SENSITIVE FLUORESCENCE-ACTIVATED SORTING OF MICRODROPLETS CONTAINING SUBCELLUAR STRUCTURES BY THERMOREVERSIBLE GELATION POLYMER

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ABSTRACT

We have developed a microfluidic-based fluorescence-activated droplet sorting system using a thermoreversible gelation polymer (TGP) as a switching material. Compared with the conventional fluorescence-activated cell sorter (FACS), TGP-based fluorescence-activated droplet sorting can be performed using a relatively simple optical setup and enables a highly sensitive detection of the fluorescent target [1, 2]. Using this system, we successfully demonstrated the quick and sufficient isolation of a fluorescently labeled subcellular structure (human artificial chromosome; HAC) from the crude cell homogenate. The results presented here constitute the first demonstration of droplet sorting using TGP. This droplet-based microfluidics platform can complement the conventional techniques in proteomics.

KEYWORDS: Droplet sorting, On-chip sorting, Thermoreversible gelation polymer, Proteomics

INTRODUCTION

Droplet-based microfluidics provide a platform for high-throughput screening of biological activities. This platform allows for the isolation of droplets of interest during the screening process. Microfluidic-based fluorescence-activated sorter using TGP has a highly sensitive detection of the fluorescent target compared with conventional FACS [2]. Thus, we employed droplet-based microfluidics on TGP-based sorting.

DEVICE DESIGN AND SORTING PRINCIPLE

Figure 1 shows the schematic representation of droplet sorting using TGP. First, the aqueous solution was encapsulated in water-in-oil (W/O) droplets by the microfluidic device with a cross-junction microchannel (Figure 1A). This device enables generation of uniform W/O droplets quickly. The droplets are then sorted in the second device. This device consists of two modules. In the first module, continuous aqueous solution containing TGP [7.5% (v/v) Mebiol gel] is used to convert W/O droplets into water-in-oil-in-water (W/O/W) droplets. In the second module, W/O/W droplets were hydrodynamically focused by the continuous aqueous solution and sorted as follows (Figure 1B). When no fluorescence is detected, the W/O/W droplets run into the waste channel. When a fluorescence signal is detected in the droplet, a sol-gel transition is induced locally at the junction of the waste and collection channels by irradiation with an infrared laser. The resulting gel formation increases the fluidic resistance in the waste channel and the droplet is sorted into the collection channel [1,2].

EXPERIMENTAL

Microfluidic devices were fabricated from polydimethylsiloxane (PDMS) using standard soft lithography and mold-replica techniques. The channel surface of W/O droplet generation device was coated with fluorinated silane, enabling the stable production of W/O droplets. W/O droplets were generated using mineral oil containing 3.0% (v/v) ABIL EM90 and 0.5% (v/v) Tween 80. Meanwhile, the channel surface of the sorting device was made hydrophilic by treatment with low-

pressure oxygen plasma, enabling the stable production of W/O/W droplets. For both the devices, solutions were introduced into the channels by air pressure regulated with electro-pneumatic transducing regulators. The fluorescent droplets were sorted in the optical setup as described previously [2].
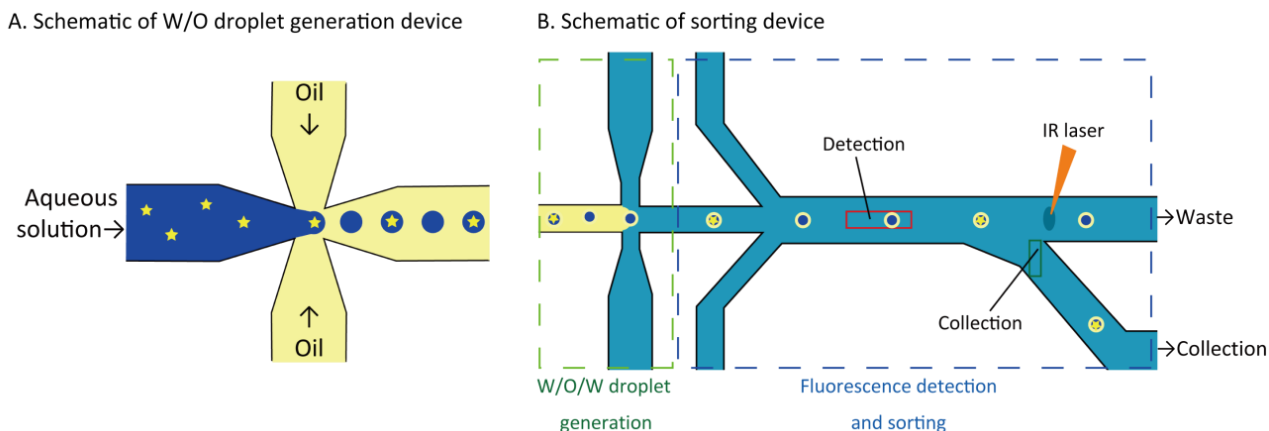

Figure 1: Schematic representation of the microfluidic devices used in this study.

