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A novel approach for drop-on-demand and particle encapsulation based on liquid bridge breakup

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ABSTRACT

A simple and robust way is devised to generate picoliter droplets out of a single microliter drop for the use of generating monodisperse droplets in droplet-based microfluidics. A single aqueous drop is placed between two hydrophilic substrates and then immersed in silicone oil, to form a liquid bridge. Then one substrate is moved away with a predefined velocity. As the distance between two glass plates increases, the liquid bridge breaks up and smaller droplets or satellites are formed. A picoliter-droplet was successfully dispensed on demand repeatedly for 100 times within 2% relative standard deviation in size under velocity-controlled system. It is found that, for the case of fixed inner and outer fluids, the droplets of nearly the same size are generated over several orders of the moving velocity. Also, the size of the maximum satellite droplet increases with the increase of the mother drop size and inner fluid viscosity, and it decreases with the increase of the outer fluid viscosity. Its feasibility of cell and particle encapsulation has been confirmed by capturing a single *Arabidopsis thaliana* protoplast as well as polystyrene microparticles successfully using this method without complex control. Based on these results, a simple forcep was designed to dispense ultrasmall droplets and its functionality was confirmed to be similar with the velocity controlled case without external devices. This simple method is expected be used to divide a small amount of bio sample on-demand into several smaller droplets for further analyses.

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1. Introduction

Droplet-based microfluidics offers a wider range of application over conventional or continuous analytical techniques [1–6]. By encapsulating reagents in droplets, advanced control of reaction kinetics can be obtained [7–10] and physical and chemical isolation of droplets eliminate the risk of cross-contamination [7] with a higher concentration of products generated confined in the droplets. Droplets of reagents can be transported faster [11–13] or more precisely [14–16] than conventional microfluidic devices with controlled contents [17–19]. These merits result in the increase of areas of applications [20–23]. However, in order to further utilize droplet microfluidics, stable generation of ultrasmall droplets with a predetermined size should be first established.

There have been many researches on the conventional droplet generating methods. To generate droplets, only when needed with a definite number of droplets or on-demand, electric pulse [24–28], piezo-electric [29–31] or other methods [32] are commonly used. These methods generally generate droplets with the size of the nozzle, i.e. smaller drops can be made using a smaller nozzle [33,34]. The operating conditions including driving pulse duration and shape, which depends on the fluidic properties, must be tuned empirically for proper operation. Among a number of papers for cell printing based on these methods, only few can stably generate single-cell-encapsulated droplets without cell rupture [35–38]. For mobile use of microfluidic devices, a smaller and simpler device is required for generating only few droplets on-demand without the need of additional equipment such as syringe pump, high-voltage power supply, laser or piezoelectric transducers and the need of precise control.

Satellites have been observed since the first drop experiment in jet breakup. Theoretical [39–41], numerical [42,43], experimental [44–46] researches and related reviews have been performed to understand jet breakup and satellite formation and to prevent satellite generation. It is now known that in common circumstances satellite formation is a universal feature of breakup, with less than 1% of the original drop volume [47,48].

Satellite formation is a multi-scale problem in both the time scale and the length scale [49]. While the original drop, or the mother drop radius is $O(10^{-3})$ m, the generated droplet radius is $O(10^{-5}) - O(10^{-6})$ m. The overall elongation motion takes place in few seconds while the breakup and collapse process take place in less than few milliseconds [50,51]. The liquid bridge breakup and satellite formation is a process balanced between viscous force and surface tension force. The overall dynamics can be characterized by two nondimensional numbers –Capillary number $\left(Ca = \frac{\mu V}{\sigma}\right)$, the relative effect of viscous force versus interfacial

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tension, and Ohnesorge number $\left(Oh = \frac{\mu}{\sqrt{\rho\sigma D}}\right)$, the relative effect of viscous force versus inertial and interfacial tension, where μ, V, σ, D and ρ are dynamic viscosity, velocity, interfacial tension, drop diameter and density, respectively.

With the characteristic length D = 1 mm, the interfacial tension between silicone oil and water σ = 40 mN/m, the kinematic viscosity ($v = \mu/\rho$) 1 cSt < v < 100 cSt, and the glass plate moving velocity 50 μm/s < V < 1000 μm/s, Ca ranges from O(10^{-4}) to O(1) and Oh ranges from O(10^{-3}) to O(1). The capillary time scale $\left(t_c = \sqrt{\rho D^3/\sigma}\right)$ is determined only by its fluidic properties (with fixed D), not by the mechanical movement. This suggests that the different mechanical movement will not dramatically affect the dynamics of the dispensing process in this regime.

In the present work, we suggest a new method of drop-on-demand using satellite formation during liquid bridge breakup using an ordinary forcep, which enables us to encapsulate single cells without other complex control or external equipment. To obtain important basis results for applications, we investigated the satellite formation again with special attention given to the effects of key parameters such as the elongation rate of liquid bridge, the mother drop size, and the outside and inside fluid viscosities. The aim of this work is to confirm the possibility to use liquid bridge breakup and satellite formation as a simple droplet generation method and a particle encapsulation method.

