Interfacial tension controlled fusion of individual femtolitre droplets and triggering of confined chemical reactions on demand

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Electronic Supplementary Information

1. Experiments

A. Chemicals

Soybean oil (Sigma Aldrich) was purified by gravity filtration through a column packed with a 1:1 mixture of fluorisil and silica gel (100-200 mesh, Sigma-Aldrich) to remove surface-active monoglycerides. Silver nitrate, NaCl and fluorescein were purchased from Sigma Aldrich. 50nm diameter fluorescent polymer beads (FS02F/9290) were obtained from Bangs Laboratories, Inc. (Fishers, IN). 8.3 mM pH 7.2 phosphate buffer (P3288, Sigma) was prepared with deionized water purified by Millipore.

B. Fabrication of PDMS devices

The Si master for PDMS molding was fabricated by a micro/nanofabrication process. Aqueous microchannels were defined on the master by electron beam lithography. 300 nm thick ZEP-520A e-beam resist (Zeon Corp.; Tokyo, Japan) was spin coated onto a 100 mm diameter Si wafer at 6000 rpm for 45 seconds, followed by soft baking for 2 minutes at 180° C. A JEOL 9300-FS Electron Beam Lithography System was used to pattern the resist-coated Si wafer at 500 μC/cm2. The exposed resist was developed in xylenes for 30 seconds and rinsed with isopropanol (IPA). After cleaning the surface with oxygen plasma (Technics RIE, 100sccm O_2, 150 mTorr, 100W for 6 seconds), a 15 nm thick Cr layer was deposited at a deposition rate of 1 Å/sec by a dual-gun electron beam evaporator. The Cr etch mask was formed

by lift-off in acetone with 8 minutes sonication. An Oxford Plasmalab 100 inductively-coupled plasma reactive ion etching system was used to etch the aqueous microchannels to 1 μm depth at an etching rate of 200 nm/min (Oxford Instruments, Concord, MA). The center oil channel was created by photolithography using SU8 2015 negative-tone photoresist (Microchem Corp., Newton, MA). SU8 2015 was spin-coated at 2000 rpm, followed by soft-baking for 6 minutes at 90° C. A contact aligner (Neutronix-Quintel, Morgan Hill, CA) was used to align the photomask, and expose the SU8-coated wafer to UV light for 18 seconds at ~13 mW/cm^2. A post exposure bake was performed at 90° C for 6 minutes, and the wafer was developed in SU8 developer (Microchem Corp., Newton, MA), rinsed with IPA and dried gently with nitrogen. The height of the center SU8 channel was 18 μm, measured with a Dektak profilometer (Veeco, Malvern, PA).

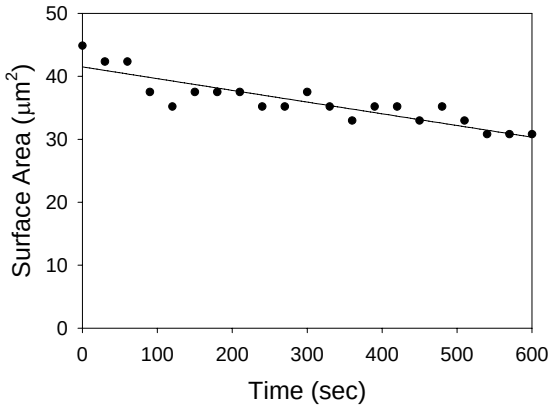
PDMS microfluidic devices were produced using a protocol modified from Schmid et al. (H. Schmid and B. Michel, Macromolecules 2000, 33, 3042-3049). To increase the mechanical stability of the device, two-layer PDMS devices were fabricated, consisting of a spin-coated thin layer of hard PDMS (h-PDMS) which was supported by a thick slab of soft PDMS (s-PDMS, Sylgard 184, Dow Corning, Midland, MI). Vinylmethylsiloxane-dimethylsiloxane copolymer (3.4 g, VDT-731, Gelest, Morrisville, PA) was mixed with 18 μL of the catalyst, platinum divinyltetramethyldisiloxane (Sigma-Aldrich), and one drop of 2,4,6,8- tetramethyl-tetravinylcyclotetrasiloxane (Sigma-Aldrich), and was degassed for 5 minutes. Methyl-hydrosilanedimethylsiloxane copolymer (1 g, HMS-301, Gelest, Morrisville, PA) was added and thoroughly mixed immediately before spin coating (1000 rpm, 45 sec) onto the Si master to make the thin layer of h-PDMS (30-40 μm). This h-PDMS layer was partially cured for 3 minutes at 85° C. s-PDMS pre-polymer with a 10:1 mass ratio of base to curing agent was poured onto the coated master to a depth of 4-5 mm, and the two-layer device was cured for 1 hour at 120° C. The now completely cured two-layer PDMS device was released from the mold and inlet holes were punched using a Uni-Core 0.75mm hole-puncher (Ted Pella, Inc. Redding, CA). PDMS was spin-coated (10 μm-thick, 6500 rpm) onto #1 cover glass slips separately, and bonded to the two-layer PDMS replica by brief plasma treatment (10.5 W for 20 seconds, Harrick, Ithaca, NY). In order to enhance adhesion and render all the channel surfaces hydrophobic, the completed chips were cured for an additional 48 hours at 120 °C. We found that the oil channel widths in the PDMS replicas (7-14 μm), determined from optical microscope images, were less than the

