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Double Emulsion Generation Using a Polydimethylsiloxane (PDMS) Co-axial Flow Focus Device

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Abstract

Double emulsions are useful in a number of biological and industrial applications in which it is important to have an aqueous carrier fluid. This paper presents a polydimethylsiloxane (PDMS) microfluidic device capable of generating water/oil/water double emulsions using a coaxial flow focusing geometry that can be fabricated entirely using soft lithography. Similar to emulsion devices using glass capillaries, double emulsions can be formed in channels with uniform wettability and with dimensions much smaller than the channel sizes. Three dimensional flow focusing geometry is achieved by casting a pair of PDMS slabs using two layer soft lithography, then mating the slabs together in a clamshell configuration. Complementary locking features molded into the PDMS slabs enable the accurate registration of features on each of the slab surfaces. Device testing demonstrates formation of double emulsions from 14 μm to 50 μm in diameter while using large channels that are robust against fouling and clogging.

Video Link

The video component of this article can be found at <http://www.jove.com/video/53516/>

Introduction

Double emulsions consist of droplets separated from a carrier phase by an intermediate, immiscible fluid layer, and are of particular interest due to their potential uses in industrial, pharmaceutical, and biological applications¹. In some cases, the ability to encapsulate high value compounds in a double emulsion's core enables material to be protected and released in a controlled manner. For example, drugs may be encapsulated under solubility conditions not appropriate for the external carrier fluid². Additionally, the intermediate oil layer can be used as a capsule template for the encapsulation and delivery of drugs, cosmetics, and nutrients³. In biology, double emulsions are also useful in high throughput screening because they allow a massive number of sub-nanoliter experiments to be carried out, then detected and sorted using a fluorescence-activated cell sorting (FACS) instrument^{4,5}.

The design of double emulsions with desired performance characteristics requires the precise control of double emulsion size, composition, and uniformity. Although bulk emulsification processes, such as membrane emulsification, are used in industry, the resulting emulsions are highly polydisperse, exhibiting a wide variety of functional properties¹. The field of droplet microfluidics is naturally suited the generation of monodisperse emulsions with carefully controlled composition⁶. Microfluidic double emulsion generation has been achieved with two main strategies, sequential drop making and glass capillary flow focusing. Double emulsions can be generated in planar PDMS devices using a two-step drop making process. First, aqueous-in-oil emulsions are created using a water-in-oil drop-making region of a device with hydrophobic channel walls. Next, the emulsion can be flowed or reinjected into a drop-making region with hydrophilic walls suited for oil in water drop-making⁴. However, hydrophilic surface treatment of PDMS requires an additional fabrication step and is often temporary⁷. The most controllable and repeatable method to form double emulsions is by co-axial flow focusing, a technique pioneered using glass capillary microfluidics, whereby a concentric jet containing the three phases is sheared through a small orifice to produce monodisperse droplets⁸. This technique allows for the production of droplets much smaller than the channel dimensions, with the precise size and composition of the double emulsion being a function of the flow rates of each phase. The large difference between droplet and channel size and the protective outer sheath flow prevents droplets from contacting the channel walls, rendering surface treatment unnecessary. However, such glass devices require custom fabrication of tapered capillary tips, along with careful assembly and sealing. Previous investigators have used 3D soft lithography to generate double emulsions using flow focusing physics, but these devices produced emulsions with diameters > 150 μm ^{9,10}, roughly an order of magnitude larger than objects typically sorted with FACS. An attractive alternative would include the robust functionality and small droplet generation of glass capillary coaxial flow focusing with the ease of manufacture of PDMS soft lithography.

