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Controlled Generation of Double Emulsions in Air

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Abstract

This communication describes the controlled generation of double emulsions in the gas phase, which was carried out using an integrated emitter in a PDMS (poly(dimethylsiloxane)) microfluidic chip. The integrated emitter was formed using a molding approach, in which metal wires with desirable diameters were used as emitter molds. The generation of double emulsions in air was achieved with electrohydrodynamics actuation, which offers controllable force exerting on the double emulsions. We developed this capability for future integration of droplet microfluidics with mass spectrometry (MS), where each aqueous droplet in the microchannel is introduced into the gas phase as a double emulsion for subsequent ionization and MS analysis.

Droplet microfluidics has shown promise in recent years in a range of chemical and biological applications [1–13]. A small sub-area of droplet microfluidics has been focused on the formation of double emulsions, which are droplets of dispersed phase containing even smaller droplets within. Double emulsions are found in diverse areas, from food, cosmetics, to pharmaceutics [14–16]. As a result, there has been interest in both the study of double emulsions and in their controlled generation. In fact, microfluidics approaches have already been devised for producing double emulsions in the condensed phase with unprecedented controllability and flexibility [17–20].

Rather than forming double emulsions in the liquid phase as has been demonstrated previously, we wanted to form double emulsions in air. Our interest in doing this is driven by our long-term goal of introducing aqueous droplets containing samples—for example, single cells—from droplet microfluidic devices into the gas phase as a double emulsion for subsequent interfacing with the mass spectrometer. In theory, this should be achievable, but in practice, there are a number of challenges to overcome. Specifically, we need to ensure that the rate at which double emulsions are generated is sufficiently low and controllable so the microfluidic sampling rate can match the duty cycle of the mass spectrometer. The double emulsions may be used to encapsulate single cells of interest in future experiments, for which droplet diameters in the tens of micrometers range is often desired. Additionally, we need to ensure the droplets do not wet the surface of the nozzle from which they are introduced into the gas phase.

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Author Contributions
D. Liu performed the experiments and wrote the manuscript; B. Hakimi, M. Volny, J. Rolfs, X. Chen, helped with the setup and the experiments; D. T. Chiu, M. Volny, and F. Turecek provided input into the manuscript. All authors have given approval to the final version of the manuscript

Notes
The authors declare no competing financial interest.

Supporting information
Additional information as noted in text. These materials are available free of charge via the Internet at http://pubs.acs.org.

Compared with the generation of double emulsions in the condensed phase, formation of double emulsions in air is more challenging because gas has a much lower viscosity, which in turn necessitates high gas flow rates to generate enough shear stress to form the droplet in air. For example, one method is based on flow focusing using two concentric tubes [21–22]: the inner tube and the outer tube contain aqueous solution and oil flowing toward the nozzle, respectively. A high pressure gas stream is forced to flow through the outer tube to shear off the oil phase into droplets, containing smaller aqueous droplets inside, in the gas phase. We were able to employ this strategy to form double emulsions in air, but the speed at which the droplets were generated was too high for interfacing with mass spectrometry.

Another electrospray based method also uses two concentric tubes for liquid introduction, but without any gas stream involved [23]. In this arrangement, the liquids are connected to a high voltage power supply and an annular electrode is positioned opposite the tube exit. When the electric field between the liquids and annular electrode is strong enough, a fine conic tip, called Taylor cone, forms at the liquid vertex. The electrified coaxial compound liquid jet is emitted from the Taylor cone and subsequently decays into double emulsions. This approach, however, is not suitable for our purpose. For our application, we need to generate firstly aqueous droplets with encapsulated biological molecules of interest in a microfluidic device so these droplets are amenable to various manipulations offered by droplet microfluidics, and then introduce these droplets as double emulsions into the mass spectrometer for analysis.

The aqueous droplets we intend to introduce into the mass spectrometer are in the range of 20–100 microns in diameter, which is a range suitable for single-cell analysis. We also wanted to form these droplets at a tunable rate of tens of Hz, so we can match droplet generation with the scanning frequency of a time-of-flight mass spectrometer (TOF-MS). Additionally, we aimed to have precisely one aqueous droplet encapsulated within each oil droplet. The approach we found that best satisfies these criteria is based on the electrohydrodynamic dispensing of double emulsions at a nozzle or emitter tip.

