size of droplets.

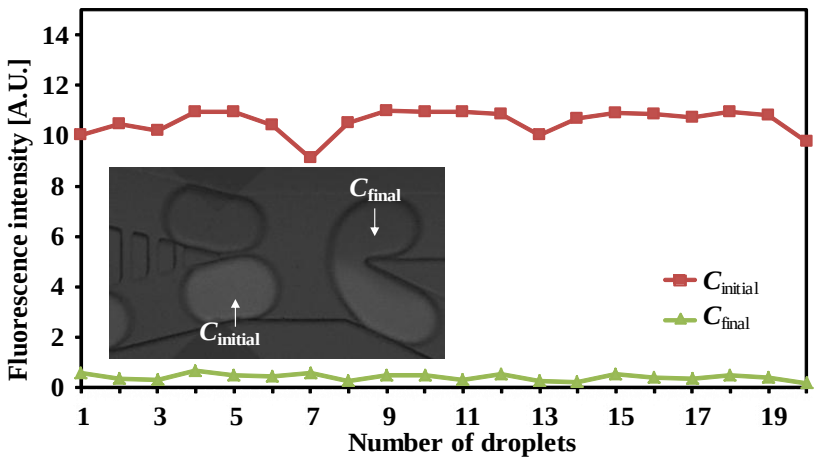

Figure 3: The intensity distribution of droplets before and after electrocoalescence. The image analysis was used to estimate the concentration of biotin with fluorescence label ($C_{initial}$) emitted from droplet and washing buffer droplet (C_{final}). The unbound excess biotin was significantly removed more than a factor of 1/16. The image inserted shows the intensity difference emitted before and after electrocoalescence.

Fig. 4 shows the sequential photographs of the magnetic particle manipulation in the droplets to separate the magnetic particles in the washing buffer droplets. In order to perform the synchronization of the two droplets coming from the bottom and top channel, the ladder network channel was used: existing pressure difference between the two main channels by the droplet flowing can enable the carrier oil to flow through the interconnection channel until the pressure in each main channel is balanced automatically, resulting in the creation of the synchronized droplets. Once the two droplets synchronized were merged by the electric field which can lead to destabilization of the surface of the droplets to be easily merged, the magnetic particles were moved into washing buffer droplets, leaving behind the unbound biotin [5]. Subsequently, the merged droplets for a while were split into two daughter droplets at the Y-shape channel geometry: one containing the majority of magnetic particles in the buffer droplets and the other containing the excess biotin. The experimental results for on-chip magnetic particle-based assay were summarized in **Fig. 5**. The optimized condition (case C) was used to generate the droplets at the junction 1 and 2. Sequentially, the mixing, synchronization, elecrocoalescence, separation and splitting were performed as mentioned above. At the outlet 1, the washing buffer droplets with magnetic particles were collected, resulting in almost devoid of the unbound biotin with the streptavidin coated on the magnetic particles as shown in the fluorescent image. At the outlet 2, the droplets containing the excess biotin were collected to be discarded.

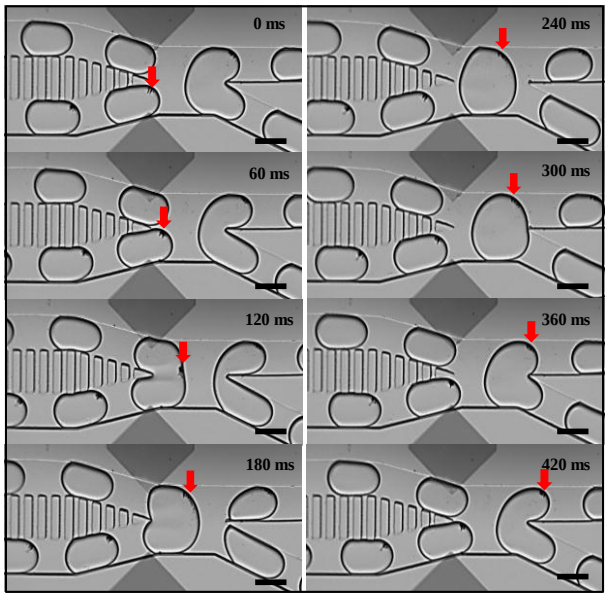

Figure 4: The movement of magnetic particles by externally applied magnetic field. The scale is 200 μm.

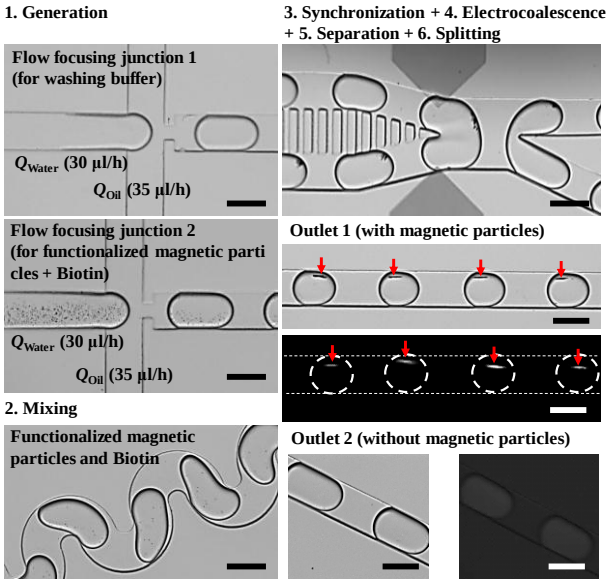

Figure 5: Experimental results for magnetic particle-based assay in the droplets. The arrows indicate separated magnetic particles. The scale is 200 μm.

CONCLUSION

We have demonstrated on-chip procedures for magnetic particle-based assay in the droplet microfluidic platform, including washing step to isolate the magnetic particles in the buffer droplets after the binding between streptavidin-coated magnetic beads and biotin labeled with fluorescence.

ACKNOWLEDGEMENTS

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