Chinese Chemical Letters 26 (2015) 1450–1454

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

A single-cell encapsulation method based on a microfluidic multi-step droplet splitting system

Chun-Guang Yang, Ru-Yi Pan, Zhang-Run Xu *

Research Center for Analytical Sciences, Northeastern University, Shenyang 110819, China

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 28 July 2015
Accepted 19 August 2015
Available online 28 October 2015

Keywords:
Microfluidic droplets
Multi-step splitting
Single cell encapsulation
Whole blood cells

ABSTRACT

Single cell analysis is of great significance to understand the physiological activity of organisms. Microfluidic droplet is an ideal analytical platform for single-cell analysis. We developed a microfluidic droplet splitting system integrated with a flow-focusing structure and multi-step splitting structures to form 8-line droplets and encapsulate single cells in the droplets. Droplet generation frequency reached 1021 Hz with the aqueous phase flow rate of 1 μL/min and the oil phase flow rate of 15 μL/min. Relative standard deviation of the droplet size was less than 5% in a single channel, while less than 6% in all the 8 channels. The system was used for encapsulating human whole blood cells. A single-cell encapsulation efficiency of 31% was obtained with the blood cell concentration of 2.5×10^4 cells/μL, and the multicellular droplet percentage was only 1.3%. The multi-step droplet splitting system for single cell encapsulation featured simple structure and high throughput.

1. Introduction

Traditional studies of cellular processes are usually carried out with a large number of cells. The difference between individual cells is mostly masked by cellular information that is measured as averages of large populations [1]. Therefore, developing the analysis at the single cell level is of significance for cell researching. A promising approach to the single-cell analysis is microfluidics, which is characterized by manipulation of small volume fluids and integration of multiple functional units [2]. Microfluidics can provide rapid, accurate, and cost-effective methods for single-cell analysis [3], including the analysis of protein [4] and genes [5] in a single cell. In some cases, the cells need to be isolated for investigating their responses to stimuli or extracellular secretion. Obviously, droplet microfluidics, which has the potential to compartmentalize individual cells into nanoliter or smaller droplets for biochemical analysis [4], can not only meet the requirement above, but also characterize the single-cell analysis by minimal sample dilution, higher sensitivity, shorter reaction time, higher throughput and no cross contamination. The single-cell encapsulation methods based on droplet microfluidics mainly include active encapsulation and passive encapsulation. The former is to actively encapsulate cells in droplets with the aid of external forces, such as optical trapping [6], electric field force [7], acoustic field force [8], pneumatic valves [9] and aspirating force *via* tapered capillary [10]. It is characterized by accurate targeting of individual cells, and therefore a higher efficiency of single-cell encapsulation in droplets is acquired. However, employing external forces increases the complexity of manipulating system without a corresponding increase in throughput. The latter method is also named hydrodynamic method, which utilizes pressure-driven flow in simple microfluidic configurations for generating auqeous droplets and at the same time entrapping single cells into droplets. The number of cells in one droplet mainly depends on the concentration of cell suspension, and the encapsulation process usually follows Poisson statistics [11]. For example, when the multiple-cell droplets was decreased to 4%, the corresponding ratio of the single-cell droplets was down to 22% and the majority of droplets contained no cell at all [11,12]. The hydrodynamic method is extremely simple; however, the efficiency of single-cell encapsulation is still insufficient. To solve this problem, some researchers tried to add a sorting step after the stochastic encapsulation of cells [12,13]. They separated the single-cell droplets from the ones with multiple cells or no cells. Although the efficiency of single-cell encapsulation of the improved hydrodynamtic methods increased to some extent, the encapsulation throughput was still kept at a low level, and the device was more complicated for it had to integrate with other functional modules.

* Corresponding author.
E-mail address: xuzr@mail.neu.edu.cn (Z.-R. Xu).

http://dx.doi.org/10.1016/j.cclet.2015.10.016

Other researchers performed special controlling processes before droplet generation, such as regulating the cell density [14], evenly spacing the travelling cells [15] and ordering cells by Dean flows in a curved microchannel [16]. Regulating the cell density before droplet formation still suffered from the low throughput. The single-cell encapsulation by the aid of spacing cells and ordering cells obained good performances both in efficiency and throughput, although special geometries and subtle regulation of flowrates were needed.

Here, we aim at developing a simple hydrodynamic method to encapsulate single cells in droplets with high efficiency and throughput. The production rate of droplets could be increased by parallelizing droplet generators [17,18]. The passive repeated breakup of droplets was a simple strategy using a splitting array [19]. Based on the array of droplet splitting structures, we explored a single-cell encapsulation method with high throughput. A large droplet with multiple cells could be repeatedly split until to form the small droplets with single cells.

