HIGH THROUGHPUT AND PICOLITER-SCALE DRUG SCREENING WITH AUTOMATED DROPLET MICTROARRAY SYSTEM

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ABSTRACT

The miniaturization of high throughput screening has been a growing trend in the field of drug discovery. This paper described a fully-automated droplet microarray system capable of performing high-throughput screening for large number of different samples with ultra-low consumption in the picoliter range. The droplet microarray was automated generated by first forming picoliter droplets from 384-well plate for storage of different samples and reagents using droplet assembling strategy, and then deposited the droplets on a picowell microchip for parallel reaction and detection. This system was applied to screen inhibitors of capases-1 from a 32-compound library with an extremely low sample/reagent consumption of 200 pL for each screening reaction. Compared to traditional 96 or 384-well plate-based drug screening systems, a 10,000-100,000 fold reduction in reaction-volume was achieved.

KEYWORDS

High throughput screening, Droplet microarray, Picoliter

INTRODUCTION

Currently, 500,000 and more discrete chemical compounds are often necessary to be screened against specific targets to identify a lead compound in drug discovery research. The miniaturization of the screening assay has become a growing trend in order to reduce the research cost [1-2]. Droplet-based microfluidics holds great potential for drug screening by minimizing biological and chemical assay in pico-nanoliter droplets. However, in most droplet system, the droplets are generated with identical composition, which are not suitable for screening of large amount of chemical compounds. To address this challenge, we have developed a flexible droplet manipulation system namely DropLab by assembling composite droplets and performing screening assay [3]. In this work, we further developed this system into two-dimensional microarray form and applied it to screening enzyme inhibitor and profiling the IC_{50} value from a large amount of chemical library.

EXPERIMENT

The operation procedure was illustrated in Figure 1. A tapered capillary connected with a high-precision syringe pump was used for droplet metering, mixing and depositing. A 384-well plate fixed on a xyz-stage served as the storage container for enzyme, substrate, and compound library. A picowell microchip covered with oil was used for droplet reaction and detection (Figure 2). Droplet assembling was performed by moving the xyz stage to allow the capillary tip to immerse into the enzyme, inhibitor and substrate solution in 384-well plate sequentially. The assembled droplet was then deposited on the multi-picowell chip to form the droplet microarray. Finally, the microarray was incubated and detected with fluorescence detector.

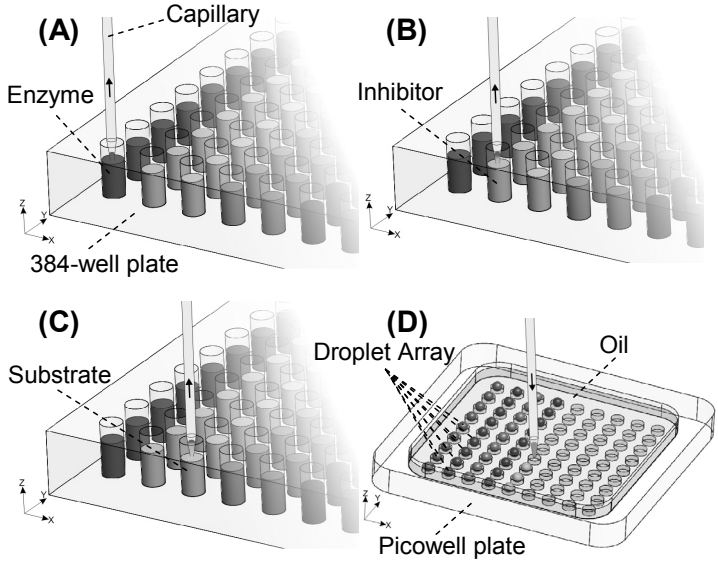

Figure 1. Schematic drawings showing the operation procedure of high throughput screening of enzyme inhibitors with droplet microarray system.

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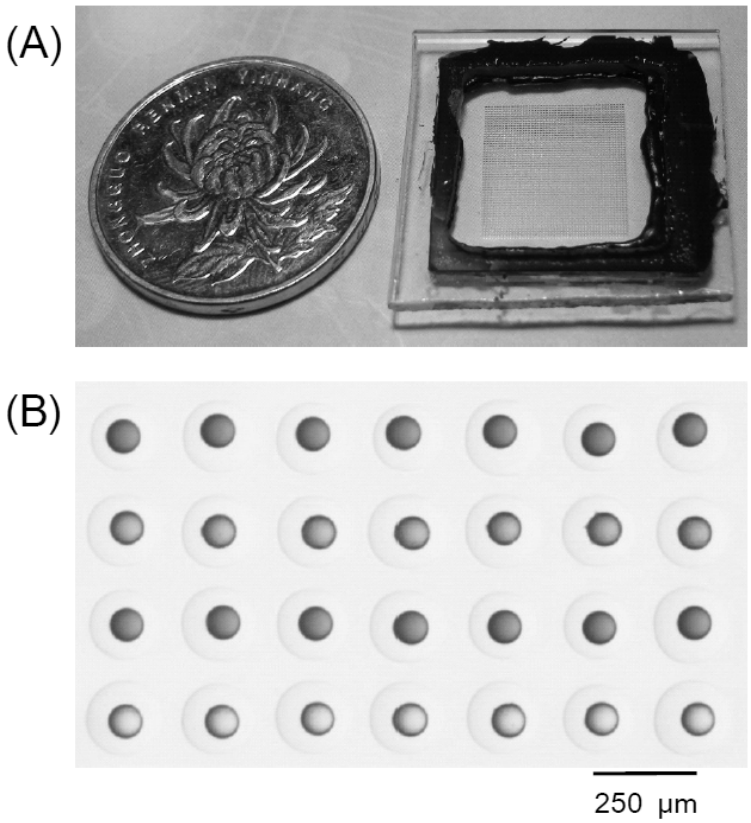

Figure 2. (A) View of the high-density picowell array microchip. (B) Microscopic images of droplet array with different dyes on the microchip. The volume of the droplets is 510 pL.

