DROPLET-BASED MICROFLUIDIC DEVICE TO ENRICH AND TO SEPARATE HYDROPHOBICALLY FUNCTIONALIZED OLIGONUCLEOTIDE IN FREE-FLOW MICRODROPLETS

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ABSTRACT

Here we present a novel droplet-based microfluidic device that enables on-chip concentration and separation of hydrophobically functionalized DNA in a free-flow microdroplet. As our previous work has discovered, specifically functionalized DNA molecules are prone to enrich at the end of a moving microdroplet. Based on this concept, we design a droplet-based micorfluidic device that enables DNA enrichment and separation in droplets. Droplets containing DNA are generated and then undergo a straight channel so that the DNA enriches at the rear of the droplets. After the droplets are divided into two parts, the rear and the front droplets, the rear droplets are collected as a solution with condensed DNA. The concentration of the condensed DNA is triple that of the original DNA. The enrichment of DNA in a moving droplet can be improved with increasing flow rate of the droplet.

KEYWORDS

DNA enrichment, separation, droplet-based microfluidic device, free-flow microdroplets

INTRODUCTION

Droplet-based microfluidics system can effectively increase the throughput of biomedical investigation and application. Providing biological and chemical isolated environment and avoiding the cross-contamination of samples, the system advances numerous applications in material and biomedical sciences, and fundamental research [1]. The investigation of enrichment of biomolecules in microdroplets is significant but scarce, especially for DNA molecules. The condensed DNA revealing several interesting properties plays an essential role in various fields such as biology, biophysics and biochemistry. The concentration of the condensed DNA primarily dominates its binding and interaction abilities with other molecules [2]. Our previous work discovered that specifically functionalized DNA molecules are prone to enrich at the end of a moving microdroplet based on a combined effect of hydrodynamic repulsion and the aqueous/oil interfacial adsorption [3]. Here we propose a novel droplet-based microfluidic device to enrich and to separate hydrophobically functionalized DNA in free-flow microdroplets without complicated design and fabrication, and without external field.

EXPERIMENT

Figure 1 shows schematically a droplet-based microfluidic device to enrich and to separate hydrophobically functionalized DNA in free-flow microdroplets, and illustrates its configuration and operating conditions (cases A and B). A droplet called a mother droplet is generated at the first junction of the device, and undergoes enrichment in which the DNA aggregates at the end of the droplet through a combined effect involving the hydrodynamic repulsion and affinity attraction. On passing through the second junction, the mother droplet becomes divided into two droplets, specifically a front droplet and a rear droplet. The rear droplets are smaller but contain greater DNA concentration than the front droplets. Based on a correlation between pressure resistance and droplet size [4], the rear droplets are separated from the whole droplets in a forked channel and collected to obtain a solution with highly concentrated DNA.

As a droplet containing the hydrophobically functionalized DNA flows through region b, the enrichment of the DNA occurs (Figure 2). On flowing through region c, the droplet is split into a large droplet (front droplet) and a small droplet (rear droplet). From a comparison of their fluorescence intensities, the small droplet possesses more DNA than the large one. Downstream, these droplets are separated in the forked channel (region e) and then flow individually into the corresponding channels (regions f and g) for collection. To quantify the DNA enrichment in droplets, we analyze the fluorescent intensity of droplets as shown in Figure 3. For a mother droplet (Figure 3(b)), the maximum intensity (4095 au) at the end is approximately four times the average intensity of the DNA solution near the inlet, $\bar{I}_{inlet}$ (1100 au), demonstrating that most DNA concentrates at the end of the droplet through the combined effect. The average intensity of the rear droplet ($\bar{I}_{rear}$) is larger than $\bar{I}_{inlet}$, whereas that of the front droplet ($\bar{I}_{front}$) is smaller than $\bar{I}_{inlet}$ (Figures 3(c) and (d)). Compared to case A (Figure 3 (e)), $\bar{I}_{rear}$ / $\bar{I}_{front}$ and $\bar{I}_{rear}$ / $\bar{I}_{inlet}$ are small for case B, indicating that a greater rate induces greater DNA enrichment. The DNA concentration of the solution collected from the rear droplets is triple that of the original solution for case B, which would be improved on enhancing the flow rate, or a modified device for multiple DNA enrichment and separation.

Using the proposed device, we have realized DNA enrichment and separation in free-flow microdroplets. This is a novel approach to purify and extract DNA from a dilute solution in Lab-on-a-Chip systems.