To achieve this, we had to develop an integrated emitter in the PDMS chip, because an integrated emitter may avoid the dead volume at the junction region between the fluidic channels and assembling external emitters. Delivery of droplets using electrohydrodynamics is easy to integrate with microfluidics chips, and is convenient for controlling droplet size and frequency, especially for the generation of larger droplets (tens of microns in diameter and above) at a lower droplet-generation frequency (tens to hundreds of Hz) that are needed for coupling with the mass spectrometer [24–25]. To achieve this, we developed a simple polymer molding method to form an integrated emitter with a three-dimensional conical shape in a PDMS chip. Although there are many existing integrated PDMS emitters, their tapered structure is mostly two dimensional because of fabrication constraints [26–29]. This is not suitable for our application because the quasi-2D shape can cause adhesion and spreading of liquids at the emitter tip. The surface of 3D integrated emitter formed by molding technique was silanized by trichloro(1,1,2,2-H_4-perfluorooctyl)silane to facilitate detachment of the double emulsion from the tip.

EXPERIMENTAL

Materials and Supplies

1-octanol, trichloro(1,1,2,2-H_4-perfluorooctyl)silane, Span80 and fluorinated oil (FC-40) were purchased from Sigma-Aldrich. Metal wires (with 40 μm diameter) used as emitter molds were obtained from SANDVIK Company.

Fabrication of Microfluidic Device and Emitter Tip

Microfluidic channels were fabricated in PDMS using standard soft lithography method, which has been described in detail elsewhere [30]. Briefly, Su8-2025 photoresist (MicroChem) was spin-coated onto a silicon wafer to form a film with desired thickness, as measured by a home-built interferometer on the finished master [31]. To fabricate the microfluidic chip, the mixer of PDMS base and curing agent (10:1; Sylgard 184, Dow Corning, MI) was poured onto the master, degassed for 30 minutes, and then cured overnight in the oven at 70 °C. After curing, the PDMS channel was peeled off the master and exposed to oxygen plasma, along with another piece of flat PDMS plate without molded structures. The PDMS channel then was sealed irreversibly against the flat PDMS plate. All the channels were 38 μm in height.

The geometry and surface chemistry of the emitter is crucial for the generation of double emulsion. We developed a polymer molding method to fabricate a fine conically shaped integrated emitter in PDMS chip. Briefly, a metal wire (surface treated with perfluorosilane) was inserted into the main channel (Ch3 in Figure 1) as an emitter mold, and freshly mixed PDMS solution was then dipped around it. After PDMS was cured, the metal wire was pulled out, and an integrated PDMS emitter was formed (see Supplementary Figure S1 and accompanying text for additional details). Figure 1e shows a picture of the PDMS emitter.

Surface Treatment

PDMS channel and emitter were treated with trichloro(1,1,2,2-H_4-perfluorooctyl) silane to make their surface fluorophilic.1-Octanol and Milli-Q water were used as continuous phase and disperse phase, respectively. In order to make water droplets more stable, 1 wt% Span80 surfactant was added to octanol. Due to the desirable surface treatment, the adhesion of water and octanol on the emitter tip was significantly diminished.

Imaging and Analysis

A high-speed CCD camera (GC640, allied Vision Technologies Inc., Canada) was used to record the process of double emulsions generation. Brightfield images of double emulsions collected in vials were acquired with an Olympus MVX10 microscope (Tokyo, Japan), along with a CCD camera (GC1380, allied Vision Technologies Inc., Canada). Images were analyzed using Image J (NIH).

RESULTS AND DISCUSSION

Figure 1a shows the setup and design of our experiment. A PDMS microfluidic chip integrated with a fine emitter was used to produce double emulsions in the gas phase. Mono-disperse aqueous droplets were generated in continuous oil phase using a flow focusing geometry (Figure 1b). The aqueous droplets flowed down the main channel (Ch3 in Figure 1a), and encountered a conical PDMS emitter at the outlet. To generate double emulsions in the gas phase, a metal wire from a high voltage power supply was connected to the aqueous phase as a positive electrode (inlet to Ch1 in Figure 1a). The ground electrode was a thin copper plate with a small 500 μm diameter hole where the double emulsion droplets passed through. The copper plate and PDMS chip were mounted onto x-y-z translation stages so their relative position can be controlled and adjusted precisely. Typically, the distance between the emitter and the ground copper plate (L2) was around 0.8 mm (Figures 1a and 1d).