In this paper, we describe a double emulsion generator that uses co-axial flow focusing to produce $\leq 50 \mu\text{m}$ emulsions and is constructed entirely using 3D soft lithography¹¹. Our device uses a clamshell approach to fabricate devices that includes a small shearing channel (**Figure 1**) to approximate the emulsion formation processes in a pulled glass capillary nozzle. More importantly, these devices require no specific surface treatment, and the all polymer construction provides easy and repeatable fabrication scalable to a large number of duplicate devices. Here, we outline the design, fabrication, and testing of the double emulsion generator. Double emulsion generation is shown to be robust and repeatable down to droplet diameters of $14 \mu\text{m}$. The coupling of functionality with ease of fabrication makes this device an appealing option for development of new double emulsion applications.

Protocol

1. SU8 Master Fabrication

1. Design the microfluidic structures for two layer fabrication using AutoCAD software and have the designs printed by a vendor on circuit board film with $10 \mu\text{m}$ resolution. The details of device design are given in an attached reference¹¹ and the channel geometries are shown in **Figure 1**. The layers should include alignment marks to help collocate features from each fabrication layer¹².
2. Place a pre-cleaned 3 inch diameter silicon wafer on a spin coater and turn on the vacuum to affix it to the chuck. Apply 1 ml of SU8-3035 in the center of the wafer and spin for 20 sec at 500 rpm, then 30 sec at 2,000 rpm, providing a layer thickness of $50 \mu\text{m}$.
3. Remove the wafer and bake on a $135 \text{ }^\circ\text{C}$ hotplate for 30 min. Allow the wafer to cool to RT before moving on to the next step.
4. Expose the coated wafer to the 1st layer mask (**Figure 2A**) under a collimated 190 mW, 365 nm LED for 90 sec. After exposure, place the wafer on a $135 \text{ }^\circ\text{C}$ hotplate for 1 min, then cool to RT before proceeding to the next step.
5. Place the wafer on the spin coater and turn on the vacuum to affix it to the chuck. Apply 1 ml of SU8-2050 in the center of the wafer and spin for 20 sec at 500 rpm, then 30 sec at 1,375 rpm, resulting in a layer that provides an additional thickness of $135 \mu\text{m}$.
6. Remove the wafer and bake on a $135 \text{ }^\circ\text{C}$ hotplate for 30 min, then cool to RT before moving to the next step.
7. Align the 2nd layer mask (**Figure 2B**) onto the geometry patterned in 1.3 and expose the coated wafer to a collimated 190 mW, 365 nm LED for 3 min. After exposure, place on a $135 \text{ }^\circ\text{C}$ hotplate for 1 min, then cool to RT before proceeding to the next step.
8. Develop the masks by immersing in a stirred bath of propylene glycol monomethyl ether acetate for 30 min. Wash the wafer in isopropanol and bake on a $135 \text{ }^\circ\text{C}$ hotplate for 1 min. Place the developed master in a 100 mm Petri dish for PDMS molding.

2. PDMS Device Fabrication

1. Prepare 10:1 PDMS by combining 50 g of silicone base with 5 g of curing agent in a plastic cup. Mix the contents with a rotary tool fitted with a stir stick. Degas the mixture inside a desiccator for 30 min, or until all air bubbles are removed.
2. Pour the PDMS to give a thickness of 3 mm over the master and place back into the desiccator for further degassing. Once all bubbles are removed, bake the device at $60 \text{ }^\circ\text{C}$ for 2 hr.
3. Cut the device from the mold using a scalpel and place on a clean surface with the patterned side up. Cut the PDMS mold in half with a razor blade to separate Master 1 from Master 2 (**Figure 3a**). On the piece containing the $50 \mu\text{m}$ fluid handling geometry imprinted by Master 1, punch the fluidic inlets and outlets with a 0.75 mm biopsy punch.
4. Plasma treat the devices at 1 mbar O_2 plasma for 60 sec in a 300 W plasma cleaner. Wet the surface of the unpunched piece of PDMS with a drop of DI water to temporarily retard PdMS-PDMS bonding and serve as a lubricant. While viewing through a stereo microscope, place Master 1 on Master 2 surface and slide the surfaces relatively until a mechanical lock is achieved when the recessed frames and protruding frames in **Figure 3A** mate.
5. Place the device in a $60 \text{ }^\circ\text{C}$ oven and bake the assembled device (**Figure 3B**) for two days at $60 \text{ }^\circ\text{C}$ to evaporate the water and complete bonding.