RESULTS AND DISCUSSION

To evaluate the performance of the droplet sorter, a mixture of W/O droplets with or without encapsulated green fluorescent protein was introduced into the sorter. Figure 2 shows micrographs of the droplets before and after sorting. Fluorescent W/O/W droplets were successfully collected and enriched in the collection channel. The sorting accuracy was greater than 96% and purity was 90%.

Using the developed sorter, we next demonstrated the sorting of a subcellular structure, HAC from a crude cell homogenate. HAC is a "mini-chromosome" that can behave as a new chromosome in human culture cells. While a lot of attention has been given to the analysis of its structure, the isolation of HAC remains challenging owing to its small size (1–2 μm), thereby yielding weak fluorescence. To overcome this problem, we used droplet-based microfluidics. HeLa cells expressing fluorescently labeled HAC [3] were disrupted and the material in the cell lysate was encapsulated in W/O droplets (Figure 3). The droplets encapsulating fluorescent materials were successfully collected using the developed sorter (Figure 4).
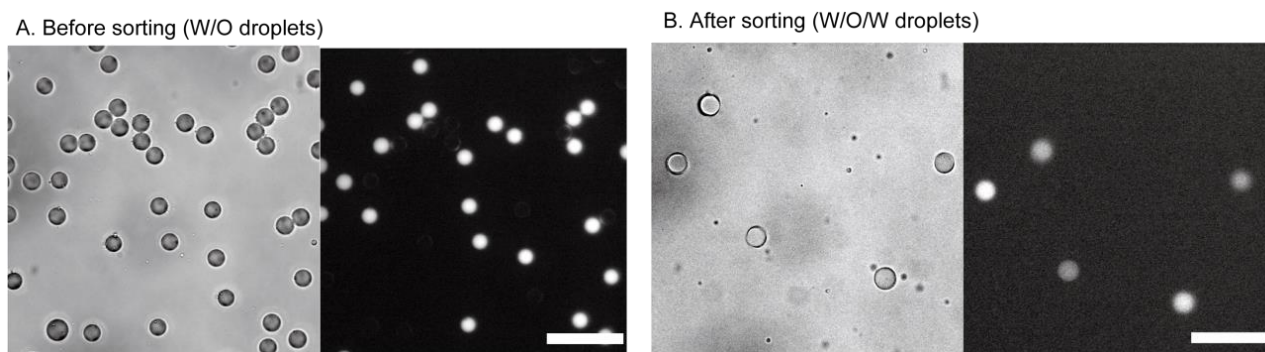

Figure 2: Bright-field (left) and fluorescence (right) images of droplets before and after sorting. Scale bars represent 50 μm.
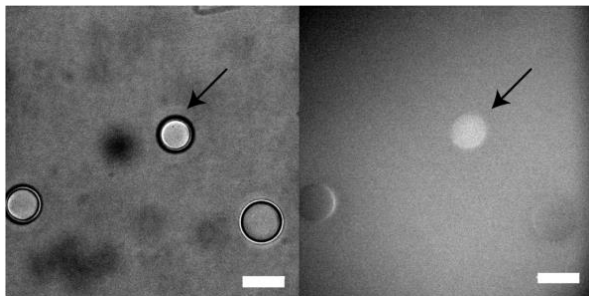
Figure 3: Bright-field (left) and fluorescence (right) images of W/O droplets encapsulating cell lysate. Since yellow fluorescent proteins were dissociated from HAC, the interior of the droplets encapsulating HACs were entirely fluorescent (indicated by arrows). Scale bars represent 20 µm.
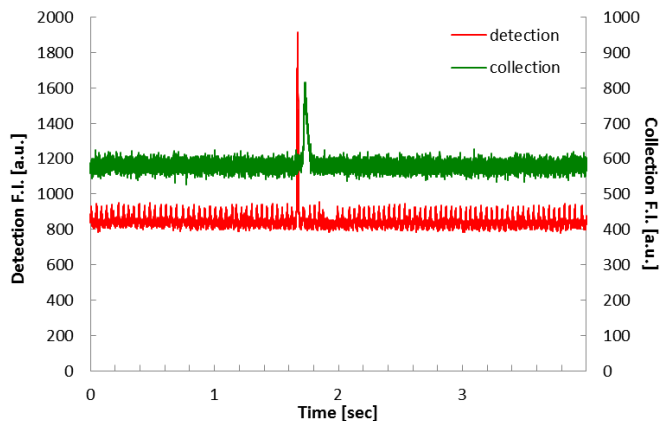

Figure 4: Time course of fluorescence signal while sorting HAC-encapsulated droplets.

CONCLUSION

We have developed a droplet-based fluorescence-activated sorting system for sorting micro-droplets using a TGP as a switching material. Fluorescing droplets are sorted with high accuracy and a purity that is greater than 90%. The fabricated sorter can be successfully used for sorting a fluorescently labeled subcellular structure (HAC) encapsulated in W/O/W droplets. A particularly significant benefit of this approach is that encapsulation prevents the irreversible dissociation of the components from the target structures during the operation. This sorting approach can complement other conventional techniques in proteomics.

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