raised SU-8 features on the silicon master (12-25 μm) used for micromolding, perhaps due to the PDMS composition used, and/or the 48 hour hydrophobic recovery curing step at 120 °C.

C. Experimental protocol and image capture

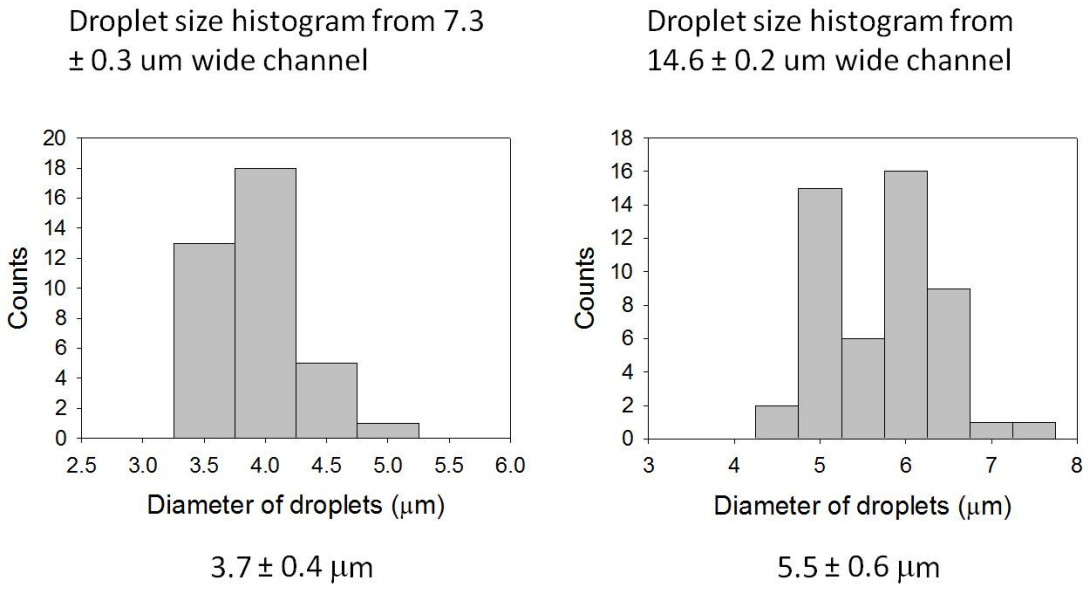
50 nm diameter fluorescent microspheres were diluted to 0.4 mg/mL concentration in phosphate buffer. 2M NaCl(aq) and 2M $AgNO_3$(aq) solution were prepared in deionized water. 100uM stock fluorescein solution was made in 20mM HCl solution (from 11.6 N HCl, VWR 3110-3). This stock solution was diluted 10 times to make 10 μM fluorescein solution. NaOH solution was prepared by diluting 10.0 N NaOH solution from VWR with deionized water. pH was measured with a S20 SevenEasy pH meter (Mettler Toledo, Columbus, OH). Three 4mL glass vials with PTFE/silicone septum lids (C4015-17W, National Scientific, Rockwood, TN) containing the aqueous and oil phases were connected to the appropriate inlets on the PDMS device through 24 gauge PTFE tubing (Small Parts, Miramar, FL) and 23 gauge stainless steel tubing (Technical Innovations, Brazoria, TX). Backing pressures for the aqueous and oil reservoirs were controlled by high precision closed-loop voltage-pressure transducers (Marsh Bellofram, Newell, WV) which forced fluids from the vials into the main flow channel. These transducers were controlled by a custom Matlab script (Mathworks, Natick, MA) through a USB-connected analog output board (16 bit resolution, 0-10 V range, USB3103, Measurement Computing, Norton, MA). The pressure response as a function of voltage was calibrated using a Dwyer Series 475 Mark III digital manometer (Michigan City, IN). An inverted optical microscope (Eclipse TE 300, Nikon Instruments, Melville, NY) equipped with a CCD camera (CoolSNAP-HQ, Roper Scientific, Tucson, AZ) and a high-speed CMOS camera (EPIX SV643, Buffalo Grove, IL) was used to acquire bright field or fluorescence images with a 60× oil-immersion objective (NA 1.4, 1 pixel = 0.108 μm). For the detection of fluorescence from fluorescein and fluorescence microspheres, a 200 W mercury-xenon arc lamp (Ushio, Japan) integrated with a computer-controlled Lambda SC smart shutter (Sutter Instrument Company, Novato, CA) was used as the light source in combination with appropriate filter sets (B-2E/C, Nikon Instruments). CCD camera and electronic shutter were controlled by Metamorph software (Universal Imaging Corp., Downing Town, PA) while the high speed CMOS camera was operated by the manufacturer-provided EPIX XCAP-Ltd v 3.7 software. Images were processed and analyzed with ImageJ software (National Institutes of Health) for subtraction of background and adjustment of contrast.

