Video Article

Generation of Size-controlled Poly (ethylene Glycol) Diacrylate Droplets *via* Semi-3-Dimensional Flow Focusing Microfluidic Devices

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Abstract

Uniform and size-controllable poly (ethylene glycol) diacrylate (PEGDA) droplets could be produced via the flow focusing process in a microfluidic device. This paper proposes a semi-three-dimensional (semi-3D) flow-focusing microfluidic chip for droplet formation. The polydimethylsiloxane (PDMS) chip was fabricated using the multi-layer soft lithography method. Hexadecane containing surfactant was used as the continuous phase, and PEGDA with the ultraviolet (UV) photo-initiator was the dispersed phase. Surfactants allowed the local surface tension to drop and formed a more cusped tip which promoted breaking into tiny micro-droplets. As the pressure of dispersed phase was constant, the size of droplets became smaller with increasing continuous phase pressure before dispersed phase flow was broken off. As a result, droplets with size variation from 1 μm to 80 μm in diameter could be selectively achieved by changing the pressure ratio in two inlet channels, and the average coefficient of variation was estimated to be below 7%. Furthermore, droplets could turn into micro-beads by UV exposure for photo-polymerization. Conjugating biomolecules on such micro-beads surface have many potential applications in the fields of biology and chemistry.

Video Link

The video component of this article can be found at https://www.jove.com/video/57198/

Introduction

Droplet-based microfluidic systems have the ability to produce highly monodisperse droplets from nanometer to micrometer diameter range[1] and hold great potential in the high-throughput drug discovery[2], synthesis of biomolecules[3,4], and the diagnostic testing[5]. Due to the unique advantages of smaller droplets, such as the greater surface area to volume ratio and the large-scale applications with consuming a few microliters of sample, the technology has attracted extensive interest in a broad range of fields. The emulsification of two immiscible liquids is one of the most typical methods to generate droplet. In previous reports in the field, researchers have developed a variety of different droplet formation geometries, including T-junction, flow-focusing and co-flowing geometries. In the T-junction geometry, the dispersed phase is delivered through a perpendicular channel into the main channel, in which the continuous phase flows[6,7]. In the typical two-dimensional (2D) flow-focusing[8,9] geometry, the dispersed phase flow is sheared from the lateral; and for the co-flowing geometry[10,11], on the other hand, a capillary introducing the dispersed phase flow is placed co-axially inside a bigger capillary for co-flowing geometry, so that the dispersed phase flow is sheared from all directions.

The droplet size is controlled by adjusting channel size and flow rate ratio, and the minimum size produced by co-flowing or T-junction is limited to dozens of micrometers. For flow-focusing droplet formation system, three modes of droplet breakup form by adjusting the pressure ratio of two-phase and surfactant concentration, including the dripping regime, the jetting regime, and tip-streaming[15]. Tip-streaming mode is also called thread formation, and the appearance of a thin the thread drawing out from the tip of dispersed phase flow cone will be observed.Previous studies have demonstrated droplets less than a few micrometers could be generated though tip-streaming process in 2D or semi-3D flow-focusing device[8,12]. However, as an aqueous solution containing a very low concentration of PEGDA was used as the dispersed phase, the shrinkage ratio of PEGDA particles was about 60% of the original droplets in diameter after photo-polymerization, while PEGDA without dilution as the dispersed phase led to unstable tip-streaming mode[12]. Interfacial tension is an important parameter of emulsion process and it will decrease due to the addition of the surfactant into the continuous phase liquid,leading to decrease in droplet size, higher generation frequency[13], highly curved tip, and preventing instability[14]. Furthermore, when the bulk surfactant concentration is much higher than the critical micelle concentration, the interfacial tension is approximately invariable in the saturated state[13] and the tip-streaming mode can occur[15].