3. Preparation of Reagents

1. Fill 1 ml syringe with distilled water for the inner phase.
2. Fill 1 ml syringe with HFE 7500 fluorinated oil with 1 wt. % biocompatible surfactant¹³ for the middle phase.
3. Fill 10 ml syringe with 10 wt. % polyethylene glycol (PEG) in water solution containing 1 wt. % Tween 20 and 1 wt. % sodium dodecyl sulfate for the continuous phase.

4. System Preparation

1. Place the microfluidic chip on the stage of an inverted microscope coupled with a digital camera capable of $< 100 \mu\text{sec}$ shutter speeds.
2. Mount all syringes on syringe pumps and attach 27 G needles. Attach $\sim 30 \text{ cm}$ lengths of PE-2 tubing on the needles and insert the loose ends into the appropriate punched holes in the device.
3. Insert a 10 cm length of PE-2 into the exit port of the device and place the other end in a waste collection container.
4. Prime the device by running the syringe pumps at high rates of speeds ($2,000 \mu\text{l}/\text{min}$) until fluid in the tubing segments reaches the inlet ports of the device.

5. Emulsion Generation

1. Focus the microscope on a region that contains the $50 \mu\text{m} \times 50 \mu\text{m}$ orifice and the downstream exit channel.
2. Set the syringe pumps to deliver fluid to the double emulsion generator at flow rates of $250 \mu\text{l}/\text{hr}$ for the inner phase, $100 \mu\text{l}/\text{hr}$ for the middle phase, and $700 \mu\text{l}/\text{hr}$ for the continuous phase and wait 10 min for equilibration.

- Maintain the flow rates of the inner and middle phases at 250 $\mu\text{l/hr}$ and 100 $\mu\text{l/hr}$, respectively. Set the flow rate of the outer phase at 1,050 $\mu\text{l/hr}$. Wait 3-5 min for the double emulsions generation to stabilize under this set of flow conditions.
- Acquire 5 sec of video images at 30 Hz for offline processing via manual image analysis.
- Repeat 5.3 and 5.4 with the flow rates given in **Table 1**. The inner and middle phase flow rates are held constant and the carrier phase flow rate is varied by adjusting the setting of the syringe pump.

Representative Results

The double emulsion generator consists of a co-axial flow focusing device created using 3D PDMS fabrication (**Figure 1A**). The geometry enables that formation of a three-phase co-axial jet to be sheared into a square, 50 $\mu\text{m} \times 50 \mu\text{m}$ orifice, allowing the formation of water / oil / water double emulsions (**Figure 1B**, **Figure 1C**). The inner aqueous phase and the middle oil phase are brought together at a junction with channel dimensions of 10 $\mu\text{m} \times 50 \mu\text{m}$ (**Figure 1D**, point "1"). Due to the hydrophobicity of PDMS, the fluorinated oil hugs the channel walls and the inner phase stays in the center of the channel as the fluids travel in a continuous jet, until a sudden expansion channel expansion is reached (**Figure 1D**, point "2"). At this location, the inner two phases are injected into the center of a 320 μm tall junction that allows the relatively concentric introduction of the aqueous carrier phase. The three phases are forced into a 50 $\mu\text{m} \times 50 \mu\text{m}$ orifice (**Figure 1D**, point "3"), whereby the high flow rate of the carrier phase shears the inner two phases into a long, thin tendril that decomposes to form uniform droplets (**Figure 1E**).

The 3D PDMS fabrication requires the coupling of two unique PDMS molds in a clamshell configuration after molding on two layer lithographic masters. A 50 μm tall layer is used to form the inner and middle fluid handling channels, along with the shearing orifice on Master 1 (**Figure 2A**), along with a complimentary protruding and recessed frames on opposing master. An additional 135 μm tall layer is used to create the carrier fluid and the exit channels (**Figure 2B**). Assembly of the double emulsion generator utilizes the recessed and protruding frames (**Figure 3A**) for geometric alignment after plasma treatment (**Figure 3B**).