2. Droplet shrinkage rate in PDMS chip

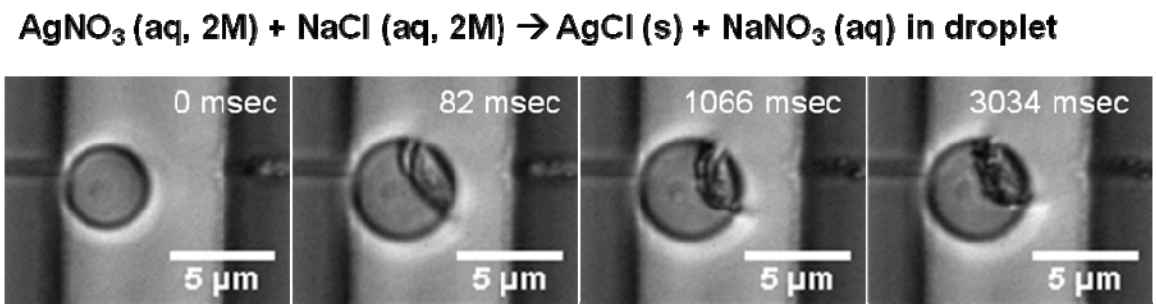


Droplet shrinkage rate for PDMS chip saturated in water overnight with purified soybean oil was 1μm^2/min.

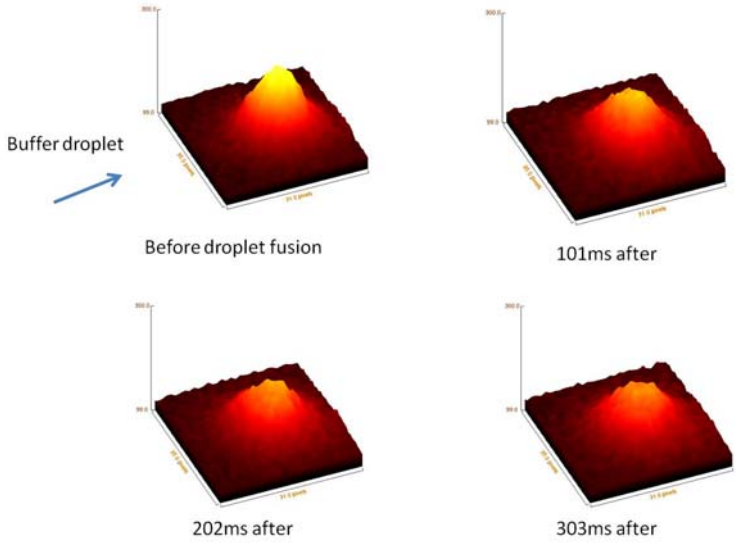
3. Droplet size distributions as functions of oil channel width.



4. Sequential CCD images of formation of Ag granules due to photoreduction from the tungsten lamp on the microscope.



5. Two dimensional fluorescence distributions rendered from the frame-by-frame sequence of CCD images from Figure 3 of the droplet containing the fluorescent microspheres, starting just before fusion.



6. Bright field movie sequences of acidic and basic droplet fusion events (Figure 4):

- 01_acid fluorescein initial_BF.avi
- 02_base added_BF.avi
- 03_acid added_BF.avi