Based on the above observations, in this paper, we developed a facile approach for PEGDA droplets generation using a semi-3D flow-focusing microfluidic device, fabricated by multi-layer soft lithography method. Different from the typical 2D flow-focusing device, the semi-3D flow-focusing device has a shallow dispersed phase channel and a deep continuous phase channel, so that the dispersed phase can be sheared from up and down beside lateral. This provides larger adjusting range for flow-focusing mode by reducing the energy and pressure required for droplet breakup. Different from the previous report[12], the dispersed phase is pure PEGDAcontaining photo-initiator, making sure that the shrinkage ratio

of PEGDA particles is lower than 10%[16]; and the continuous phase is the mixture of hexadecane dissolving with a high bulk concentration of the silicone-based nonionic surfactant. Size-controllable and uniform droplets were produced by adjusting the pressure ratio of two phases. The diameter of the droplets changes from 80 μm to 1 μm as the droplet breakup processes changes from the jetting mode to tip-streaming mode. In addition, the PEGDA particle was synthesized through photo-polymerization process under UV exposure. The droplet generation microfluidic system with ease of fabrication will provide more possibilities for biological applications.

Protocol

1. Mold Fabrication

1. Design two photomasks using a drawing software. Describe the outline of microchannel structure and use two separate layers for mask 1 and 2 in the same drawing file, so ensure all connections between different channels. Print different layers independently to chrome plate on the glass by a vendor with 1 μm resolution. Ensure that the photomasks are dark with transparent designed structures, as a negative polarity.
   Note: The mask 1 contains the dispersed phase inlet channel and an orifice. The mask 2 contains the continuous phase inlet channel, the filter, and the outlet.
2. In designated photolithography lab, clean a 3-inch diameter silicon wafer. Place the wafer on a spin coater, turn on the vacuum to affix the wafer to the spin chuck. Spin-coat 2-3 mL of SU-8 2025 negative photoresist onto the wafer for 10 s at 1,000 rpm, then 30 s at 3,000 rpm, providing the first layer thickness of 20 μm.
3. Soft bake on a 95 °C hotplate for 6 min. After the coated wafer cools to room temperature (RT), expose it via the mask 1 under a collimated 15 mW/cm^2, 365 nm of UV for 18 s. Post-exposure, bake on a 95 °C hotplate for 6 min, then allow the wafer to cool to RT.
4. Repeat the spin-coating process. Apply 2-3 mL of SU-8 2100 negative photoresist onto the wafer for 10 s at 1,000 rpm, then 30 s at 2,000 rpm, providing the second layer thickness of 130 μm. After soft bake on a 95 °C hotplate for 35 min, place the mask 2 on the second layer photoresist to expose for 30 s, which was aligned with the dispersed phase channel layer by a UV aligner. Post-exposure, bake on a 95 °C hotplate for 7 min.
5. Develop the wafer by immersing in a stirred bath of 50 mL of propylene glycol methyl ether acetate until features become clear on the wafer, then wash it with ethyl alcohol. Finally, place the wafer on the thermostatic platform for hard-baking for 2 h.

2. Semi-3D Flow-focusing Microfluidic Chip Fabrication

1. Mix PDMS monomer and its curing agent in a slightly different weight ratio for the top and the bottom layers, typically 10:1 for the top layer, and 8:1 for the bottom layer, using automatic ointment agitator for 4 min.
   Note: The top and bottom PDMS slabs are prepared in a slightly different ratio (10:1 and 8:1 respectively) of PDMS base to curing agent, enhancing the bonding strength. When 5:1 ratio is chosen for the bottom layer, the stiffer bottom PDMS slab makes alignment difficult and reduces bonding strength due to lack of flexibility. In addition, the thickness of the bottom PDMS slab is about 1 mm for adapting the working distance of microscope. The chip easily deforms under high pressure when 15:1 ratio is chosen.
2. Pour the mixture into the completed silicon mold in a 90-mm petri dish and provide a thickness of 2~3 mm. Place it in a vacuum chamber and degas until all air bubbles disappear. Cure at 80 °C for 1 h in an oven.
3. Allow the PDMS to cool to RT. Use a scalpel to cut the device at least 3 mm away from the features and slowly peel off the PDMS layer from the silicon wafer. Punch the dispersed phase inlet, the continuous phase inlet and the outlet in the top PDMS layer using a 0.75 mm diameter punch.
4. Clean the PDMS with adhesive tape to remove dust particles. Plasma treat both the top and bottom PDMS layers simultaneously for 2 min in a 300 W plasma cleaner. Place the top layer on the bottom layer surface and slide the surfaces relatively until features are aligned viewing through a stereo microscope.
5. Cure the device in an oven at 120 °C for one day to enhance the strength and complete bonding.