The double emulsion device was tested at a variety of flow conditions to demonstrate the formation of varied size, monodisperse double emulsions. For these experiments, the inner and middle phase flow rates were held constant and the carrier phase flow rate was modified to affect the shearing force during droplet generation. Experimental conditions are parameterized by the ratio of the carrier phase flow (Q_c) to the sum of the inner two phases flows (Q_{sum}). Images of droplet generation for experiments performed at Q_c/Q_{sum} from 3 to 57 are shown in **Figure 4**. An elongated region containing the inner two phases is observed to protrude into the 50 $\mu\text{m} \times 50 \mu\text{m}$ orifice and breaks into droplets that are convected downstream. Increasing the flow of the carrier phase (increasing Q_c/Q_{sum}) leads to the inner phases being sheared into progressively thinner regions that produce smaller droplets. Double emulsions produced by the device at different flow rates show an average diameter coefficient of variation of 5.2%. Histograms of droplet diameters for select values of Q_c/Q_{sum} also show the relative uniformity in the size of the droplets generated (**Figure 5**). The device demonstrates an ability to form double emulsions significantly smaller the orifice width, and shows a clear decreasing trend with increased Q_c/Q_{sum} (**Figure 6**). At highest carrier phase flow tested, 14 μm double emulsions were formed using the 50 $\mu\text{m} \times 50 \mu\text{m}$ orifice.

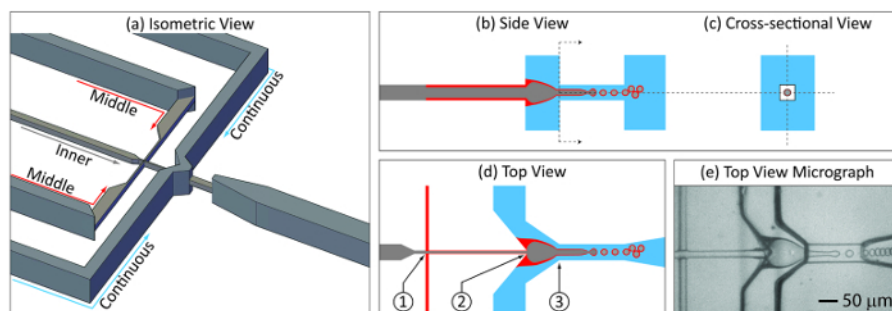
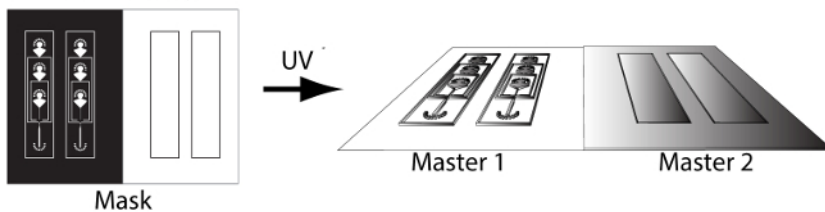


Figure 1. Geometry of the double emulsion generator. (A) 3D model of the fabricated device. (B) Vertical cross section of the central channel showing introduction of the inner (gray), middle (red), and carrier (blue) phases. (C) Cross-section showing the jet containing the inner two phases entering the square orifice. (D) Top view of emulsion generation in the device. At junction (1) the injection of the hydrophobic middle phase is aided by the hydrophilic PDMS, which causes it to coat the channel walls. At junction (2) the channel expands and a jet of the inner two phases is sheared into the orifice (3) by the higher rate of flow of the continuous fluid to a point where the physics cause droplet formation. (E) A microscope image of double emulsion generation in the device. [Please click here to view a larger version of this figure.](#)

