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From single phase to multiphase: single cell encapsulation in a droplet

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Abstract

Single cell analysis is of great significance to understand the activities of organisms. We present here a microfluidic device capable of precisely capturing single cells by changing from single phase to multiphase with encapsulation efficiencies of 90%. Our device uses hydrodynamic trapping structures for cell trapping.

1. Introduction

Microfluidics has been proved to be a promising approach for single cell studies. Cellular analysis has a major application in biology and other research areas and thus single cell trapping has become one of the popular area of research [1]. Various active and passive cell capturing methods have been discussed in recent years. However, droplet microfluidics has emerged out as the more successful because of various advantages [2]. Droplet microfluidic has potential to characterize single cell analysis by minimal sample dilution, higher throughput and no cross contamination as the droplets are separated. However, the droplet generation is limited by the Poisson's statistical limit [3,4]. We present a method capable of capturing single cells with high precision by removing Poisson's statistical limitation.

2. Device design

The cell trapping structure is a flow resistance based design capable of capturing single cells. The device consists of one inlet and one outlet. The pairing channels can be arrayed over larger area for trapping higher number of single cells. Homogeneous or heterogeneous cells can be trapped using this microfluidic device. Chip is bonded on the glass slide for closing the channels. (Figure 1)

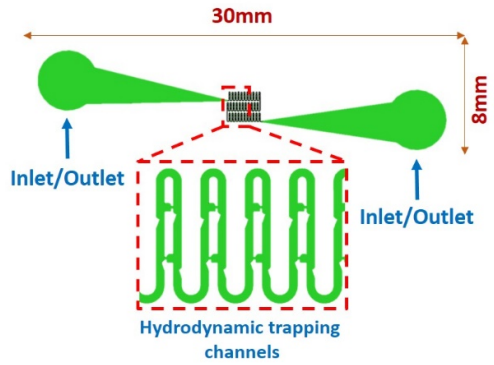

Fig. 1 Illustration of microfluidic chip with closer look of trapping channels

3. Experimental method

Device fabrication

Microfluidic device was developed using soft lithography technique. Master mold for rapid cell trapping microchannel was created using SU8 which is hard-baked at 150°C for 30 mins. Microstructures were casted by using PDMS, mixed in the ratio of 10:1, and degassed in vacuum chamber to remove the bubbles. The mixed PDMS was then poured onto master mold and heated at 60°C in an oven for two hours. PDMS chip was finally plasma bonded on glass wafer with patterned electrodes (Figure 2). Holes were punched to make the connections for inlet using Teflon tubing.

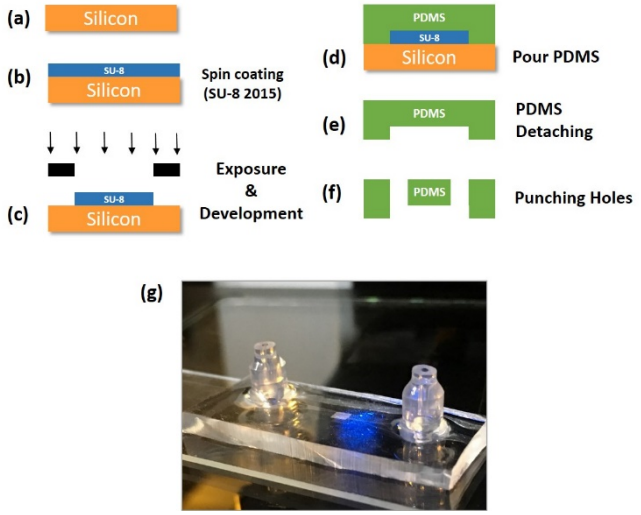

Fig. 2 (a-f) Fabrication process flow for soft lithography technique (g) fabricated microfluidic chip bonded on glass slide

Cell trapping and encapsulation

The microchannels were first washed with 75% ethanol followed by channel cleaning with distilled-deionized (DD) water and removal of air bubbles. In order to reduce the adhesion of cells, 1% Bovine Serum Albumin (BSA) with DD water solution was injected into the chamber. The surface was treated using 3M Novec HFE-7500. THP1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 4.5 g/L glucose, 10 mM HEPES and 1.0 mM sodium pyruvate, supplemented with 0.05 mM 2-mercaptoethanol. The first step consists of flowing THP-1 cells. The cells are captured by hydrodynamic trapping sites. In the next step, 3M

Novec 7500 oil mixed with surfactant is injected to change the phase. The area near the trapping sites forms a droplet. Finally, the droplets are released from the trapping sites by flowing the oil in the reverse direction that is from outlet to inlet (Figure 3).

