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Encapsulation of single cells into monodisperse droplets by fluorescence-activated droplet formation on a microfluidic chip

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ABSTRACT

Random compartmentalization of cells by common droplet formation methods, i.e., T-junction and flow-focusing, results in low occupancy of droplets by single cells. To resolve this issue, a fluorescence-activated droplet formation method was developed for the on-command generation of droplets and encapsulation of single cells. In this method, droplets containing one cell were generated by switching on/off a two-phase hydrodynamic gating valve upon optical detection of single cells. To evaluate the developed method, flow visualization experiments were conducted with fluorescein. Results indicated that picoliter droplets of uniform sizes (RSD < 4.9%) could be generated. Encapsulation of single fluorescent polystyrene beads demonstrated an average of 94.3% droplets contained one bead. Further application of the developed methods to the compartmentalization of individual HeLa cells indicated 82.5% occupancy of droplets by single cells, representing a 3 fold increase in comparison to random compartmentalization.

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1. Introduction

Droplet-based microfluidics allows the miniaturization of reactions by compartmentalizing reactions in aqueous droplets, separated by immiscible oil. The compartmentalization of reactions in droplets provides rapid mixing of reagents, flexible control of sample volumes, and prevention of water evaporation and cross contamination between samples [1–3]. These advantages of droplet-based microfluidics have shown benefits to a wide range of chemical and biological applications, such as enzymatic kinetics [4–6], protein crystallization [7–9], polymerase chain reaction [10–12] and clinical diagnosis [13].

In addition to compartmentalizing reactions, droplet-based microfluidics can also be used to encapsulate prokaryotic [14–18] and eukaryotic cells [19–23], and even the embryos of multicellular organisms [24,25], which opens up a new avenue for cell analysis. Recently, Brouzes et al. [19] developed a droplet-based viability assay that permitted quantitative analysis of cell viability and growth within compartmentalized aqueous droplets. By encapsulating human monocytic U937 cells, they screened a drug library for its cytotoxic effect against the U937 cells. Clausell-Tormos et al. [24] reported a droplet-based microfluidic platform, in which human cells were compartmentalized and grown in aqueous droplets separated by carrier oil with biocompatible surfactants. Droplets containing cells could then be collected and incubated for several days. Injection of these droplets into a microfluidic detecting device after incubation enabled the measurement of the expression of a reporter gene.

T-junction [26] and flow-focusing [27] are the two common approaches to encapsulate cells in two-phase systems. In the T-junction method, the disperse phase of the cell suspension is injected from the side-channel to the continuous phase of oil in the main channel. Droplets containing cells are generated as a result of the shear force at the fluid–fluid interface. In flow-focusing, the continuous phase of oil is injected through two side-channels and the disperse phase of the cell suspension is injected through a central channel into a narrow orifice. Droplets containing cells are produced in the channel downstream the orifice. Both methods have been proven effective in encapsulating cells. However, the compartmentalization of cells in droplets is a random process dictated by Poisson statistics, yielding a majority of empty droplets and droplets containing multiple cells [28,29].

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Since high occupancy of droplets by single cells is critical for it to effectively generate a pool of single-cell-bearing droplets for applications such as single-cell analysis, efforts have been made to resolve the random compartmentalization issue in droplet-based microfluidics and to improve the occupancy of droplets by single cells. Previously, Abate et al. [28] proposed a close-packed ordering strategy. Particles were closely packed so that they were organized with a regular spacing before the formation of droplets. By matching the periodicity of the particles and the formation of the droplets, they successfully loaded every droplet with one particle. Edd et al. [30] demonstrated the use of a high aspect-ratio microchannel to self-organize cell suspensions prior to encapsulation. By matching the droplet generation rate to the flow rate of the cells, they were able to encapsulate single cells into monodisperse droplets. Alternatively, it is possible to control the droplet generation process for on-command droplet formation and encapsulation of single cells. He et al. [31] reported a strategy combining optical trapping with microfluidic-based droplet generation. In this approach, an optical laser was used to manipulate individual cells to the interface of two immiscible fluids. Application of a slight positive pressure to the aqueous phase would trigger the generation of one droplet containing a cell. However, it is difficult to implement due to the requirement for sophisticated optical equipments.

In this paper, we demonstrated a new on-command droplet formation method, i.e., fluorescence-activated droplet formation based on two-phase hydrodynamic gated injection for encapsulating single cells with high repeatability and occupancy. Droplets containing one cell were generated by switching on/off the two-phase hydrodynamic gating valve upon optical detection of single cells. Flow visualization experiments with fluorescein showed that picoliter droplets of uniform sizes could be generated with relative standard deviations of less than 4.9% ($n=20$). Encapsulation of single fluorescent polystyrene beads was conducted to evaluate the developed method. Results indicated an average occupancy of 94.3% for droplets containing one bead. Further application to individual HeLa cells showed 82.5% occupancy of droplets by single cells, which is about 3 fold higher than those obtained by random compartmentalization [28,29]. The developed method provides an effective means for on-command generation of droplets and encapsulation of single cells on microfluidic chips.