When the high positive voltage was applied to the device, an electric field gradient was present along Ch1 and Ch3. As a result, more negative charges were drawn back to upstream by the positive potential at the end of Ch1, and more positive charges were left in the

droplets in the oil phase. Under the applied electric field, octanol also became polarized. Because of the net positive charges in the water droplets and the polarization of the liquids, electric force developed between the droplets and the ground electrode. When the electric field was high enough, double emulsion formed at the emitter tip was ejected toward the copper plate (ground electrode). Figure 2 is a series of images captured by a high speed camera showing the above described process. The arrows point to the front (red) and back (blue) ends of the aqueous droplets. In Figure 2a, a previous double emulsion droplet just passed through the hole on the copper plate and a new droplet begins to form at the tip. Figures 2b–2f show the formation and ejection of a new double emulsion droplet (see Supplementary Material for a video of the whole process).

We used a glass vial filled with fluorinated oil to collect the ejected double emulsion droplets so we could characterize these droplets. The adding of 1 wt% surfactant Span80 in octanol may further increase the stability of double emulsions, and therefore avoid the instability encountered during droplet collection. The interfacial tension between water and octanol was low enough to generate water droplets in octanol. In other word, if double emulsions were not collected for characterization in a vial, a process that destabilized the emulsions because of sudden changes in momentum when the emulsions impinged on the oil in the vial, no surfactant was needed in the system for simply generating double emulsions in air. The presence of positive charges on the droplets caused them to repel each other when they landed into the fluorinated oil. Most droplets were observed at the bottom of the vial. To maintain the stability of these droplets in contact with the vial bottom, the surface of the glass vial was treated with trichloro(1,1,2,2-H_4-perfluorooctyl)silane. Figure 3 shows the collected double emulsion droplets at the bottom of the vial, as produced under different flow rates and voltages. To quantify the mono-dispersity of the double emulsions, we measured the distributions of the diameters of the inner and outer droplets (Figure 3d–f). It is evident that the distributions of droplet diameter are very narrow; the standard deviation is below 4% of the mean diameter in each of Figure 3d–f. In addition, by changing the voltage, successive double emulsions encapsulating two (1.12 kV), three (1.10 kV), or four (1.07 kV) small droplets were formed in the gas phase, which were shown in the videos (ESI2A, 2B and 2C, respectively) given in the Supporting Information. In the above three experiments, the liquid flow rates did not change, although the droplet-generation frequency changed slightly. When the applied voltage was lowered, the electric field acting on the emulsion at the emitter tip was correspondingly weaker. As a result, the frequency of double emulsion generation became slower, which in turn allowed for longer time for encapsulating more aqueous droplet inside the oil droplet. Figure 4 shows mono-dispersed double emulsions each encapsulating two small droplets inside (also see Supplementary Material for videos showing the processes).

CONCLUSIONS

The new electrohydrodynamic method allows for the controlled generation of water-in-oil double emulsions in air using an integrated PDMS emitter tip. This integrated emitter tip minimized issues associated with dead volume, which is often encountered when coupling microfluidic chips to an external emitter. Using this technique, we were able to generate droplets at a frequency range suitable for interfacing with MS and over a droplet size range tailored for single-cell analysis. Because this approach encapsulates preformed aqueous droplets, it allows us in principle to employ droplet microfluidics for various droplet manipulations prior to the generation of double emulsions and introduction into the MS instrument. The double emulsions formed using this method are mono-disperse with the added flexibility of allowing us to control the number of aqueous droplets encapsulated per oil droplet. We believe this method will be a useful tool for coupling droplet microfluidics to mass spectrometers for sensitive droplet analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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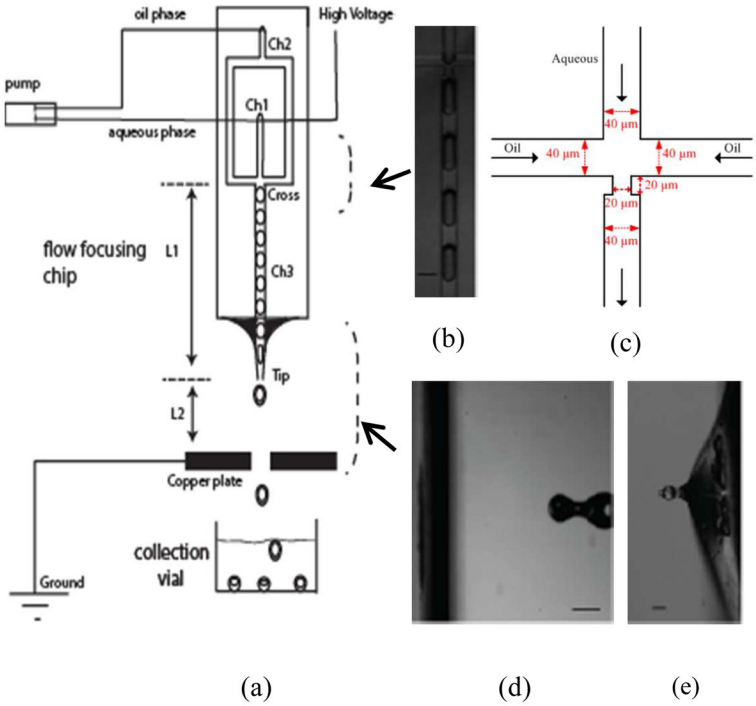

