UNIFORM AND HIGH THROUGHPUT AGAROSE GEL MICRO DROPLET GENERATION DEVICE FOR SINGLE CELL ANALYSIS

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ABSTRACT

We designed and fabricated high throughput agarose gel micro droplet (GMD) generation device for single cell function analysis. Temperature control of each elements of the device is important issue because of the thermo-oriented sol-gel transfer of agarose gel. Uniform GMD of 50 μm in diameter which is suitable size for cell culturing media is achieved with size deviation less than 1.58% and generation rate of about 190deoplets/sec. Single *E.coli* cell encapsulation and cultivation for 5 hours are successfully achieved in an agarose GMD.

KEYWORDS

Microfluidic, Agarose Gel, *E.coli*, Gel Micro Droplet (GMD)

INTRODUCTION

Microfluidic devices for single cell analysis have been reported and evaluated in recent years. [1] However, some problems remain in actual use, for example, cell adhesion to channel wall and diffusion of cell products during handling. Encapsulating cell method in a spherical gel micro droplet is useful for efficient single cell analysis. (Figure 1) Microfluidic systems for single-cell analysis using larger cells (mammalian, yeast etc.) have been introduced. However the encapsulation of bacteria at the single cell level is still a challenge. [2] Uniform and high throughput GMD generation has been requested for this purpose. For cell cultivation media, agarose gell is a suitable material but uniform droplet generation was not realized yet in a micro fluidic device. For cell analysis, GMD of about 50 μm is one of the optimum sizes in actual use. Once agarose gel changes sol state at about 80 degree C, agarose gel keeps sol state even about 30 degree C. (Figure 2) If a living cell is encapsulated in GMD, the temperature during GMD formation should be at less than certain temperature; less than 45 degree C in the case of *E.coli*. The precise temperature control of droplet generation zone is critical issue of the micro flow device.

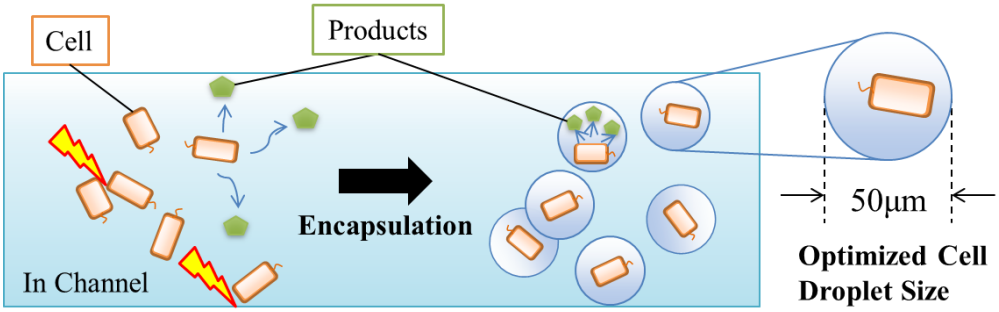

Figure 1. Advantage of single cell encapsulation in GMD

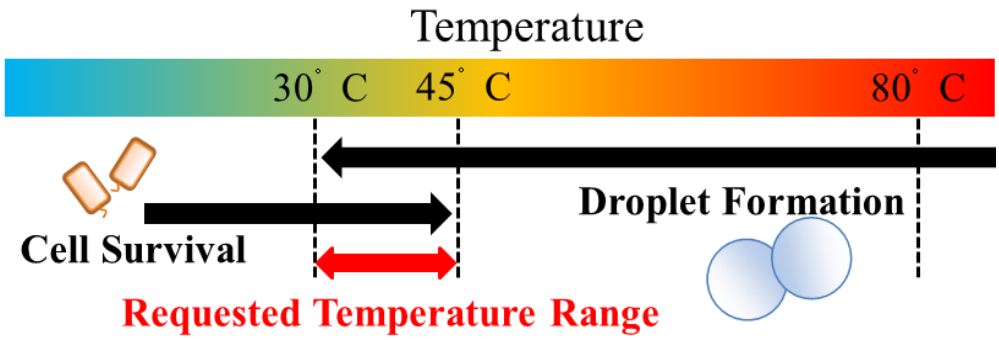

Figure 2. Chart of requested temperature range for cell include agarose GMD generation

DEVICE FABRICATION

Figure 3 shows the device design. There are two inlet channels to introduce sample and carrier respectively and one outlet channel. The sample inlet channel has long and winding structure having a heater underneath to keep it in certain temperature. A cross-junction of the sample and the carriers are used for droplet generation. The device was fabricated by polydimethylsiloxane (PDMS) using a SU-8 mold. The mold structure which has a height of 50 μm was patterned on a glass substrate (49 mm: 40 mm) by spinning SU-8 (SU-8 3050, MicroChem) photoresist and photolithography. The PDMS is bonded to the glass substrate coated with PDMS after O_2 plasma treatment.