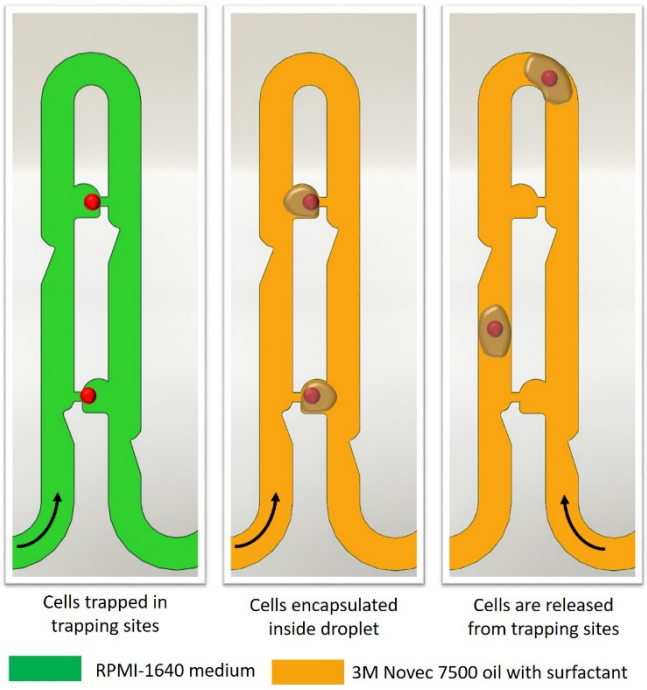

Fig. 3 Illustration of working of microfluidic chip

4. Results and Discussion

A cell can only be trapped in the trapping structure if the trapping site resistance is higher than the bypass channel resistance (Figure 4a). One of the most important parameter for trapping of single cell is the flow rate. We also observed that oil flow rate also has significant effect on the droplet formation that is cell encapsulation (Figure 4b).

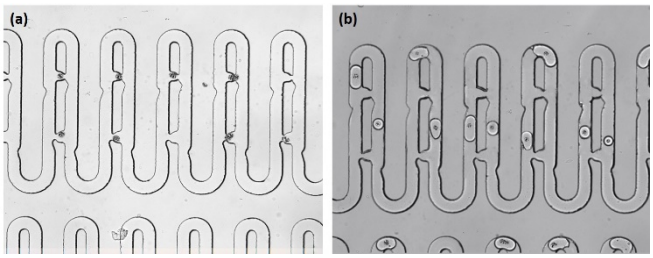

Fig. 4 (a) THP-1 cells captured at trapping sites (b) Droplet retrieval from the trapping sites

We performed the study for flow rates ranging from 5µl/hr to 10 µl/hr and observe the effect on trapping of cells. We found that at lower flow rates multiple cells were trapped. As we increased the flow rate, the trapping efficiency was increased which was maximum at 10µl/hr (Figure 5). We also observed effect of oil flow rate on encapsulation efficiency. The flow rate was varied from 7µl/hr to 10µl/hr. We observed that as the oil flow rate increased, the encapsulation efficiency is reduced. The maximum encapsulation efficiency of 90% was observed at flow rate of 7µl/hr (Figure 6)

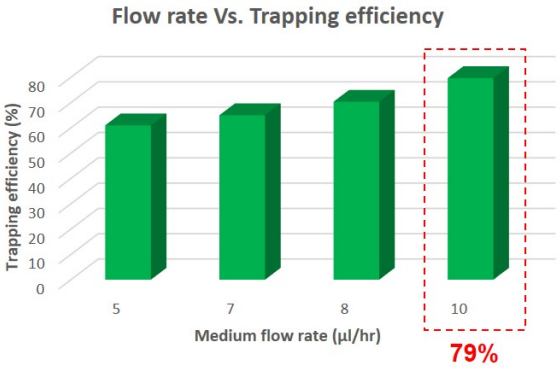

Fig. 5 Effect of medium flow rate on trapping efficiency

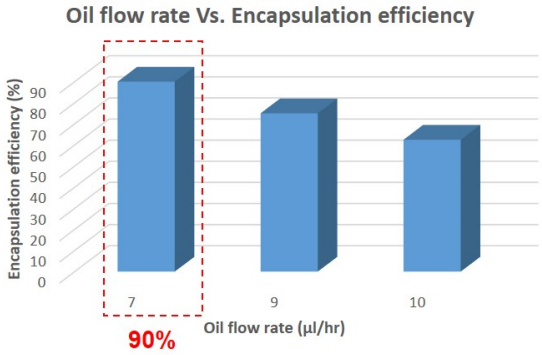

Fig. 6 Effect of oil flow rate on encapsulation efficiency

5. Conclusion

We observed efficiency of about 90% for single cell encapsulation. We also observed that medium flow rate and oil flow rate has significant effect on the trapping efficiency and encapsulation efficiency.

Acknowledgement

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