HIGH THROUGHPUT SINGLE CANCER CELL ENCAPSULATION AND SELF SORTING FOR PROTEASE ASSAY BY USING JETTING MICROFLUIDICS

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ABSTRACT

In this work, a droplet microfluidic device integrated with droplet sorting function was presented for single cancer cell encapsulations and protease activity measurements. Individual cells were encapsulated into aqueous droplets at flow focusing junction under jetting conditions. Droplets with cells encapsulated inside are larger than other empty droplets, enabling effective droplet sorting through a deterministic lateral displacement micro-pillar design. All droplets with cells are therefore collected by an observation chamber for enzymatic activity monitoring. Here we focused on measuring matrix metalloproteinase (MMPs) secretion intensities at the individual cell level to obtain further insights into the malignant characteristics of these cells .

KEYWORDS: Microfluidics, Single cell encapsulation, Droplet sorting, Enzymatic assay

INTRODUCTION

Direct biochemical analysis of rare cancer cells, such as circulating tumor cells and primary cancer cells from tumor mass, is becoming increasingly important in cancer diagnostics. However, because of the heterogeneity of cancer cells, conventional bulk measurement methods based on cell population can only reveal the bulk cell responses but fail to capture potentially important genotypic and phenotypic behaviors of individual cells. Therefore, assays conducted at the single cell level have become essential for systematic cancer studies. Recently, microfluidic cell trapping and encapsulation technology has been demonstrated as a promising method for a wide range of single cell assays [1-4]. Among them, droplet based microfluidics has the advantage of high throughput encapsulations and high assay sensitivity from small cell confinement [3-5]. With high cell concentration input, individual cells can be ordered in the micro-channel and then trapped in the droplets with frequency up to 10^4 cells per second as reported in previous works [3]. However, it becomes very challenging to perform single cell assay at low cell density, due to unsatisfactory cell encapsulation rate. Extensive cell culturing might be able to provide sufficient cell number for performing droplet based single cell assay, but it might remove some valuable biological information from that of the original cell samples. A jetting microfluidic device was reported previously to address the challenges of droplet based cell encapsulations in which droplets with cells encapsulated can be distinguished from empty droplets by size [6]. Based on this jetting microfluidic design, the presented system utilizes an array of micro-pillar structures to enhance separation efficiency. This not only further extends the applications of cellular enzymatic protease assay, but also enables rare single cancer cell assays without intensive cell culturing steps for diagnosis.

OPERATING PRINCIPLES

In brief, a solution with cells dispersed was emulsified into many picoliter droplets by co-flowing two continuous oil phase solutions. The flow rate of cell and oil solutions were carefully controlled to achieve jetting so that the generated droplets are 15um in diameter. If a cell was captured inside a droplet, the diameter of this droplet would be about 25um. The inherent size difference between empty and cell droplets enabled downstream micro-pillar structure to automatically select larger cell droplets from the smaller empty droplets. The micro-pillars were aligned according to the principle of deterministic lateral displacement with 20um as the critical dimension for separation. Any droplets above 20um diameter were deflected to cell droplet outlet. On the other hand, those droplets below 20um diameter can flow straight to the waste outlet without undergoing deflection. By making use of jetting droplet generation and passive droplet sorting, single cell encapsulation rate can now be greatly enhanced without strict requirements on cell sample concentration or complicated active-sorting technique. The operation schematics is demonstrated in Figure 1. Each cell encapsulated droplet can be treated as an individual cell compartment with an isolated environment that is suitable for further single cell study. In this work, a Y-junction inlet was incorporated to introduce reagents into cell solution before encapsulation process. Fluorescent signals from single cell droplets can be continually monitored to observe various cellular metabolism processes (e.g., enzyme secretion).