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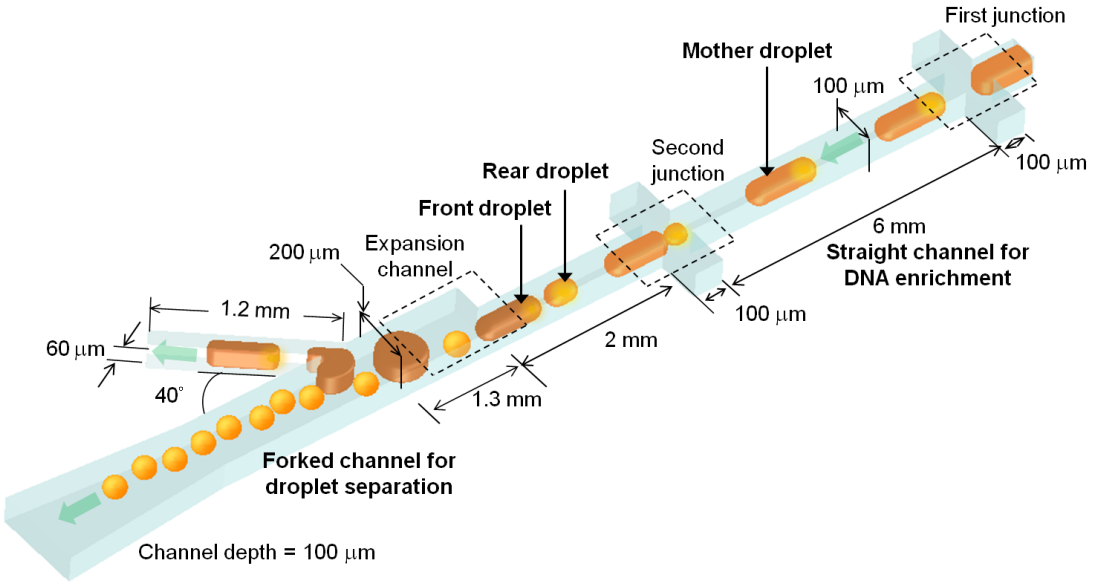


Concentration of TAMRA-labeled DNA solution = 2 μM
DNA sequence : TAMRA-5'-ACCTGACCTG-3'
TAMRA λ_{ex} | λ_{em} = 560 | 583 nm
Kinematic viscosity (ν) of silicone oil = 100 cm^2/s
Surface tension between silicone oil and water (γ) = 0.042 N/m
Droplet velocity (V) and Capillary number (Ca):
Case A: $V \sim 3.12$ mm/s; Ca $\sim 5.6 \times 10^{-3}$
Case B: $V \sim 5.96$ mm/s; Ca $\sim 1.1 \times 10^{-2}$

Flow rate Q (μL/min)	Silicone oil (1st crossing)	Silicone oil (2nd crossing)	DNA solution
Case A Re = 0.00312	0.6	2.3	0.9
Case B Re = 0.00596	1.2	4.8	1.6

Figure 1. Schematic illustration of the droplet-based microfluidic device for DNA enrichment and separation in a free-flow droplet. The continuous phase and dispersion phase of this droplet-based system are silicone oil and TAMRA-labeled DNA solution respectively. Two cases (A and B) under distinct flow rates are performed in this work.