2. Experimental section

2.1. Materials

The dynamics of liquid bridge breakup and satellite formation are mainly determined by the fluid interfacial tension and the viscosities of the inner and the outer fluids. In this experiment, DI water, and its mixture with polyethylene glycol (PEG #200, Samchun Chemicals) were used as inner fluids. For particle encapsulation, polystyrene microparticle suspension with an average particle diameter of 3, 11, 30 μm (Sigma Aldrich) were used with 0.1% (w/v) solid concentration in 1X PBS. For the cell encapsulation experiment, W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, 5 mM glucose, and 1.5 mM Mes-KOH, pH 5.6) was used to carry and encapsulate plant protoplast. *Arabidopsis* (*Arabidopsis thaliana*; Columbia ecotype) protoplast was used. Since high intensity light must be used in high-speed imaging, cells which can be observed under visible light is needed. Plant cells have chloroplasts that are visible under visible light. In addition, protoplasts are larger in size than *E. Coli.* and has better size uniformity. *Arabidopsis* was grown according to the standard protocol. The protoplasts were pelleted by centrifugation at 55 g for 5 min and resuspended in 20 mL of W5 solution. The protoplasts were incubated on ice for 30 min. Silicone oil (Shin Etsu KF-96, 50 cSt, Dow Corning DC200F, 6 cSt), and n-dodecane (Alfa Aesar) were used as outer fluid in this experiment. Silicone oil with very low viscosity (kinematic viscosity v < 5 cSt) was also available but due to its low vapor pressure, alkane hydrocarbon was used instead.

Microslide glasses (Corning, single frosted, pre-cleaned) and copper plates were cut into 5 mm by 25 mm strips, each bonded to t forcep, as hydrophilic plate to hold the mother drop and split the liquid bridge. All the plates were cleaned with isopropyl alcohol and air-dried.

2.2. Method

Schematic diagram of the experimental setup is shown in Fig. 1. Sample mother drop and two glass plates are immersed into silicone oil, each connected to a motorized stage. Observation is made from the bottom unless specified.

The droplet dispensing procedure is as follows. On one side of the glass plate, a single drop of given volume is placed using a micropipette (Eppendorf, single channel). By applying force to a forcep or using a motorized stage, either plate approaches the other plate or a drop. A drop contacts the other plate, forming a liquid bridge. Depending on the viscosity of the outer fluid, stabilization time of few seconds is needed to form a symmetric liquid bridge. After the liquid bridge is stabilized, plates move apart. After it reaches the critical length depending on the surface tension, the liquid bridge breaks up, and satellites are formed, leaving two mother drops attached on each plate. The whole process is repeated for further dispensing. Representative snapshots are shown in Fig. 1C.

Velocity can be applied in different profiles, i.e. constant velocity, constant elongation rate, constant acceleration. However, the breaking and collapsing process takes place in capillary time scale, which is less than O(10^{-3}) seconds, any of the above velocity profiles can be approximated as a constant velocity profile for the short time interval. Triple contact line movement shows a nonlinear dynamics with dependency on the plate elongation rate, so to minimize its effect, the plate movements were kept as minimum by using appropriate spaces. Additional process or small circular discs could be used to pin the liquid bridge on each end and eliminate the triple contact line movement. However, initial positioning and aligning of the mother drop needs extra process than a single-drop-on-plate case. Also, by having a mechanical confinement on the surface, the use of the device is limited to a single droplet size. However, once the dispensing conditions and required resulting droplet size are settled, pinning the liquid bridge can be a way to increase the stability of this device.

To confirm the elongation velocity effects on the generated satellite size, plates were moved with a constant velocity using a motorized stage. Then, similar elongation velocity was applied on a forcep manually. Two types of forceps were used. One is an ordinary forcep that is closed when force is applied (Fig. 4C), and the other is an inverted forcep that is closed naturally and opened when force is applied (Fig. 4B). Experimental setups for the forcep droplet dispensing experiments are shown in Fig. 4A with representative snapshot shown in Fig. 4D.

High-speed images were captured using Photron PCI 1024X, which is capable of capturing up to 109,500 frames-per-second (fps). The full-frame resolution was possible only for the speed up to 1000 fps. Higher capturing frequency reduces the size of the region-of-interest (ROI) and also requires more light. To capture the whole system 1000 fps with full frame were used and for detailed dynamics up to 18,000 fps were used.

The obtained images were analyzed using LabVIEW with Vision Assistant and ImageJ. For accurate analysis, droplet volume and moving distance were crosschecked using both programs. All the measurements were averaged over 3 times of measurement.

Velocity controllable motorized stages (Sigma Koki, SGSP2020) were used with LabVIEW via RS-232 protocol. Due to the high magnification microscope on the high-speed camera, a 5 W white light emitting diode (LED) light source was used to provide enough backlight without heating instead of the front-side mercury light source. All the experiments were done in room temperature, and mechanical movement was slow enough not to heat up the oil by viscous dissipation.

3. Results and discussion

To confirm its reliability, single droplet generation was tested by dispensing droplets repeatedly from 1 μL mother drop for

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