3. Reagents Preparation

1. Prepare the solution ofthe continuous phase: Hexadecane by dissolving 18 vol% silicone-based nonionic surfactant.
2. Prepare the solution of the dispersed phase: hydrophilic poly (ethylene glycol) diacrylate (PEGDA, MW 255,) containing 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (98%, MW 224) at a concentration of 5 mg/mL as the photo-initiator, and rhodamine B (95%, MW 479.01) at a concentration of 1 mmol/L as the fluorescent dyes.
3. Fill the 1 mL reservoirs of pneumatic pressure controller with the continuous phase. Fill the 200 μL gel loading tip with the dispersed phase.

4. System Preparation

1. Place the semi-3D microfluidic device on the stage of an inverted optical microscope, viewing with a high-speed camera.
2. Connect the fluorinated ethylene propylene (FEP) tube to the punched hole of the continuous phase by attaching a short stainless-steel tube, then insert the end of a gel loading tip into the punched hole of the dispersed phase. Insert a 20-cm length of FEP tube into the outlet in the device and place the end in a 15-mL centrifuge tube.
   Note: The configuration of the system is illustrated in **Figure 1**.

5. Droplets Formation

1. Place the device on the workbench of an inverted microscope, and ensure that the junction of different channels is roughly located in the position of the light source of the microscope. Focus the inverted microscope on a region that contains two-phases intersection, the orifice region, and the downstream channel.

2. Set pressures of two phases using pneumatic pressure controller to deliver fluid slowly to the intersecting region, with 15 mbar for the dispersed phase and 30 mbar for the continuous phase. Wait 3 min for stabilization and equilibration until stable fluid flow carrying no bubbles and PDMS residue.
3. Input the parameters on the user interface of the control software. Set the pressure of the dispersed phase as the base-level pressure of the system to a constant, for example, maintain it at 45 mbar. Increase the pressure of continuous phase until the emulsion breakup mode is changed from the jetting to the tip-streaming mode, then wait 5 min for stabilization.
4. Place the FEP tube end connecting the outlet hole to the centrifuge tube to collect the droplets.

6. PEGDA Particles Collection and Characterization

1. Fix the centrifuge tube aslant in the fixture. Obtain the PEGDA particles through the rapid solidification by UV exposure when liquid phase flow to the tube.
2. When finishing the collecting process, sample and observe the PEGDA particles through the fluorescent microscope with 20X or 60X objects respectively.
   Note. The digital fluorescent images captured by the camera are analyzed by a custom-made software routine. The image is first deconvolution based on the R-L algorithm[17] to eliminate the blurring effect of out-of-focusing light, and the sphere objects in the image are extracted based on the Canny edge detection method; finally, the diameter of each sphere object can be calculated using Hough transformation[18]. As a result, mean and standard deviation of the diameters of sphere objects in each image can be estimated.

Representative Results

The semi-3D flow-focusing microfluidic chip was fabricated using multi-layer soft lithography techniques as described above. The fabrication process and results for master mold in the protocolare shown in **Figure 2**. The first layer, which provides a 65 μm wide channel for introducing the dispersed phase and a 50 μm wide orifice (**Figure 2a**), is 20 μm in thickness. An addition 130 μm thickness layer is used to provide the continuous phase channel and the exit channel (**Figure 2b**). **Figure 2c** shows a finished mold. A filter in the inlet is designed to prevent debris of the holes punched in the PDMS from entering. This is done to overcome clogging in the orifice (**Figure 2d**).