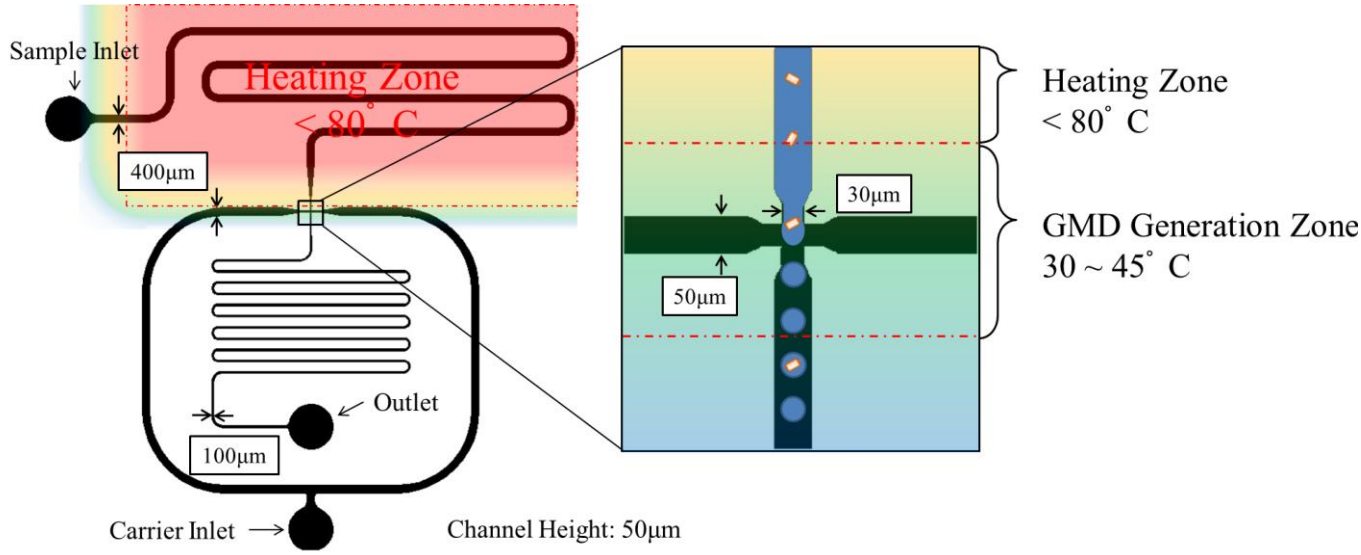

Figure 3. Schematic of device design (sample inlet and carrier inlet channel width: 400μm, cross-junction channel width: 30μm and 50 μm, outlet channel width: 100 μm, channel height: 50μm)

EVALUATION OF GMD GENERATION

In order to evaluate GMD generation, we use 1.0% agarose (UltraPure L.M.P. Agarose, Invitrogen) and mineral oil (SIGMA) includes 3.0% (v/v) nonionic surfactant Span80 (SIGMA) as a sample and carrier respectively. The sample and carrier were introduced into the channel by using syringe pumps (Model 210, KD Scientific).

First, the device was used at room temperature (less than 25 degree C) without a heater. Without temperature control, agarose was gelled in microchannel and change its viscosity. This causes turbulent flow and the droplet size is not uniform (Figure 4(a)). Then, the device was heated at the optimum temperature with a heater. Under optimum temperature control, stable generation of 50 μm diameter agarose GMD is achieved (Figure 4(b)). In addition, use of surfactant included oil as carrier prevents GMD fusion at the GMD collection part (Figure 5). The generation rate of GMD is about 190/sec and the size deviation is smaller than 1.58%. By heating the device, stability and uniformity of agarose GMD generation is increased.

