Electronic Supplementary Information (ESI) for Analytical Methods

Electronic Supplementary Information

Heat-Shock Transformation of *Escherichia coli* in Nanolitre Droplets Formed in a Capillary-Composited Microfluidic Device

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Abstract. This Supplementary Information includes all the additional information as noted in the manuscript and more detailed discussion on the current study.

Conventional Tube-based *Escherichia coli* Transformation. To evaluate the reliability and transformation efficiency of the droplet-based transformation of *Escherichia coli* (*E. coli*) cells, conventional tube-based *E. coli* transformation was also carried out in the current study following the method reported previously.[1,2] Briefly, 100 μL competent cells and 5 μL plasmid pGLO are mixed in a chilled 1.5-mL polypropylene tube. After incubation at 0 °C for 30 min, the mixture in the tube was treated by a heat-shock step without agitation in a 42 °C water bath for 45 s and cooled on ice for 2 min. After adding 400 μL fresh LB medium, the solution is placed in a 37 °C shaking incubator for 45 min with a rotation speed of 100 rpm to allow expression of antibiotic resistance genes. Next, 100 μL of the resulting culture was then spread on an agar-LB plate (10 g/L bacto-tryptone, 5 g/L yeast extract, 10 g/L sodium chloride, 15 g/L agar, and 3g/L arabinose; adjusted to pH 7.0 with 1M NaOH solution) with ampicillin (100 μg/mL) and incubated at 37 °C for 18 h. Fluorescence images of the transformed *E. coli* DH5α cells were recorded using an inverted fluorescence microscope (Olympus, CKX41) equipped with a high-resolution CCD camera (QIMAGING, Micropublisher 5.0 RTV) and a mercury lamp (Olympus, U-RFLT50). Each test was repeated at least three times. Blank controls were run simultaneously during each experiment. All the steps above were performed in a laminar flow hood to maintain sterility of all reagents.

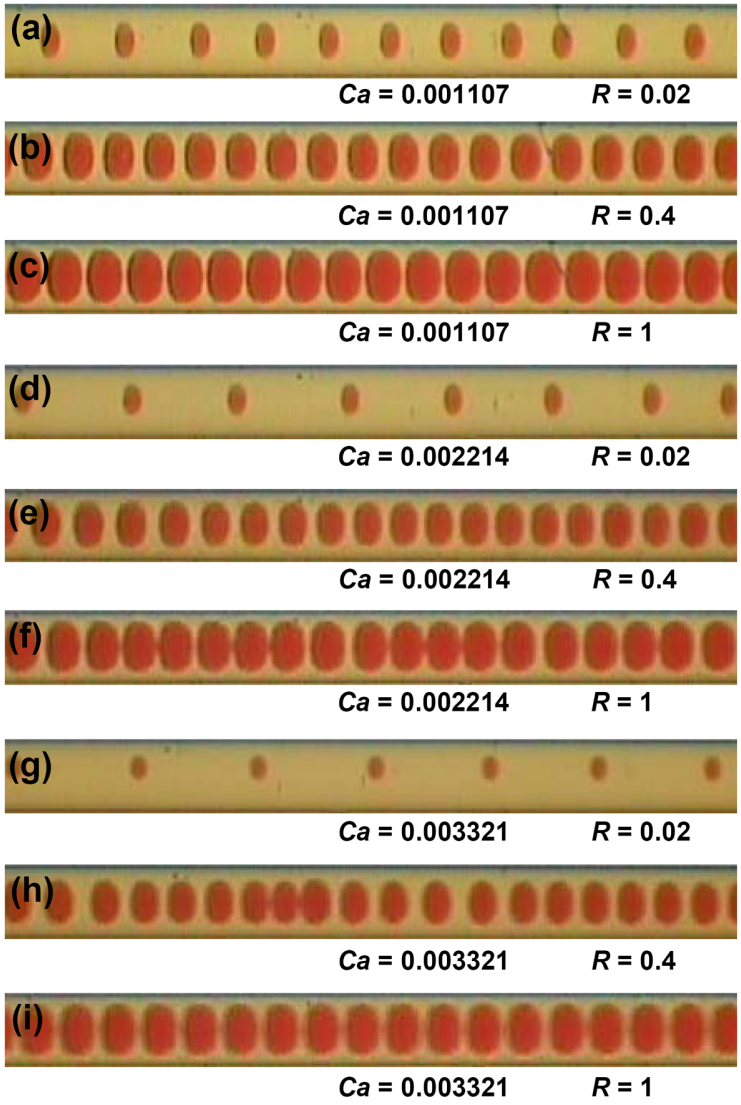

Figure S1. (a)–(i): Typical results of experimental micrographs of droplet generation in the PCM device as a function of the capillary number *Ca* and the ratio of the two phases *R* (*R* is defined in the text). The *Ca* values are calculated as Eq. S1. As shown in Fig. S1, a direct qualitative comparison was made by visual inspection of the change in drop size. The droplets were shown flowing in a single droplet stream manner in the quartz capillary with tunable and uniform size. The most notable phenomenon was that as the flow rate ratio *R* was increased, the drop size increased significantly. However, the droplet size changed slightly as the capillary number was increased for a fixed flow rate ratio *R*.