RESULTS AND DISCUSSION

We applied the droplet microarray system to screen inhibitors of caspase-1 to demonstrate its potential in drug screening. Caspases have shown to play key roles in apoptosis induced by various deleterious and physiologic stimuli. Inhibition of caspases could be a possible treatment of apoptosis related disease. We performed the screening assay with a library containing 32 compounds (100 μM). Each droplet containing 200-pL enzyme, 200-pL compound, and 200 pL substrate. As shown in Figure 3, three compounds were identified as inhibitors (Num. 28, 30, 32). We also applied the droplet array in the determination of IC_{50} value for an identified inhibitor (Num. 28). Eight different concentrations of the inhibitor ranging from 0 nM to 100 μM were tested. The IC_{50} value was calculated to be 46.8 ± 3.1 nM (Figure 4).

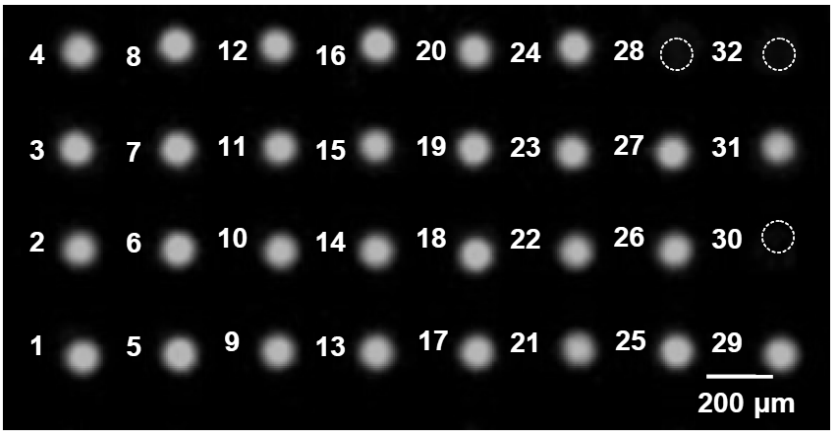

Figure 3. Screening of caspase-1 inhibitors from a 32-compound library with the droplet microarray system. The low fluorescence intensity spot shows high inhibition efficiency.

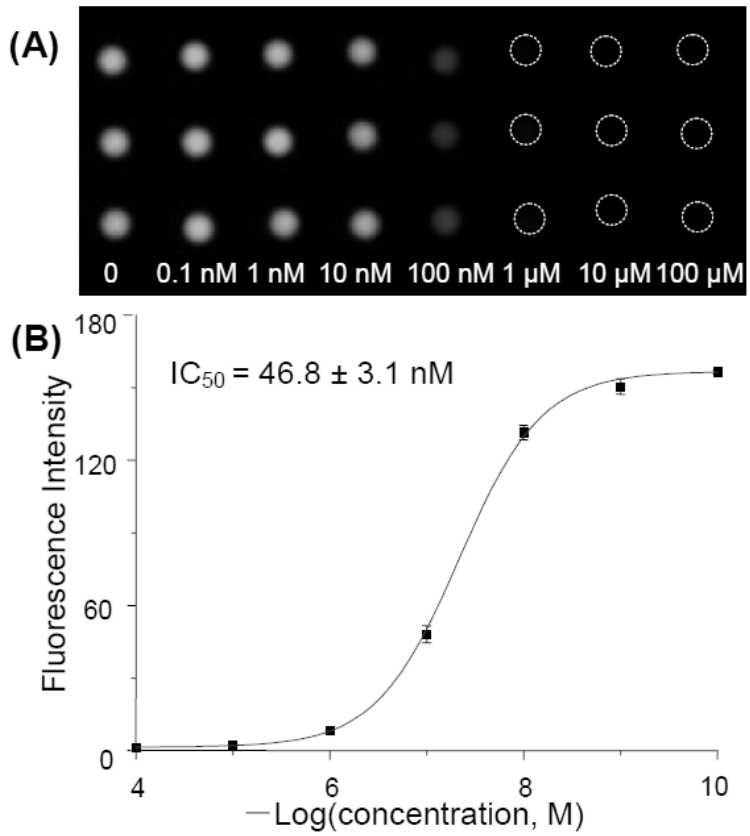

Figure 4. Determination of the IC_{50} value for an identified inhibitor (Num. 28 as shown in Figure 3). (A) Fluorescent image of the droplet array with 8 different concentrations of the inhibitor ranging from 0 nM to 100 μM. (B) Dose-response curves of the inhibitor. The IC_{50} value was calculated from the fitted curves (red line). Each droplet containing 200 pL inhibitor, 200 pL enzyme and 200 pL substrate.

CONCLUTION

In summary, the droplet microarray system is proven to be a promising platform for ultra-small volume and high throughput screening. Compared with other droplet-based screening system, the present droplet microarray system is naturally suited to perform screening experiments with large amount of samples. Further applications of the droplet microarray system include protein crystallization screening, enzyme evolution, combinatorial chemistry, and single cell analysis.

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