a) 50 μm SU-8 layer



b) 135 μm SU-8 layer

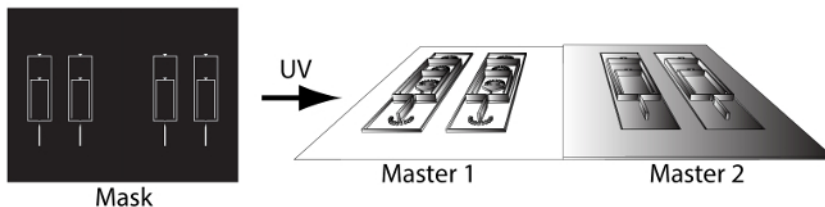
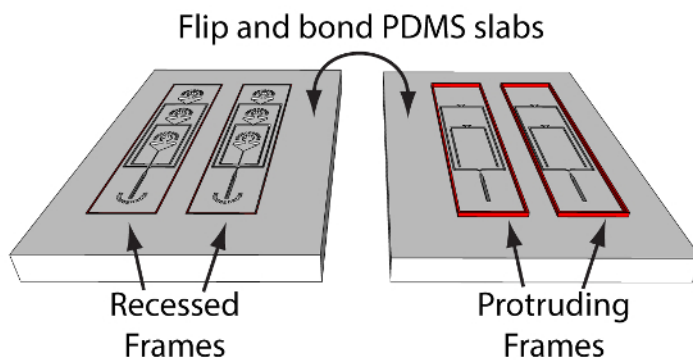


Figure 2. Lithographic production of the masters. (A) The mask used for preparation of 50 μm features. Master 1 is used to mold the fluidic inlets, the inner / middle phase junction, the emulsion generation orifice, and a recessed trough for alignment. Master 2 contains a raised ridge used for alignment. (B) The mask used for preparation of 135 μm features. The masters are mirror images that contain the carrier fluid routing channels and the exit channel. [Please click here to view a larger version of this figure.](#)

a) PDMS molding and bonding



b) Final PDMS device

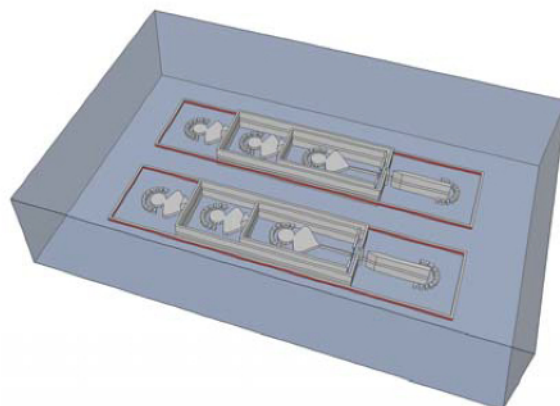


Figure 3. Assembly of the PDMS device. (A) Fabricated PDMS slabs contain complimentary recessed and protruding frames. (B) Assembled, the frames interlock to provide optimum alignment of features. [Please click here to view a larger version of this figure.](#)