Calculation of Capillary Number (*Ca*) of Droplets Formed in the PCM Device. According to a previous study,[3] the dimensionless capillary number (*Ca*) can be described as follows:

$$Ca = U\mu / \gamma \qquad \text{Eq. S1}$$

where U (ms^{-1}) is the velocity of the continuous flow (here, the oil phase), μ ($kgm^{-1}s^{-1}$) is the viscosity of the continuous fluid (here, the oil phase), and γ (Nm^{-1}) is the interfacial tension at the aqueous and oil interface. U is determined by dividing the continuous volumetric flow rate (Q_o) by the cross-sectional area (A) of the channel (here, the capillary). The viscosity of mineral oil M5904 (2 wt% Span80) is 23.8 mPas, the viscosity of ultra-pure water is 1 mPas, and interfacial tension between them is 3.65 mN/m.[4] Hence, the *Ca* of the droplet-based PCM device is 0.001107 when Q_o is 0.5 μL/min, 0.002214 when Q_o is 1.0 μL/min, and 0.003321 when Q_o is 1.5 μL/min.

Acquisition of Equation 1 (Eq. 1 in the text). Taking the two variables (R, *Ca*) into consideration, the droplet size is then described by the equation as follows:

$$D_{aq} / D_i = kR^{\alpha}(1/Ca)^{\beta} \qquad \text{Eq. S2}$$

where D_{aq} (μm) is the diameter of the droplet; D_i (250 μm) is the inner diameter of the quartz capillary; R is the flow rate ratio of the two phases ($R = Q_{aq}/Q_o$); and k, α, and β are constants that depend on geometrical characteristics of the device, viscosities, and other physical parameters except for *Ca* and R. As shown in Figure 3 of the text, Eq. S2 was utilized to fit the experimental data in our work. A fitted equation was obtained as follows:

$$D_{aq} / D_i = 0.5913R^{0.1869} \times (\frac{1}{C_a})^{0.0405} \qquad \text{Eq. S3 (corresponding to Eq. 1 in the text)}$$

Theoretical Calculation of Heat Transfer Time of the Capillary. To obtain the value of the heat transfer time from capillary surface to central area of the capillary, Fourier's law of heat conduction was introduced.[4]

$$q = \lambda \frac{\partial T}{\partial x} \qquad \text{Eq. S4}$$

where q is the heat flow density (the vector of specific energy flow rate), λ is the thermal conductivity tensor, and $\partial T / \partial x$ is the temperature gradient vector through a thickness x. The rate of heat flow (H) is defined as the following equation:[5]

$$H = qA = \frac{\partial Q}{\partial t} \qquad \text{Eq. S5}$$

where A is the total cross sectional area of conducting surface, $\partial Q / \partial t$ is defined as the quantity of heat ∂Q and heat transfer time ∂t. Combining Eq. S4 and Eq. S5, the heat transfer time can be described as

$$\partial t = \frac{\partial Q \partial x}{\lambda A \partial T} \qquad \text{Eq. S6}$$

The inner diameter (ID) of the capillary is 250 μm and the outer diameter (OD) 370 μm. Thus, the radius of the capillary is 185 μm, consisting of 60 μm-thick quartz capillary and 125 μm-thick mineral oil. The thermal conductivity of quartz and mineral oil is 1.147 W/(m·K) and 0.145 W/(m·K), respectively.[5,6] Calculated results from Eq. S6 are as follows: transmitted time through the thickness of 60-μm quartz is 0.0048 s, and that through the thickness of 125-μm mineral oil droplet is 0.3785 s. That is to say, the time of temperature equilibrium of the quartz capillary is 0.3833 s.

Movie S1. Droplet formation at the junction of the microchannels and the capillary (bottomside view). The movie shows the bottomside view at the junction of microchannels and capillary within the PCM device (Q_o = 0.5 μL/min, Q_{aq} = 0.1 μL/min). Clearly, there is no dead volume at the junction and no droplet fusion occurs.

Figure S2. Schematic representation of plasmid pGLO.

REFERENCES

(1) M. Mandel and A. Higa, *J. Mol. Biol.* 1970, **53**, 159–162.

(2) J. Sambrook and D. W. Russel, *Molecular cloning: a laboratory manual*, CSHL press, New York, 3rd ed., 2001.

(3) C. N. Baroud, F. Gallaire and R. Dangla, *Lab Chip* 2010, **10**, 2032–2045.

(4) J. Sivasamy, Y. C. Chim, T. N. Wong, T. T. Nguyen and L. Yobas, *Microfluid. Nanofluid.* 2010, **8**, 409–416.

(5) T. J. Ahrens, *Rock physics & phase relations: a handbook if physical constants*; Americal Geophysical Unlon, Washington, DC, 2000.

(6) S. Goodhew and R. Griffiths, *Appl. Energ.,* 2004, **77**, 205–223.