After fabricating the master molds, the casting process and results in **the** protocol are shown in **Figure 3**. Top and bottom half-pieces with mirrored structures are prepared using PDMS. Using a 0.75 mm punch to drill the inlet and outlet holes into the top layer of PDMS. After oxygen plasma treatment simultaneously, the features of the top and the bottom PDMS slabs are aligned with less alignment error which does not significantly affect the device performance. The length of the whole semi-3D device is about 5 cm. We tried the 10 cm long chips adding the downstream channel. However, the larger the chip, the more difficult the alignment process due to increasing alignment region. In addition, the shorter chip (such as 2.5 cm long chip we used) also makes alignment process difficult due to lack of flexibility.

The semi-3D flow-focusing microfluidic device and the typical droplet formation process are illustrated in **Figure 4**. Because of the difference of depth of the dispersed phase channel and the continuous phase channel, the dispersed phase flow is expected to be squeezed from all the directions by the continuous phase flow. As a result, symmetric conical liquid tip forms to produce droplets continuously. The size of droplets is changed by the pressure ratio of dispersed and continuous phases flow. For our experiments, the pressure of dispersed phase (P_D) is maintained constant as the base-level pressure, and the pressure of continuous phase (P_C) is modified to affect the shearing force, so that the droplet breakup processes change from the jetting mode to the tip-streaming mode, as shown in **Figure 5**. The droplets are solidified by photo-polymerization forming particles. UV exposure polymerizes the monomer in the droplets. **Figure 6** shows fluorescent images of particles with different pressure ratio; and image analysis reveals the droplet size, which is plotted as a function of the pressure ratio in **Figure 7a**. By analogy with the electric circuit method[24], the equivalent fluidic circuit is shown in the following **figure 7b**. We roughly calculated the hydraulic resistance of three parts: the dispersed phase channel is 1.26 X $10^{14} Pa \cdot s \cdot m^{-3}$ (R3); the sum of the orifice and downstream channel is 6.08 X $10^{12} Pa \cdot s \cdot m^{-3}$ (R4+ R5); the continuous phase channel and the filter are 2.19 X $10^{12} Pa \cdot s \cdot m^{-3}$ (R2)and 1.10 X $10^{12} Pa \cdot s \cdot m^{-3}$ (R1). The relationships between all hydraulic resistances and flow rates are shown as following:

$$P_C - P_B = (R_1 + R_2) \cdot \frac{Q_C}{2}$$

$$P_D - P_B = R_3 \cdot Q_D$$

$$P_B - P_{out} = (R_4 + R_5) \cdot (Q_C + Q_D)$$

P_B is the pressure of intersection of two phases microchannel. When the pressure of dispersed phase (P_D) is maintained at 45 mbar, the pressure ratio is converted to corresponding flow rate ratio:

$Q_C = 0.8859 P_C - 1.62891$

$Q_D = 2.1302 - 0.0217 P_C$

The droplet size is plotted as a function of the flow rate ratio in **figure 7c**. The figure indicates that the increasing pressure ratio (P_C / P_D) leads to the dispersing phase flow being sheared into the spindlier tip and the decreasing droplet. The size range of the PEGDA particles varies from 1 μm to 80 μm with an average coefficient of variation (CV) below 7%. The smaller droplets were observed through the fluorescent microscope with 60X object, so there were only a dozen or so droplets in the view of the microscope. In addition, smaller droplets were approximately twenty or thirty pixels in radius. It was hard to characterize the coefficients of variance of smaller droplets, and the small base would lead to an inaccurate calculation, so the CVs of those smaller droplets were not indicated.