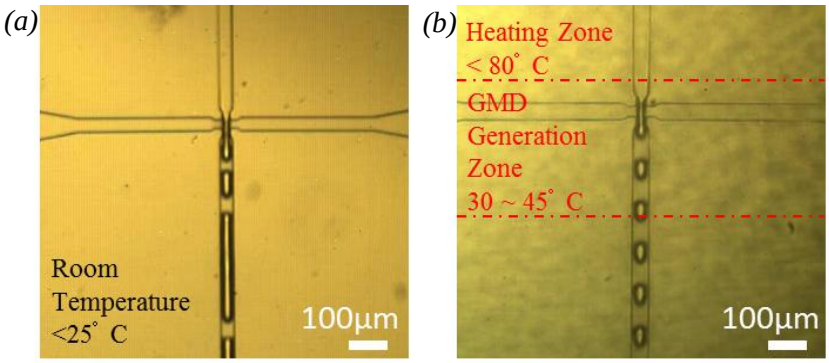

Figure 4. Result of agarose GMD generation (sample flow rate: 1.0μl/min, carrier flow rate: 1.5μl/min) (a) Image of turbulent flow without temperature control (b) Image of uniform agarose GMD generation with temperature control

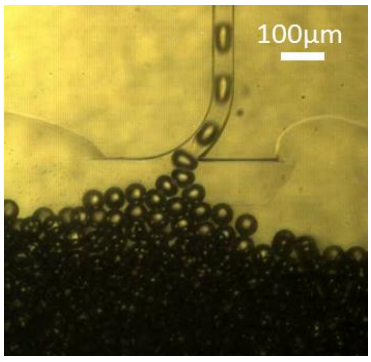

Figure 5. Image of collection part of agarose GMD

SINGLE CELL ENCAPSULATION

To encapsulate single cell in an agarose GMD, we use 1.0% agarose includes *E.coli* cells as a sample. We demonstrated encapsulation and cultivation of *E.coli* cell expressing green fluorescence protein (GFP) in agarose GMD.

E.coli cells were cultured overnight at 37 degree C and added to the agarose prior to GMD generation. Single *E.coli* cell was encapsulated in an agarose GMD, and was cultivated with shaking in Luria-Bertani (LB) medium at 37 degree C. Figure 6 shows growth of GFP expressing *E.coli* in agarose GMD after 3 hours (a) and 5 hours (b). This result shows that agarose GMD can be used for cell cultivation and successive cell functional analysis.

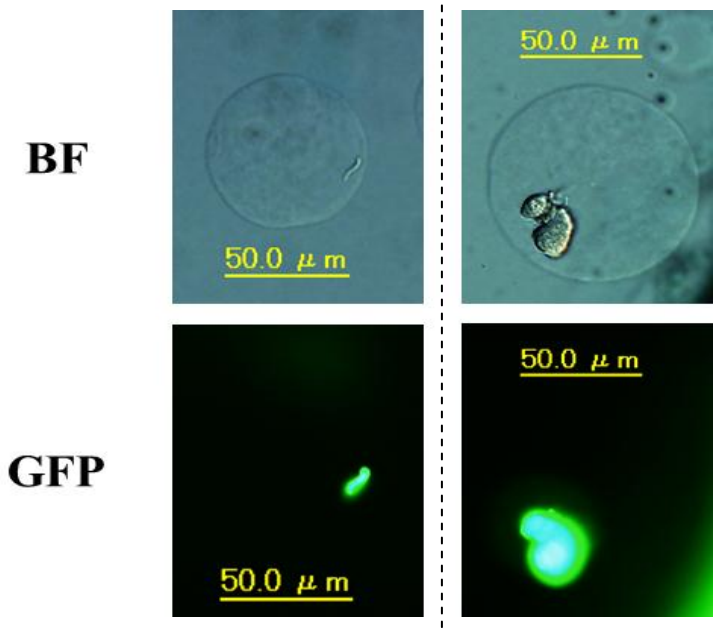

Figure 6. Images of E.coli in agarose GMD after (a) 3 hours and (b) 5 hours cultivation (bright field (BF) and gfp fluorescence (GFP))

CONCLUSIONS

Using the proposed micro fluidic system, we achieved uniform and high throughput agarose GMD generation with size deviation less than 1.58% and generation rate of about 190deoplets/sec. Single *E.coli* cell encapsulation and cultivation for 5 hours are successfully achieved in an agarose GMD.

This uniform and high throughput agarose GMD generation device can be applicable for other practical single cell analysis by optimizing the channel structures and temperature control.

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