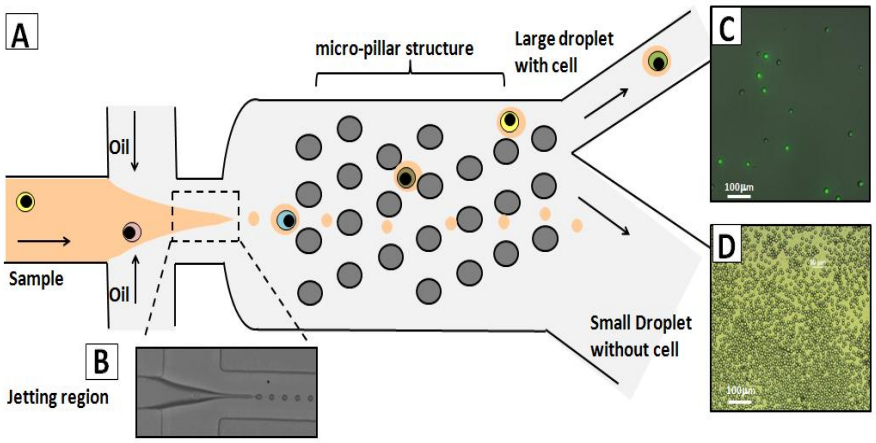

Figure 1. (A) 2D schematics demonstrating the process of cell encapsulation and passive droplet sorting, where droplets are generated by jetting and then large droplets with cell encapsulated inside are separated from other empty droplets in the micro-pillar array. (B) The high-speed camera image of droplet generation by jetting with a cell inside the jetting tip. (C) The image of GFP-labeled MDA-MB-231 cell encapsulated into droplets collected at cell droplet outlet. Scale bar:100um. (D) The image of empty droplets collected at waste outlet. Scale bar: 100um.

EXPERIMENTAL METHODS AND MATERIALS

The micro channel used in this work had been produced in PDMS from standard soft lithography. The silicon wafer mould was fabricated via deep reactive-ion etching to achieve good surface profile for PDMS micro-pillar casting. The micro channel substrate was made from PDMS-coated glass slide. PDMS (with curing agent and base) was first mixed with hexane in a ratio of 1:1. After that, it was spin-coated onto the glass slide with uniform thickness. After baking, the PDMS coated glass slide was bonded with a PDMS micro channel with oxygen plasma treatment. Four side surfaces of the resulting microfluidic channel were now hydrophobic.

To calculate single cell encapsulation rate, GFP-labeled MDA-MB-231 breast cancer cells were suspended in DMEM culture medium as a dispersed phase. Cell solution and oil solution (mineral oil with 3% w/w Span80 and 2% w/w EM90) were loaded into microfluidic device by syringe pumps. The flow rate of the dispersed phase and continuous oil phase were adjusted to 0.5uL/min and 25uL/min respectively in order to achieve proper size of empty droplets for separation. After flow condition had stabilized, cell droplets were collected onto a PDMS-coated glass slide for imaging. The single cell encapsulation rate was determined based on droplet images by dividing the number of droplets containing one cell inside by the total number of droplets. To conduct single cell MMP assay, two dispersed phases, which are PC-9 non-small lung cancer cells in RPMI cell culture medium and MMP-9 substrate (40uM) in Tris buffer (pH 7), together with a continuous oil phase were individually loaded into the microfluidic device by syringe pumps. Droplet collection started at cell droplet outlet after flow had stabilized. When enough cell droplets have been collected, the flows of aqueous and oil solutions were stopped. The cell encapsulated droplets were imaged using high sensitive sCMOS camera. The fluorescence intensities of each cell droplet were then determined by software ImageJ. The fluorescence intensity data obtained from cell droplets were compensated for photobleaching and diffusion effects, which were calibrated from pure MMP enzyme test.

RESULTS AND DISCUSSION

Cell Encapsulation: A population of GFP-labeled MDA-MB-231 breast cancer cells was first harvested from culture flask and re-suspended into DMEM cell culture medium. The cell solution was then introduced into the microfluidic device. The GFP-labeled cells were encapsulated into aqueous droplets at the jetting region. Subsequently, all droplets were passed through micro-pillar structure for sorting. The droplets collected from cell droplet outlet were directly imaged under fluorescent camera as illustrated in Figures 2(A) and 2(B). The enumeration results were used to calculate the cell encapsulation rate. The single cell encapsulation rate was greatly enhanced by incorporating jetting mechanism and size-based sorting technique as demonstrated in Figure 2(C). The empty droplets might be generated due to the instability of jetting which could randomly produce a few large droplets. The multiple-cell droplets might be resulted from cell clusters formed in the cell harvesting process.