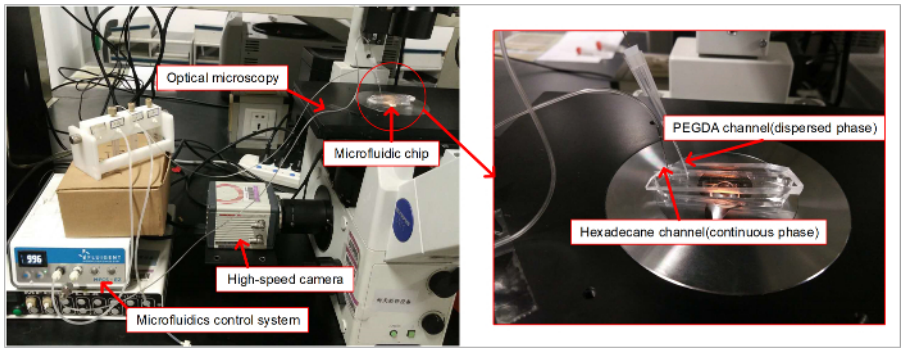

Figure 1: The configuration of experimental system Please click here to view a larger version of this figure.

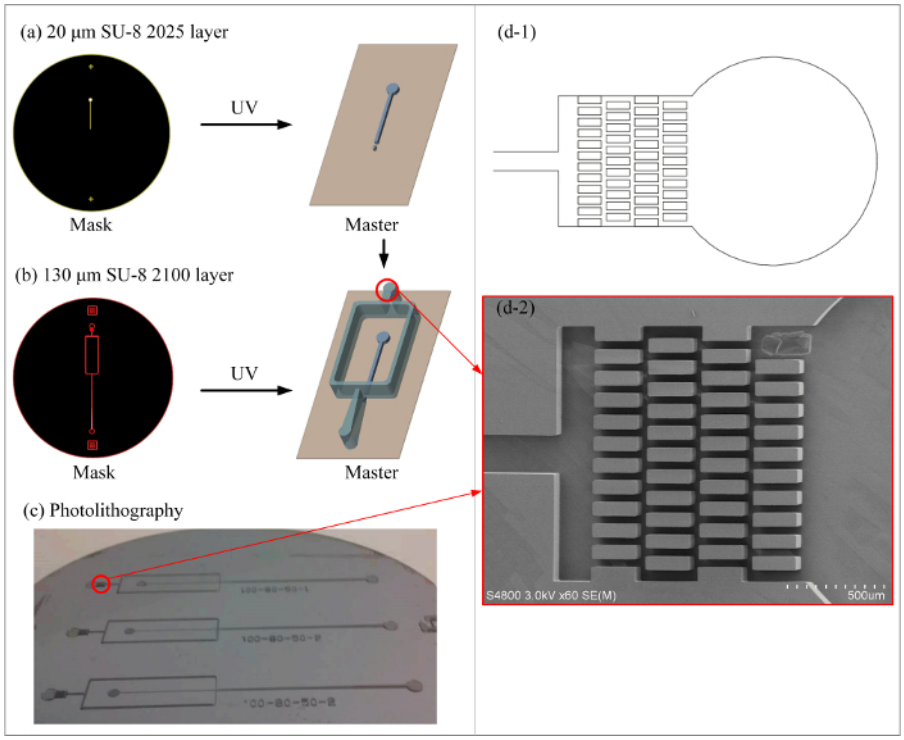

Figure 2: **Mater molds for multi-layer soft lithography. (a)** The mask 1 used for formation of 20 μm features. The master contains the dispersed phase channel and an orifice. **(b)** the mask 2 used for formation of 130 μm features. The master contains the continuous phase channel and the exit channel. **(c)** Monolithic master. **(d)** CAD drawing and SEM of the filter, located at the inlet to prevent clogging. Please click here to view a larger version of this figure.

