ABSTRACT
We introduce a scheme of breaking a large droplet containing high concentrations of cells into smaller droplets, which increases the proportion of single-cell (*Escherichia coli*) containing droplets in the outlet with the aid of the hydrodynamic sorting method.

KEYWORDS: Single cell, Cell encapsulation, Droplet breakup, Droplet sorting

INTRODUCTION
Single-cell isolation is important for biochemical screening platform in which each cell can be genetically varied and observation of individual reaction level is necessary. Microfluidic droplet-based technology enables the isolation of cells in individual aqueous chamber, but single-cell encapsulation efficiency is usually as low as 22% [1]. Several reports have been presented to enhance the efficiency of single-cell encapsulation into droplets by hydrodynamic self-organizing or close-packed ordering of cells [1,2]. However, these methods are only applicable to relatively large mammalian cells in certain flow rate conditions. In this work, we use a micro-groove design for breakup of droplets, which creates size differences between single-cell containing droplets and the ones with multiple cells or no cell, and overcomes the problem of stochastic distribution of cells in the inlet.

SCHEMES
The series of simple groove structures which repeats expansion and contraction of the channel area along the flow yields disruption of droplets by elongating and splitting them. The dimension of the grooves sets the range of the droplet diameters after breakup. Since the height of the whole channel ($h_1 + h_2$) and the distance between the protrusions ($w_3$) are 30 μm, droplets of diameter less than 30 μm will restore their spherical shape and will not be easily deformed due to high surface tension. Therefore, most of the droplets will have diameters 10−30 μm, along with satellite droplets. Larger droplets are more likely to contain multiple numbers of cells as shown in Table 1. To eliminate the large droplets with multiple cells, we had to break the initial droplet completely to have diameter less than 20 μm or remove them by sending them to the top outlet with the aid of the slanted (30°) groove structures.

EXPERIMENTAL
The microchannel was fabricated from two layers of poly(dimethylsiloxane) (PDMS) slabs engraved with channel structures. To verify the single-cell encapsulation, we used enhanced green fluorescence protein expressing *E. coli* DH5α. The continuous oil phase used was mineral oil with 1 wt.% Span80 as surfactant, and the aqueous phase containing cells was Luria-Bertani broth (LB medium). First, the larger monodisperse droplets with multiple cells were created at the T-junction (Fig. 2a). These initial droplets induce the internal flow and the mixing effect reduces the problem of clustering of cells in the inlet. Then the groove structures induce deformation of droplets and shear forces to break them (Fig. 2b),

![Figure 1. Multiple groove structures for breakup of aqueous droplets flowing in the oil phase.](image_url)

Table 1. Number of cells encapsulated in droplets after breakup

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>Droplet diameter</th>
<th>Droplet volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.4 ± 2.1 μm</td>
<td>1.6 pL</td>
</tr>
<tr>
<td>1</td>
<td>19.9 ± 3.3 μm</td>
<td>4.1 pL</td>
</tr>
<tr>
<td>2</td>
<td>24.1 ± 4.4 μm</td>
<td>7.3 pL</td>
</tr>
<tr>
<td>3</td>
<td>29.0 ± 3.0 μm</td>
<td>12.8 pL</td>
</tr>
<tr>
<td>4</td>
<td>31.9 ± 3.8 μm</td>
<td>17.0 pL</td>
</tr>
</tbody>
</table>

(Initial loading concentration of cells: 12×10^7 cells/mL)
and the resulting small droplets were separated by hydrodynamic pinched-flow method (Fig. 2c). Satellite droplets flow out to the uppermost outlet, and larger droplets to the next branch of the outlet. The droplets of interest were collected to the outlet A.

RESULTS AND DISCUSSION

Figure 3 shows the volume distribution of droplets before and after the pinched flow sorting, when initial droplet volumes were 220 pL (Fig. 3a) and 400 pL (Fig. 3b), respectively. In the outlet A, after pinched-flow sorting, more than 85% of the resulting droplets had volumes in the range of 2−5 pL, 15.5−22 \( \mu \)m in diameter of spheres. As a result, droplets of certain sizes with high probability of containing one cell can be selectively collected. When the cell-loading concentration is as high as \( 20\times10^7 \) cells/mL, the ideal droplet volume for single-cell encapsulation should be 5 pL, and it matches close to the collected volume of droplets in the outlet A.
Figure 4. (a) Fluorescence images showing droplets containing GFP-expressing E. coli right after random breakup. (b) The sorted droplets containing single E. coli after passing through the pinched-flow sorting part. Scale bars are 50 μm. (c,d) Percentage of single-cell containing droplets at different initial loading concentrations of cells, collected in the outlet of (c) the conventional T-channel and (d) the device integrated with the random breakup and sorting modules.

Figure 4a and 4b compares the images taken right after the random breakup of droplets and after pinched flow sorting at the reservoir A and the outlet A, respectively. The reservoir A includes satellite droplets containing no cell and some larger droplets having multiple cells inside. In the outlet A, high proportions of single-cell containing droplets could be observed. To verify the device works at various cell concentration, we prepared cell media of three different concentrations, 5, 10, and 20 × 10⁷ cells/mL. In the conventional T-channel producing droplets of 25 μm in diameter (Figure 4c), the proportions of single-cell droplets did not exceed 30%, and the percentage of multiple-cell containing droplets increased as the initial loading concentrations of cells increased. However, using the scheme of random breakup and sorting, we were able to collect droplets of which more than 50% are containing single E. coli at various initial cell concentrations, keeping the proportions of multiple-cell containing droplets to be lower than 10% (Figure 4d).

CONCLUSION

We have presented a method to break up droplets into the sizes in the range of certain dimensions with simple groove structures along the channel. The advantages of this method include that it can sort out the single-cell droplets regardless of the initial droplet sizes generated from the T-junction as well as the flow rate conditions, and it is independent of the cell concentration of the aqueous phases. If supported by more efficient hydrodynamic sorting method, the efficiency and monodispersity of single-cell droplet encapsulation can be further increased.

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REFERENCES


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