**Electronic Supplementary Information (ESI)**

**Mesh-integrated microdroplet array for simultaneous merging and storage of droplets**

Eujin Um, Eugene Rha, Su-Lim Choi, Seung-Goo Lee and Je-Kyun Park*

*a Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon, 305-701, Republic of Korea. Fax: +82-42-350-4310; Tel: +82-42-350-4315; E-mail: jekyun@kaist.ac.kr.

*b Systems and Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRBIB), 125 Gwahak-ro, Yuseong-gu, Daejeon, 305-806, Republic of Korea. Fax: +82-42-860-4489; Tel: +82-42-860-4373; E-mail: sglee@kribb.re.kr.

1. Secondary breakup of droplets for single-cell encapsulation

![Diagram](image)

**Fig. S1.** (a) Schematic representation of the secondary breakup of droplets with an enlarged image of the pinched-flow section. The main channel from the T-junction which has width $w_1 = 120 \mu m$, tapers to $w_2 = 20 \mu m$. We can estimate the diameter of droplets, $d$, produced from the pinched-flow section using equation 1.1

$$d = w_2 \left(1 + \alpha \frac{Q_d}{Q_o}\right)$$

With $\alpha \sim 1$, the rate of the oil flow $Q_o$ was set to be 40 $\mu L/h$, and $Q_d$ (flow rate from the T-junction) was set to be 20 $\mu L/h$, in order to break droplets into those having 30 $\mu m$ diameter. The satellite droplets, produced during the second breakup of droplets flow out to the upper waste. (b) Fabrication of the single-cell droplet generation module by bonding two PDMS substrates engraved with the microchannels. The height of the channels patterned in both of the PDMS substrates is 20 $\mu m$, which results in 40 $\mu m$ of total height in the waste region and outlet channels after aligning. The outlet channel is designed to be deeper and wider after the pinched-flow region, thereby increasing the sorting effect of differently sized droplets. (c) Microscopic image of the pinched-flow section, where 30-$\mu m$ droplets flow toward the outlet channel and satellite droplets are removed to the waste. Scale bar is 100 $\mu m$. (d) Volume distribution of the collected droplets in the outlet. (e) Fraction of droplets collected in the outlet according to the number of cells encapsulated, when initial loading concentration was $1.2 \times 10^8$ cells/mL.
2. Procedure for droplet trapping in mesh-integrated microwell array

A. Preparation of the mesh-integrated microwell array

Droplets generated from a separate device such as a single-cell droplet generation module were transferred to the mesh-integrated microwell array using a glass capillary tube, 500 μm in diameter. Direct contact of the glass capillary tube, which contained a 1-μL volume of the droplet suspension, ensured the droplets entered through the mesh grid. The flow of oil passing through the mesh grid, and the space between the grid and microwells, is important for inducing droplet trapping. The flow can be induced by placing absorbing material such as absorbent paper or cotton balls beside the grid. Using the same method, the removal of excess oil or droplets can be facilitated after all microwells contain trapped droplets. Grid and microwell dimensions should be designed to be compatible with droplets for trapping. Droplets that are too large will not pass through the grid and into the wells. Small droplets will be trapped more than one per microwell. They also will easily flow through the space between the grid and the array, resulting in more empty wells. Because the density of the oil phase is less than that of droplets in this experiment, droplets trapped in the wells will stay in their positions.

B. Droplet trap

Droplets generated from a separate device such as a single-cell droplet generation module were transferred to the mesh-integrated microwell array using a glass capillary tube, 500 μm in diameter. Direct contact of the glass capillary tube, which contained a 1-μL volume of the droplet suspension, ensured the droplets entered through the mesh grid. The flow of oil passing through the mesh grid, and the space between the grid and microwells, is important for inducing droplet trapping. The flow can be induced by placing absorbing material such as absorbent paper or cotton balls beside the grid. Using the same method, the removal of excess oil or droplets can be facilitated after all microwells contain trapped droplets. Grid and microwell dimensions should be designed to be compatible with droplets for trapping. Droplets that are too large will not pass through the grid and into the wells. Small droplets will be trapped more than one per microwell. They also will easily flow through the space between the grid and the array, resulting in more empty wells. Because the density of the oil phase is less than that of droplets in this experiment, droplets trapped in the wells will stay in their positions.

C. Droplet addition

A well-known material for demulsification of the two-phase system is butanol.\textsuperscript{4} We did not inspect any cell damage with the use of butanol, but we used octanol (40% (v/v) in mineral oil) instead because it is more insoluble in water and is assumed to have a smaller effect on water-phase droplets. With butanol, the merging event is much faster, even at concentrations as low as 5% (v/v) in mineral oil.
D. Droplet recovery

Fig. S3. (a) Glass capillary tubes, 30 μm in diameter, were used to remove droplets from the microwells. (b) After transferring a single-cell droplet from the well array to an agar plate containing ampicillin (the *E. coli* were resistant to ampicillin), single-colony formation from one parent cell was observed.

3. Implementing droplets for a cell-based assay

Fig. S4. (a) Schematic of the interaction between enzyme-producing cells and the reporter cells. We detected the presence of enzyme-producing cells by co-culturing with reporter cells, which display a fluorescence signal in response to the enzyme product. (b) Co-encapsulation of the enzyme (TPL)-producing cell and reporter cell by merging single-cell-containing droplets.

The enzyme-producing cell was *E. coli* XL1-Blue/pTrc99a-TPL expressing tyrosine phenol-lyase (TPL; EC 4.1.99.2), which produces phenol, pyruvate and ammonia by the hydrolytic reaction of L-tyrosine. The reporter cell was designed so that phenol (as the enzyme product) is recognized by DmpR, a transcriptional activator of phenol-catabolizing *Pseudomonas*, which activates the expression of the reporter protein. The interaction between the TPL cell and reporter cell was examined by co-cultivation in M9 minimal medium supplemented with 1 mM tyrosine, at 37°C. After 16 h, specific cellular fluorescence was measured using a fluorescence plate reader.
(Victor™ V, Perkin-Elmer, MA) with an EGFP filter set (ex: 488 nm, em: 530/20). Optical density of the cells was determined by measuring absorbance at 600 nm with a UV/visible spectrophotometer (Pharmacia Biotech, Sweden). Only the cultures with both the enzyme-producing cell and the reporter cell exhibited high fluorescence emission, as shown in Fig. S5.

To encapsulate enzyme-producing cells into droplets, we used 3% (w/w) Abil EM 90 as a surfactant, instead of Span 80, in the oil phase, because Span 80 has been reported to show high leakage of entrapped water-soluble molecules. The initial loading concentration of cells into the microchannel for secondary breakup of droplets was 1.2 × 10⁸ cells/mL for all experiments.

Fig. S5. Fluorescence intensity per cell was measured using a fluorescence plate reader. High fluorescence emission was observed for reporter cells in the presence of TPL-producing cells. The inset is the fluorescence microscopic image of five wells from the 96-well plate. The respective wells contained cell culture broth with, from left to right, control cells only, TPL-producing cells only, reporter cells only, both control cells and reporter cells, and both TPL-producing cells and reporter cells.

4. Captions for video clips

Video S1: Merging of multiple droplets into one droplet inside microwells under the mesh grid, while the surrounding oil phase (mineral oil with 2 wt % Span 80) was exchanged with 40% (v/v) octanol in mineral oil.

Video S2: Using a glass capillary tube to select three droplets from the mesh-integrated array.

References