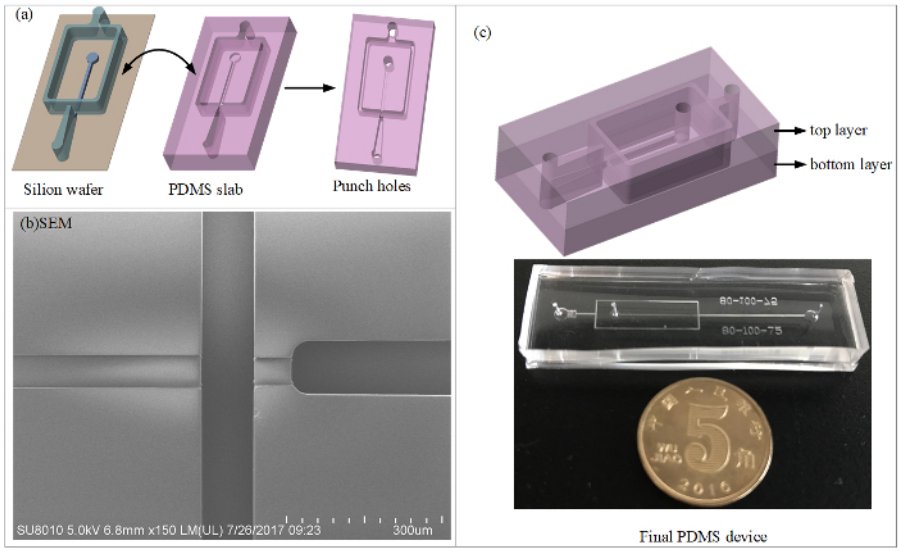

Figure 3: **Casting and bonding processes for the PDMS microfluidic chip. (a)** Schematic diagram of the assembly of the semi-3D PDMS device. **(b)** Structures of PDMS slab under SEM.**(c)** Monolithic microfluidic device. Please click here to view a larger version of this figure.

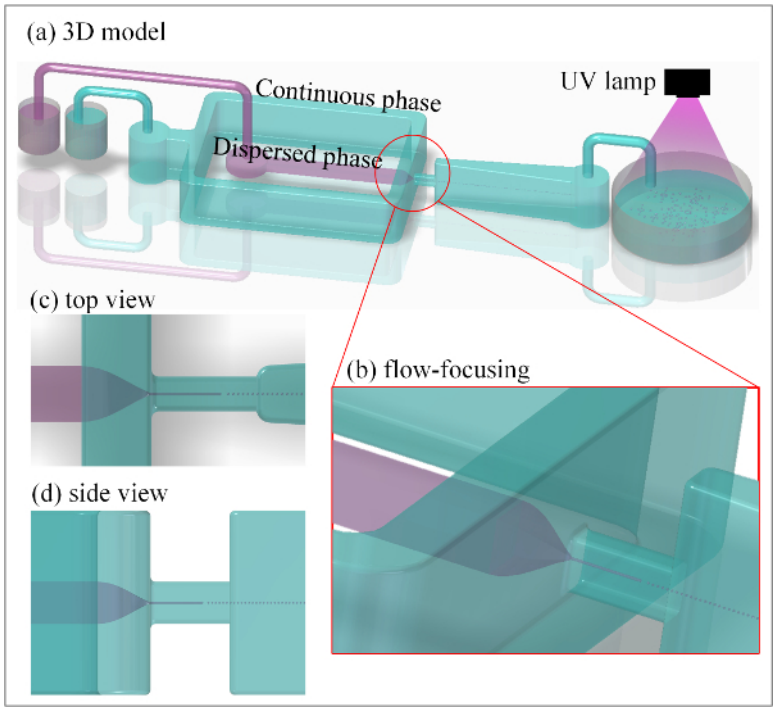

Figure 4: **An Illustrative working principle of the semi-3D flow-focusing microfluidic systems**. Please click here to view a larger version of this figure.