Figure 1.

(a) Schematic of the set up. (b) Micrograph showing generation of aqueous droplets in the flow focusing channel. The scale bar represents 50 μm. (c) Schematic of the flow focusing geometry. (d) Micrograph of copper plate, PDMS emitter, and the formation of double emulsion in air. The scale bar represents 100 μm. (e) A micrograph of the PDMS emitter. The scale bar represents 100 μm.

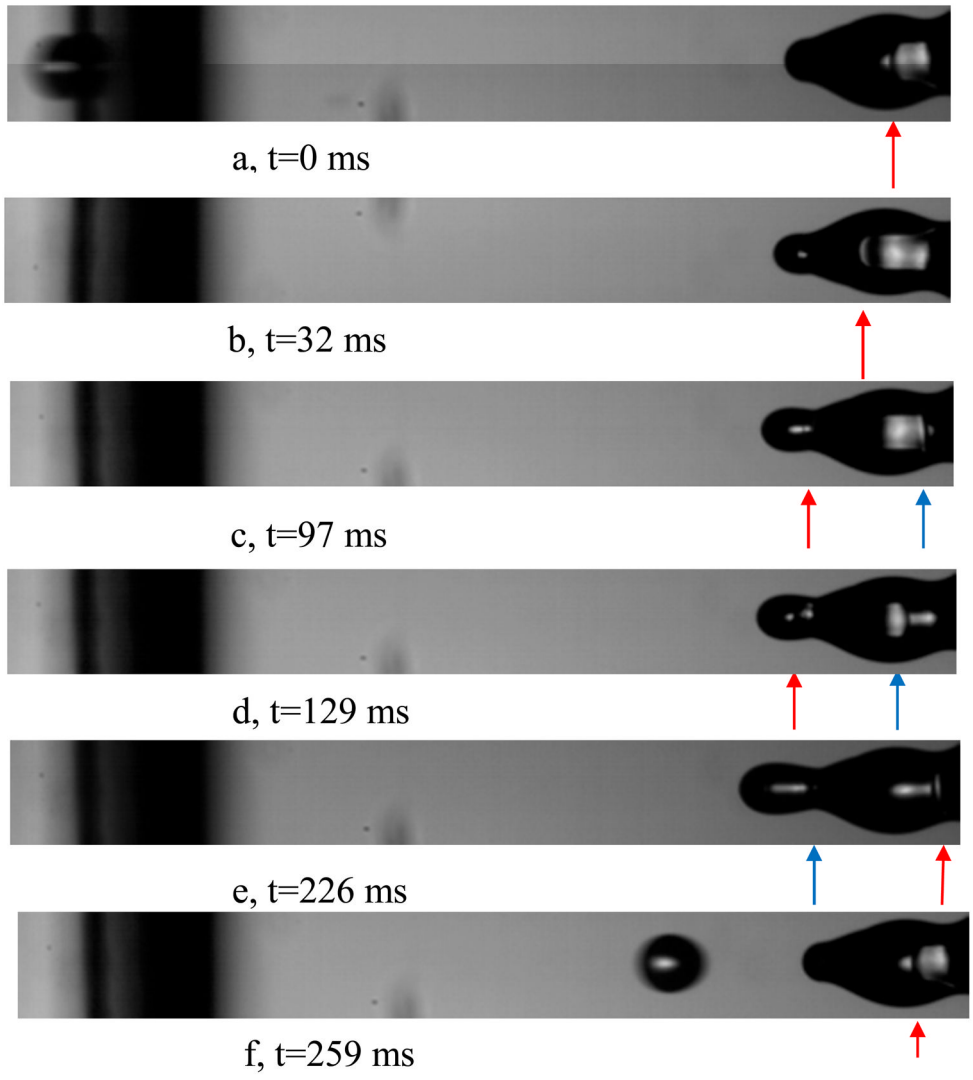

Figure 2.
A series of images showing the generation of double emulsions in air. The red and blue arrows point to the front and back ends of the aqueous droplets, respectively. Voltage applied, 1.01 kV; oil-phase flow rate, 0.06 μL/min; aqueous-phase flow rate, 0.06 μL/min.