2. Experimental

2.1. Chip design and fabrication

The design of the microfluidic chip is shown in Fig. 1a with indicated lengths for all the microchannels. The width of all channels is 80 μm. The height of all channels is 50 μm. The microfluidic chip was fabricated using rapid prototyping methods with polydimethylsiloxane (PDMS) as the structural material [32,33]. SU-8™ (GM 1070, Gersteltec Sarl, Switzerland) mold was fabricated on a silicon substrate. PDMS structures (base oligomer and curing agent in a ratio of 10:1) were produced by prototyping the SU-8™ mold. After curing at 65 °C for 4 h, the patterned PDMS sheet was peeled off from the silicon substrate and perforated at each end of the microchannels using a metal puncher with an inner diameter of 2.5 mm (Rubicon no. 18, Japan). The PDMS sheet with structures was irreversibly bonded to a plain PDMS sheet to form microchannels using oxygen plasma treatment (PDC-GC-M, Weike Spectrum, Chengdu, China). The entire PDMS structure was then irreversibly bonded to a clean glass slide with quartz tubes (inner diameter, 4 mm; height, 12 mm) attached to the punched holes to form the final devices. Before the experiments, microfluidic chips were incubated at 120 °C for 1 h to remove residual water from the PDMS microchannels.

2.2. Chemicals and reagents

Sodium fluorescein, sodium alginate, Span-80 and hexadecane were purchased from Sigma-Aldrich (MO, USA). Fluorescent polystyrene beads with a diameter of 10 μm were purchased from Research Institute of Chemical Engineering and Metallurgy (Beijing, China). All reagents were of analytical grade unless otherwise specified. All solutions were prepared with water purified by the Direct-Q system (Millipore, USA) and filtrated with 0.45 μm microporous membrane filters before use.

For droplet generation, 2% (w/v) sodium alginate solution was used as the aqueous phase, and hexadecane containing 3% (v/v) Span-80 was used as the immiscible phase. For flow visualization experiments, 2% (w/v) sodium alginate solution containing 10 μM fluorescein was used as the aqueous phase. For encapsulation of microbeads, fluorescent polystyrene beads were suspended in 2% (w/v) sodium alginate solution with a concentration of 1.5×10^5 beads mL^{-1}.

2.3. Cell culture

DsRed-transfected HeLa cell line (HeLa-DsRed) was maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% (v/v) newborn calf serum (NCS, Gibco), 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 37 °C under 5% CO_2 atmosphere saturated with water. For cell harvest, HeLa cells were treated with 0.25% (w/v) Trypsin-EDTA solution (Gibco) for 1–2 min and then collected into a 1.5 ml EP tube with fresh cell culture medium. The collected HeLa cells were centrifuged at 1000 RPM for 8 min, and resuspended in 2% (w/v) sodium alginate solution before experiments. The final density of HeLa cells was adjusted to 1×10^6 cells mL^{-1} before use.

2.4. Optical imaging system

All experiments were conducted on an inverted fluorescence microscope (IX71, Olympus, Japan) with an EMCCD (EvolveTM, Photometrics) camera for image acquisition and a photon counter (PMS 400A, B&H GmbH, Berlin, Germany) for recording photon counts over time. Fig. 1b illustrates a schematic of the optical imaging system. Excitation light from a mercury lamp (100 W, USH-1030L, Olympus, Japan) was shaped by a field stop, filtered by a band-pass filter, reflected by a dichromatic filter, and focused on the microchannel by a 60 × objective (NA 0.7, LUCPLFLN, Olympus, Japan). The excited light was collected through the same objective with a high-pass filter. A filter set U-MWIB2 (excitation filter 460–490 nm, dichroic mirror 505 nm, high-pass filter 510 nm) was used to detect fluorescein and fluorescent polystyrene beads. A filter set U-MWG2 (excitation filter 510–550 nm, dichroic mirror 570 nm, high-pass filter 590 nm) was used to detect HeLa-DsRed cells.

2.5. Pressure control system

A custom-built pressure control system was used for two-phase hydrodynamic gated injection (Fig. 1b). Negative pressures were supplied through 1 L air containers, which were generated by micropumps (FM2002, Ruiyi, China) and monitored by pressure sensors (PSE541, SMC, Japan). A sensor controller with a resolution of 0.1 kPa (PSE200, SMC, Japan) was used to display the pressure in each air container and toggle on/off the corresponding micropump to maintain the pressure according to the signals from the pressure sensor. A three-way solenoid operated valve was used to switch between negative pressure and the atmosphere at BW. A

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