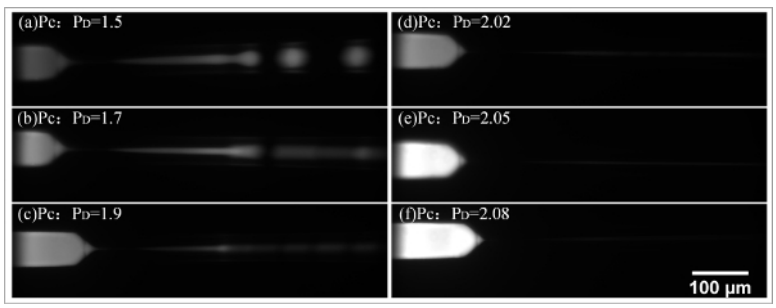

Figure 5: An Illustrative fluorescence mages of various droplet breakup processes. (a-d) the jetting mode and **(e-f)** the tip-streaming mode. P_C is the pressure of continuous phase, and P_D is the pressure of dispersed phase. Please click here to view a larger version of this figure.

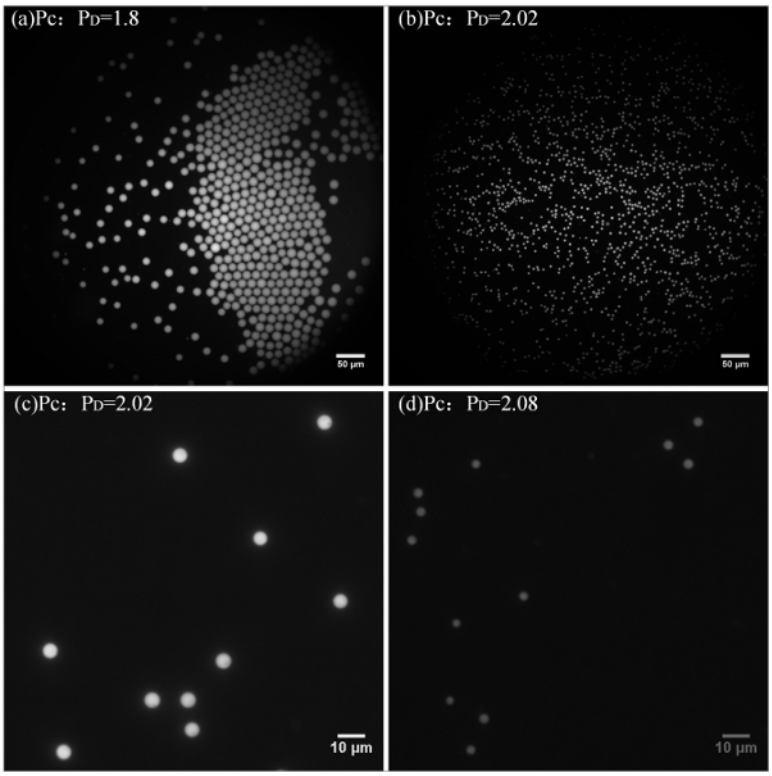

Figure 6. PEGDA particles under different pressure ratio. Fluorescence images of particles in different sizes and particles under **(a-b)** the optical microscope and **(c-d)** confocal laser scanning microscopy. P_C is the pressure of continuous phase, and P_D is the pressure of dispersed phase. Please click here to view a larger version of this figure.