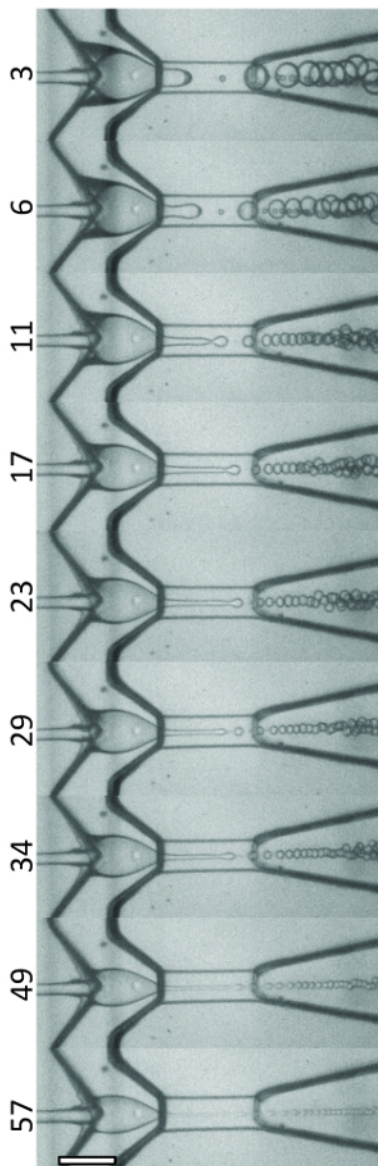


Figure 4. Images of generated double emulsions at different flow rates. The flow rate of the outer phase is changed to alter Q_c/Q_{sum} , which is given to the left of each image. Increasing Q_c/Q_{sum} narrows the jet of the inner fluids being sheared through the orifice, creating increasingly small droplets. [Please click here to view a larger version of this figure.](#)

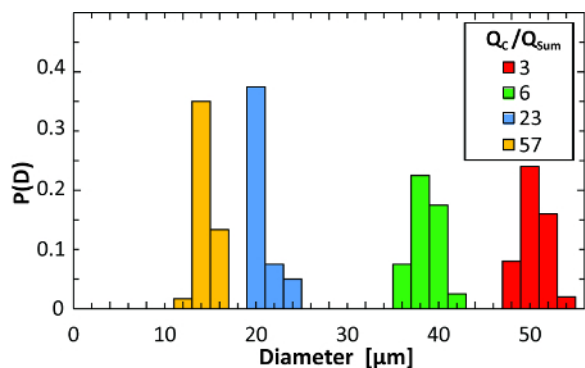


Figure 5. Histograms of double emulsion droplets sizes at different flow rates. The average coefficient of variation of the diameter of emulsion droplets produced at a given set of flow conditions is 5.2%. [Please click here to view a larger version of this figure.](#)

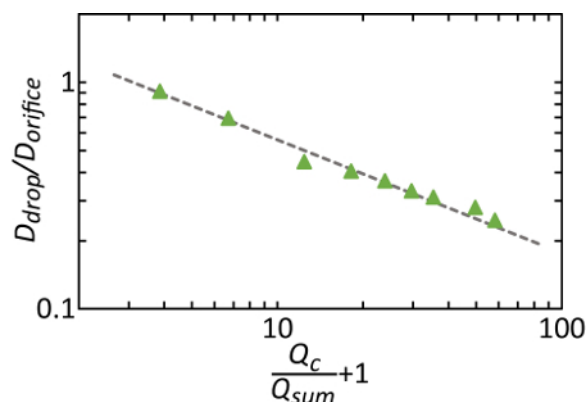


Figure 6. Droplet diameter versus normalized flow rate parameter. Adjusting the flow rate of the continuous phase allows production of double emulsions that are 30%-100% of the orifice diameter. [Please click here to view a larger version of this figure.](#)

Q_i [$\mu\text{l/hr}$]	Q_m [$\mu\text{l/hr}$]	Q_{sum} [$\mu\text{l/hr}$]	Q_c [$\mu\text{l/hr}$]	Q_c / Q_{sum}
100	250	350	1050	3
100	250	350	2100	6
100	250	350	3850	11
100	250	350	5950	17
100	250	350	8050	23
100	250	350	10150	29
100	250	350	11900	34
100	250	350	17150	49
100	250	350	19950	57

Table 1. Flow rate parameters used for the experiments. The inner phase and middle phase flow rates (Q_i , Q_m) are held constant, giving a constant combined flow rate (Q_{sum}). The carrier phase flow rate (Q_c) is varied to produce double emulsions with different diameters. The ratio Q_c / Q_{sum} is the main nondimensional parameter describing experimental conditions.