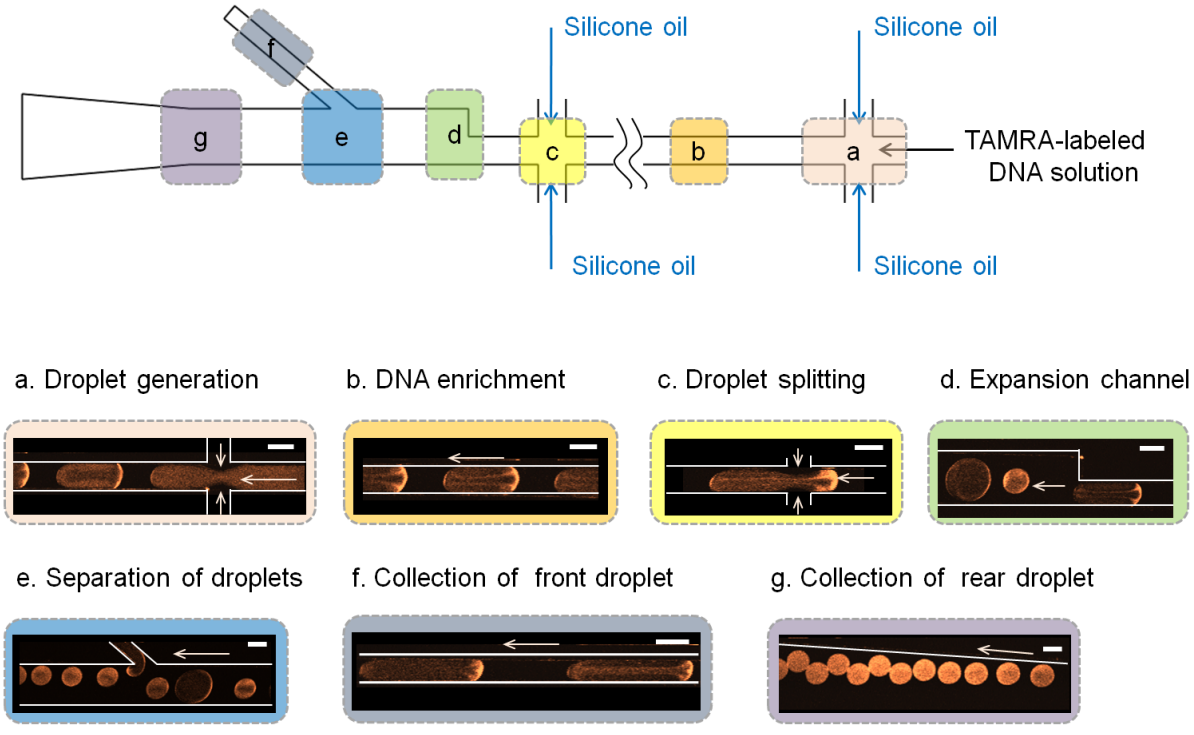
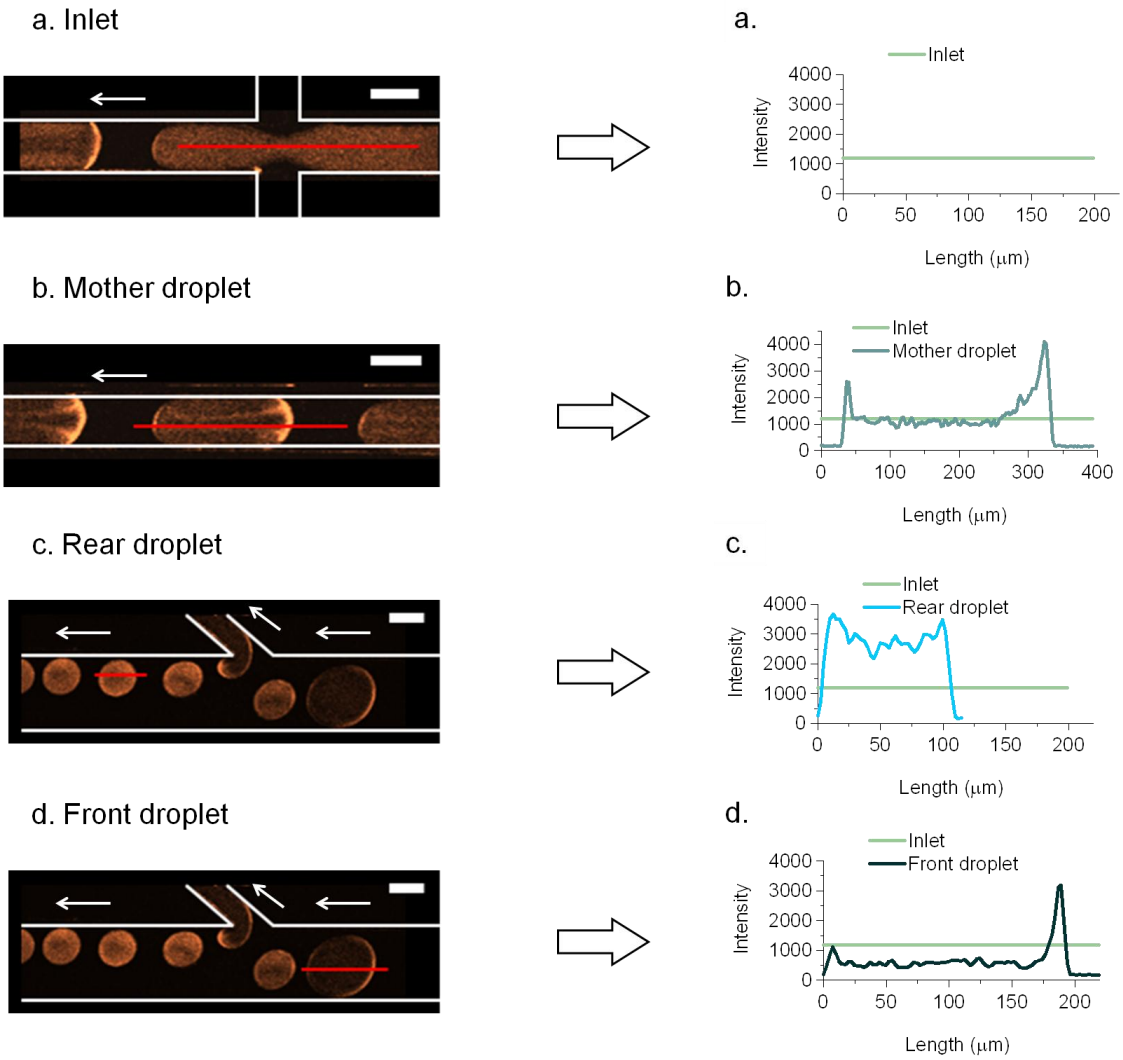


Figure 2. Experimental photographs of various regions (a to g) of the device (Case B). Scale bar = 100 μm.



Scale bar = 100 μm
White arrows indicate flow directions.

Intensity Ratio	Rear droplet / front droplet	Rear droplet / inlet
Case A	3.57	1.86
Case B	4.29	2.46

Figure 3. DNA intensity profiles near the inlet (a), in the mother droplet (b), the rear droplet (c) and the front droplet (d); intensity profiles are plotted along red lines (case B). (e) Average DNA intensity ratios of the rear droplet to the front droplet ($\bar{I}_{rear}/\bar{I}_{front}$) and of the rear droplet to the region near the inlet ($\bar{I}_{rear}/\bar{I}_{inlet}$) for the two cases.

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