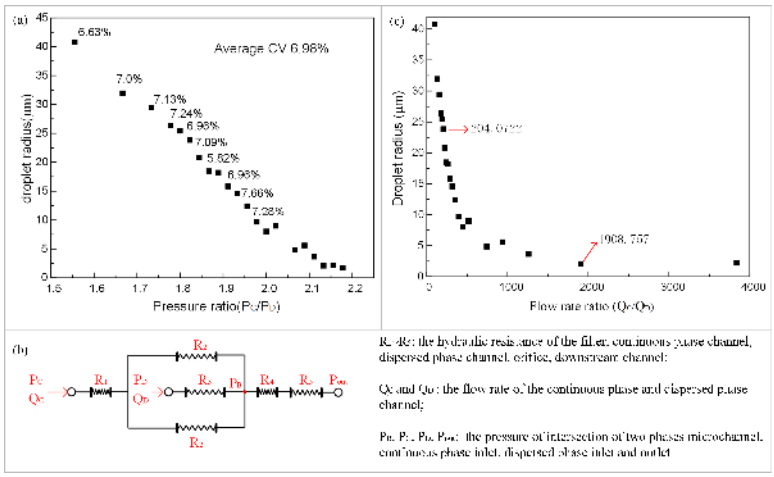

Figure 7. Droplet size. (a) Corresponding sizes on the basis of the pressure ratio. Black square represents the droplet size distribution, and the superscript numbers are the corresponding coefficient of variation. Smaller droplets are hard to characterize the coefficients of variance of smaller droplets, so the CV of those smaller droplets were not indicated. **(b)** Illustration of fluidic circuit. **(c)** The relationship between droplet size and flow rate ratio. Please click here to view a larger version of this figure.

Discussion

The generation of droplets in the flow-focusing mode using 2D and semi-3D microfluidic device has previously been developed in a variety of reports[8,9,15,19,20,21]. In these systems, the aqueous liquid that could not be solidified was chosen as the dispersed phase, such as deionized water[8,15,20,21], an aqueous solution of sodium hydroxide[19] and the formation of stable tip-streaming mode needs the support of high voltage electric field[8,21]. In addition, such flow-focusing droplet formation system is similar to the one of pure water, with a low concentration aqueous solution of PEGDA[12], which is more stable than the one of using PEGDA without dilution as the dispersed phase.

In our semi-3D flow-focusing microfluidic system without high voltage electric field, undiluted PEGDA solution was used as the dispersed phase liquid, increasing the difficulty to form stable droplet breakup process. We found that the tip-streaming mode was more stable by increasing the concentration of surfactant; and also, increasing the concentration of surfactant decreased the local surface tension and formed a more cusped

tip, leading to the droplet size decrease. As a result, size-controllable (1 μm to 80 μm in diameter) droplets can be obtained by only adjusting the pressure ratio, in an ease of fabrication and high reproducibility manner.

However, there is a major restriction for our semi-3D flow-focusing microfluidic system. PDMS is a kind of flexible material so that the flow-focusing mode would become unstable under high pressure due to deformation of the microchannel. In addition, although it was reported that hexadecane would cause PDMS swelling[22], we didn't observe significant deformation of our microchannel causing by such effect.The 80 μm and 100 μm wide channel for the dispersed phase were selected, and slight deformation was observed when the pressure increased. So, we suggest that the flow rate in the orifice region is too high under such high pressure, leading to the inevitable deformation, but not due to the swelling effect of hexadecane. A whole flat device will bend slightly after continued use for 7 hours. It takes about 4 hours to measure a group of practical data, and the device has not been deformed remarkably. Furthermore, it is worth exploring whether the fiercer breakup process using T-junction resulted in the unstable flow-focusing mode. Y-junction, the flow-focusing structure with an angle between two phase channels (including 15°, 45°, 65°) was selected to make a gentle flow-focusing for a more stable mode. However, no tip-streaming mode occurred under those microfluidic devices, and only bigger droplets formed under jetting mode. It also was reported that the full width of dispersed phase flow was about 30 μm under high-pressure ratio using Y-junction[23]. Finally, the base-level pressure applied on the dispersed phase was somewhat low, and low pressure reduces the generation frequency, especially for the smaller droplets. Higher production rate is expected to be acquired through the paralleled structure in our future work.

Smaller droplet causes higher surface-volume ratio, leading to higher reaction rate and efficiency. In biology, the small droplet will be used for antibody screening and drug discovery by surficial decoration, encapsulation by adding biological molecules, such as targeted genetic and cells, and producing functional particle by adding magnetic and fluorescent material. We hope that our protocols, relating to fabrication of semi-3D flow-focusing PDMS device and small droplet generation, will contribute to continuous and deeper studies in such field, and be used in wide range of biological applications.

Disclosures

The authors have nothing to disclose.

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