Video Article

Capillary-based Centrifugal Microfluidic Device for Size-controllable Formation of Monodisperse Microdroplets

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Abstract

Here, we demonstrate a simple method for the rapid production of size-controllable, monodisperse, W/O microdroplets using a capillary-based centrifugal microfluidic device. W/O microdroplets have recently been used in powerful methods that enable miniaturized chemical experiments. Therefore, developing a versatile method to yield monodisperse W/O microdroplets is needed. We have developed a method for generating monodisperse W/O microdroplets based on a capillary-based centrifugal axisymmetric co-flowing microfluidic device. We succeeded in controlling the size of microdroplets by adjusting the capillary orifice. Our method requires equipment that is easier-to-use than with other microfluidic techniques, requires only a small volume (0.1-1 µl) of sample solution for encapsulation, and enables the production of hundreds of thousands number of W/O microdroplets per second. We expect this method will assist biological studies that require precious biological samples by conserving the volume of the samples for rapid quantitative analysis biochemical and biological studies.

Video Link

The video component of this article can be found at https://www.jove.com/video/53860/

Introduction

W/O microdroplets[1-5] have many important applications for the study of biochemistry and bioengineering, including protein synthesis[6], protein crystallization[7], emulsion PCR[8,9], cell encapsulation[10], and construction of artificial cell-like systems[5,6]. To produce W/O microdroplets for these applications, important criteria are control of size and monodispersibility of the W/O microdroplets. Microfluidic devices for making monodisperse, size-controllable W/O microdroplets[11] are based on the co-flowing method[12,13], flow-focusing method[14,15], and the T-junction method[16] in microchannels. Although these methods produce highly monodisperse W/O microdroplets, the microfabrication process requires complicated handling and specialized techniques for the preparation of microchannels, and also requires a large amount of sample solution (at least several hundred µl) because of the inevitable dead volume in the syringe pumps and tubes that conduct the sample solution to the microchannels. Thus, an easy-to-use and low-dead-volume method to generate monodisperse W/O microdroplets is needed.

This paper, along with videos of experimental procedures, describes a centrifugal capillary-based axisymmetric co-flowing microfluidic device[17] for generating cell-sized, monodisperse W/O microdroplets (**Figure 1**). This simple method achieves size monodispersity and size controllability. It requires just a tabletop mini-centrifuge and a capillary-based axisymmetric co-flowing microfluidic device fixed in a sampling microtube. Our method needs only a very small volume (0.1 µl), and does not waste any significant volume of the sample.

Protocol

1. Fabrication of a Capillary-based Microfluidic Device

1. **Set up of the holders**
   Note: The holder design is presented in **Figure 2A**.
   1. Cut out each of the four discs of the holders (**Figure 2A(i)-(iv)**) from 2-mm-thick polyacetal plastic plate using a milling machine. Use the following dimensions for each of the four discs of the holder: (i) disc 1 diameter 8.5 mm, capillary hole (CH) diameter 1.3 mm, screw hole (SH) diameter 1.8 mm; (ii) disc 2 diameter 8.7 mm, CH diameter 2.0 mm, SH diameter 1.8 mm; (iii) disc 3 diameter 8.7 mm, CH diameter 0.5 mm, SH diameter 1.8 mm; and (iv) disc 4 diameter 9.1 mm, CH diameter 1.0 mm, SH diameter 1.8 mm.

2. Assemble the holders using M2 × 40 screws (**Figure 2B**). A bottom part of the holder (**Figure 2B**) consists of the disc 1 and the disc 2 in **Figure 2A(i), (ii)** and an upper part (**Figure 2B**) of the holder consists of the disc 3 and the disc 4 in **Figure 2A(iii), (iv)**.
    1. To construct the bottom part of the holder, insert the screw in three SH of each disc 1 and 2. Shorten the screws by nipping off a piece of the thread portion. Keep the length of screw at 0.9 cm (the same length as the bottom part of the holder).
    2. To construct the upper part of the holder, insert screws into the two SH of each disc 3 and 4. Shorten the screws by nipping off a piece of the thread portion. Keep the length of screw at 0.7 cm (the same length as the upper part of the holder).
    3. To assemble the holder, join the bottom and upper parts of the holder using a long screw.
    Note: Keep the length of the each part of the holder exact: the bottom part is 0.9 cm; the upper part is 0.7 cm (**Figure 2B**).