Single Cell Enzymatic Assay: In order to demonstrate the device capability of monitoring single cell enzymatic activities based on fluorescent signal change, PC-9 cells were pumped with fluorescent MMP-9 substrate solution into the microfluidic device. They were mixed by a Y-junction inlet before being encapsulated into droplets. After cell encapsulated droplets were collected, the monitoring process was initiated immediately without any loss of early critical cell signals. Figure 3(A) shows the fluorescent signals extracted from eight individual PC-9 cells. Based on this figure, MMP kinetics of different cells could be identified from the gradients of signal curves. Since standard cell line was used as assay target, the resulting signal patterns were relatively similar with each other. However, it is believed that clinical samples acquired from cancer patients should be much more heterogeneous and may be categorized into several subpopulations.

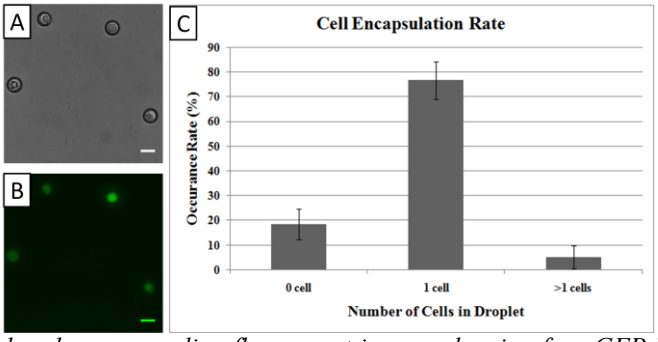

Figure 2: (A) and (B) Brightfield and corresponding fluorescent images showing four GFP-labeled MDA-MB-231 cells encapsulated into individual aqueous droplets. Scale bar: 20um (C)A bar chart demonstrates the cell encapsulation ratio obtained at cell droplet outlet. The single cell encapsulation ratio is much higher than the empty and multiple-cell droplets ratio.

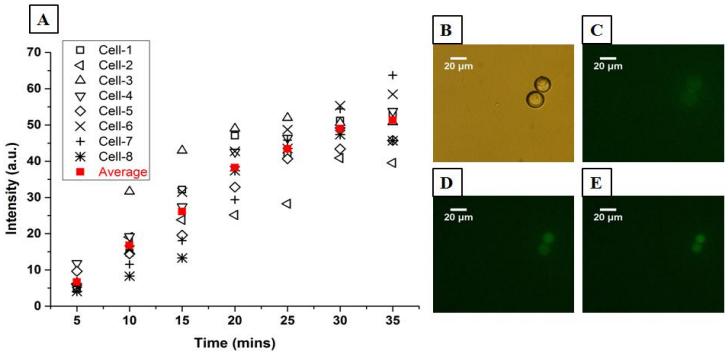

Figure 3: (A) Results from single PC-9 cell enzymatic assays. The slope of cell response demonstrates the MMP secretion activity at the individual cell level. (B) The brightfield image of two PC-9 are encapsulated into droplets. The sequence (C-E) shows the fluorescent images taken from these two cells at 5, 20, and 35 minutes of observation. Scale bar: 20um.

CONCLUSIONS

We demonstrated our droplet-based microfluidic system of combining a droplet jetting technology and an array of micro-pillar structures to passively sort cell droplets for downstream single cancer cell analysis. This system is capable of encapsulating single cells into individual picoliter aqueous droplets, sorting cell droplets from empty ones automatically and detecting intercellular enzyme kinetics within individual droplets compartments. The single cell encapsulation rate is greatly enhanced from prior arts by introducing the passive droplet sorting technique without strict requirement on cell sample concentration. Therefore, the system is applicable of studying low-concentration clinical cell samples. The profile of enzymatic reaction extracted from every cell elucidates the heterogeneous cell activities in terms of MMP secretion. These results may potentially be linked with the invasiveness of cancer cells and may provide further insights in understanding cancer progression. Future work will be focused on using this system for clinically relevant studies such as performing drug tests on clinically derived tumor cells.

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