Discussion

The double emulsion generating geometry described here is designed to mimic the physics of glass capillary devices⁸. In these, aligned cylindrical glass capillaries are used to create a three phase coaxial jet that is sheared into uniform double emulsion droplets. The function of our 3D PDMS device is dependent on the central alignment of small features formed with 50 μm tall fabrication with carrier phase channels that are 320 μm in total height. There is a significant potential for misalignment of the taller features patterned by the 2nd layer mask in step 1.7 with respect to the 50 μm tall geometry if masks are not accurately aligned. Proper alignment can be aided by designing alignment marks, such as concentric circles into masks to be co-located during photo patterning. The plasma bonding of the two PDMS halves of the device is a second process that can lead to significant misalignments of the final device. Plasma bonding of PDMS to PDMS is generally instantaneous, so in step 2.4 we describe the wetting of a device surface with DI water to retard bonding and allow manipulation so that the alignment frames shown in **Figure 3A** can be allowed to lock. If this is attempted without sufficient wetting, the PDMS surfaces will irreversibly bond before coming into proper alignment, and device must be disposed of and new PDMS molds made.

The double emulsion device is designed to take advantage of the fabrication techniques that lead to uniformly hydrophobic surface properties. However, operation outside of the parameters described in the protocol requires some understanding of the required fluidic processes. At the junction of the inner and middle phases (**Figure 1D**, point "1"), a relatively high flow of the inner phase and low flow of the middle phase create a two phase jet, with the hydrophobic middle phase coating the channel walls. If the proportionate flow of the middle phase is increased, the generation of discrete water-in-oil droplets will begin to occur, eliminating the ability to form a coherent three-phase jet for drop formation in the orifice (**Figure 1D**, point "3"). After the channel expansion (**Figure 1D**, point "2"), a significant amount of carrier phase flow is required to create geometric separation between the middle phase and the hydrophobic channel walls. Reductions in carrier phase flow will eventually lead to the middle phase wetting the hydrophobic device walls. Significant reductions in the carrier phase flow may create flow conditions that are insufficient to shear the inner phases into a long, thin filament, thereby radically altering the physics of double emulsion droplet formation.

Once built, this device is designed to produce double emulsions from 14 to 50 μm , a convenient size for sorting using commercial FACS instruments. If double emulsions outside this size range are desired, the orifice dimensions need to be scaled from the 50 μm x 50 μm size used here. Because the device is designed to produce water / oil / water double emulsions with uniformly hydrophobic surface properties, oil / water / oil double emulsions cannot be created unless there were a surface treatment applied to make the device uniformly hydrophilic.

This work demonstrates an easy-to-fabricate PDMS device capable of the robust formation of water / oil / water double emulsions. Although previous investigators have reported the formation of double emulsions in devices created using 3D lithography^{14,15}, the double emulsions

formed in their devices had diameters that were measured in 100s of μm . The device reported here is suited to producing double emulsions an order of magnitude smaller than this, providing volumes similar to mammalian cells and well suited to sorting by FACS.

Although these results may also be attained using glass capillary microfluidics, fabricating glass devices is laborious and requires many hands-on steps per device. For our all PDMS device, fabrication largely consists of molding, bonding, and baking PDMS slabs, processes that are simple, repeatable, and easy to scale to large numbers.

The utility of a lithographically fabricated device to generate double emulsions using co-axial flow focusing has been demonstrated. We hope that the straightforward fabrication and robust functionality of this double emulsion generator design should lead to its adaptation for scientific and industrial applications. Investigators previously deterred by the specialized skills required to work in glass capillary microfluidics, should be more comfortable using PDMS soft lithography, now a common laboratory technique. Furthermore, the small droplet sizes that can be produced is well suited to perform cell and biological assays in droplets, and quantification and sorting using FACS. For industrial applications, it has already been shown that these types of devices can be fabricated into arrays and parallelized¹⁰, enabling double emulsion generation rates to increase by orders of magnitudes as compared to single devices. In addition, the ability to form small double emulsions in large coaxial flow focusing channels should make the device resistant to fouling and clogging, which is critical when parallelizing the devices intended to run for long durations without intervention.

Disclosures

The authors have nothing to disclose.

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