2. Experimental

2.1. Microfluidic chip design and fabrication

Two types of microfluidic chips were fabricated, respectively, in the experiment. Both of them consisted of flow-focusing water/oil phase channels and a multi-step droplet splitting geometry, in which a main channel was sequentially divided into two branch channels 3 times to form 8 parallel branches (Fig. 1). All of the channels are about 30 μm deep. The width of the main aqueous channel is 120 μm, and the width of the branch channels is, respectively 100 μm, 80 μm and 60 μm after each dividing. The oil phase channels contract from 120 μm to 80 μm in width at the flow-focusing junction, and each Y-shaped splitting junction was designed with a nozzle contracting from the width of the upstream channel to the width of the downstream channel. The whole microchannel network has only one aqueous phase (cell suspension) inlet, and one oil phase inlet and 8 outlets. In one type of the microfluidic chips, winding channels were added into the upstream of each junction (Fig. 1b). The microfluidic chip was made of polydimethylsiloxane (PDMS, RTV615, Dow Corning, USA) using the soft lithography technique [20]. Master molds of photoresist (AZ P4620, Clariant, Japan) were fabricated in clean-room facilities. PDMS layers were replicated from the master molds and bonded with a plain PDMS substrate after air plasma treatment (PDC-32G, Harrick Scientific Co., USA). The bonding was strengthened by placing the chip on a heating plate at 120 °C for 1 h.

2.2. Multi-step droplet splitting

We used yellow food dye solution as the aqueous phase and mineral oil (Sigma, USA) containing 2 wt% Span 80 as the continuous oil phase. Syringe pumps (Model MD-1001, Bioanalytical Systems, USA) were used to introduce the fluids into the microfluidic chip. When the two phases met at the flow-focusing junction, mother aqueous phase droplets were formed. And then the mother aqueous phase droplets were broken into two branch channels at the first Y-shaped splitting junction. After three times of splitting, the mother droplets were finally divided into 8 lines of daughter droplets. The images and movies of droplet generation under different conditions were taken by a high-speed camera (AOS Technologies, Switzerland) mounted on a stereo microscope (Stemi 2000-C, Carl Zeiss, German). The length of the droplets along their central axes was measured by using software of Nis-Elements 3.2.

2.3. Single-cell encapsulation

Human whole blood (supplied and endorsed by volunteers working in a lab of China Medical University) used as the model cell type was diluted by cell suspension medium (pH 7.4) containing 0.85% (w/v) NaCl and 30 mmol/L Tricine-NaOH. And the concentration of cells in human whole blood was regulated using cell separating medium (OptiPrepTM, Axis-shield, Norway). All the reagents were of analytical reagent grade unless specified, and all the solutions were prepared with deionized water and filtered by 0.22 μm filter membrane before used. Two types of microfluidic chips (Fig. 1a and b) were used for the single cell encapsulation experiments. Droplet arrays of food dye solution were formed in continuous oil phase at a flowrate of 1 μL/min for both aqueous phase and oil phase. When the system reached a steady state, the food dye solution was quickly replaced with cell suspension, and the flowrates of two phases were regulated carefully to form single-cell droplets. The process of cell encapsulation was recorded by the high-speed camera mounted on the stereo microscope mentioned above.

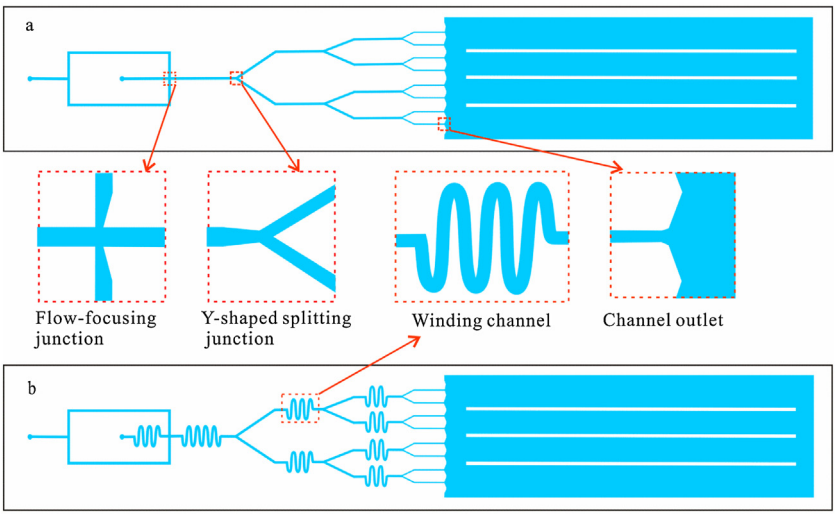

Fig. 1. Schematic diagram of the microfluidic droplet splitting chips. (a) Chip without winding channels; (b) Chip with winding channels.