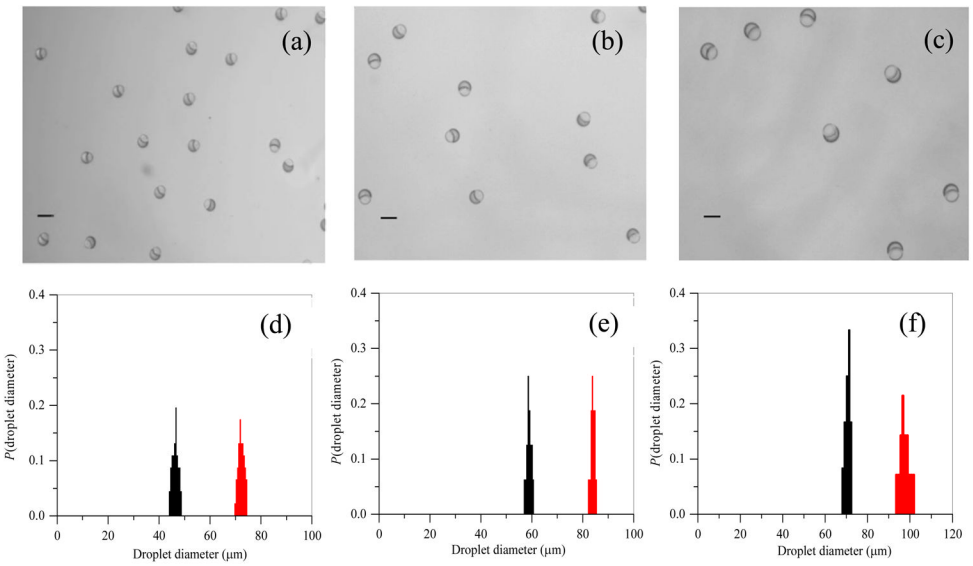

Figure 3.

(a)–(c) Micrographs of double emulsions. Scale bars are 100 μm. (d)–(f) Distributions of the diameters of the inner and outer droplets of the double emulsions. (a) and (d), aqueous flow rate, 0.2 μL/min; oil flow rate, 0.2 μL/min; voltage applied, 1.10 kV; (b) and (e), aqueous flow rate, 0.12 μL/min; oil flow rate,0.12 μL/min; voltage applied, 1.01 kV; (c) and (f), aqueous flow rate, 0.06 μL/min; oil flow rate, 0.06 μL/min; voltage applied, 1.01 kV.

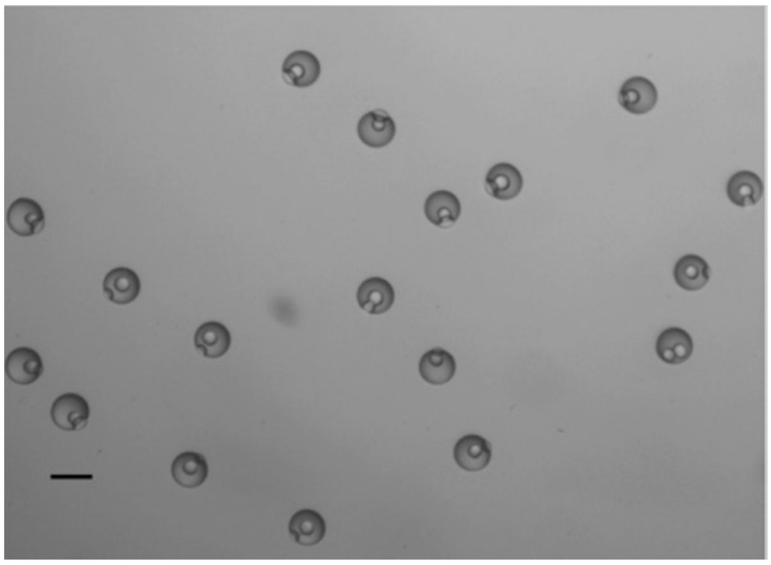

Figure 4.
Micrograph of double emulsions encapsulating two small droplets. Aqueous flow rate, 0.1 μL/min; oil flow rate, 0.8 μL/min; voltage applied, 1.12 kV; The scale bar represents 100 μm.