2. **Fabrication of the glass capillaries**
    1. Use two types of glass capillaries: an inner glass capillary (Outer diameter (OD)/ Inner diameter (ID): 1.0/0.6 mm), and an outer glass capillary (OD/ID: 2.0/1.12 mm).
    2. Use a glass cutter to divide the outer glass capillary into three equal parts, and then use the glass cutter to divide the inner glass capillary into two equal pieces.
    3. Sharpen each divided inner and outer glass capillaries using a glass capillary puller (**Figure 3A**). Set the weight of the puller at max. Set the heat level of the puller at 60 degrees for the outer glass capillary and 70 degrees for the inner capillary. Carefully sharpen the glass capillary.
        1. Keep the length of the tip within the constricted part of the glass capillary: the inner capillary is 1.5-1.8 cm; the outer capillary is 0.8-1.0 cm (**Figure 3C**). If this length is shorter or longer than the described length, please adjust the heat level of the puller.
    4. Fix the inner or outer glass capillaries to the microforge stand using tape (**Figure 3B**).
    5. Cut off the tip of the glass capillary using the microforge in three steps (**Figure 3B**): (i) touch the tip of the glass capillary to the glass beads on a platinum wire, (ii) heat the platinum wire by stepping on a foot switch for 1-2 sec, and (iii) after 1-2 sec, cut off the tip of the glass capillary by cooling the platinum wire.
        1. Adjust the diameters of the inner (d_i) and outer (d_o) capillary orifices, respectively. The orifice diameter of the inner glass capillary is 5, 10, and 20 μm ($d_{i=}$ 5,10, 20 μm) and the outer glass capillary (d_o) is 60 μm ($d_{o=}$ 60 μm) in this experiment.
        Note: The glass capillary is disposable. Repeat the fabrication of the glass capillaries.

2. Procedure for Generating W/O Microdroplets

1. Fill an outer glass capillary with oil containing surfactant. The mixture of oil and surfactant is hexadecane containing 2% (w/w) sorbitan monooleate in this experiment (**Figure 4A**).
Note: There are many combinations of oils and surfactants (*e.g.*, oils may be fluorinated or carbonated; surfactants may be ionic, nonionic, or fluorochemical).
    1. Introduce 10 μl of hexadecane containing sorbitan monooleate into an outer glass capillary. In **Figure 4A**, the orifice diameter of the outer glass capillary (d_o) is 60 μm ($d_{o=}$ 60 μm). To adjust the orifice of glass capillary, return to steps 1.2.4-1.2.5.
2. Set the outer capillary in the bottom part of the holder (**Figure 4B**).
3. Draw about 0.1 μl of an aqueous solution into an inner glass capillary (**Figure 4C**) by capillary action. In **Figure 4C**, the orifice diameter of inner glass capillary (d_i) is 10 μm ($d_{i=}$ 10 μm). To adjust the orifice of the glass capillary, return to steps 1.2.4-1.2.5.
4. Set the inner capillary in the upper part of the holder (**Figure 4D-a**). Insert the inner capillary into the outer capillary (**Figure 4D-a**). Looking at the white dot circle as in **Figure 4D-a**, observe the position of the inner capillary inside the outer capillary (internal diameter of the outer capillary (w) = 130 μm) (**Figure 4D-b,c**) using a digital microscope. The position of the inner capillary in the outer capillary must be set to w = 100-150 μm.
Note: To change the position of the inner capillary in the outer capillary, please turn the screw in the upper part of the holder. Thereby, distance w can be controlled precisely.
5. Introduce 100 μl of hexadecane containing sorbitan monooleate (2% w/w) into the bottom of a 1.5 ml sample microtube. Install the holder, with the inner and outer capillaries, in the sample microtube (**Figure 4E-a**). Be sure to check the outer capillary to keep it away from the air-oil interface (**Figure 4E-b**).
6. Centrifuge the sample microtube using a tabletop swinging-out-type mini-centrifuge at a gravity of 1,600 x g for 1-2 sec to generate microdroplets (**Figure 4F**). Carry out all experiments at RT.
Note: Use a swinging-out-type centrifuge. A droplet may collide with a sidewall of the sample microtube and disintegrate when a fixed-angle-type centrifuge is used.
7. Slowly draw up the W/O droplets by pipette, and then, put them on a glass slide.
8. Capture images of the microdroplets generated using a digital microscope (magnification, 200X).

Representative Results

In this study, we present a simple method for the generation of cell-sized W/O microdroplets by using a capillary-based centrifugal microfluidic device (**Figure 1**). The microfluidic device was composed of a capillary holder (**Figure 2B**), two glass capillaries (inner and outer glass capillaries in **Figure 3C**), and a microtube containing an oil including surfactant. We injected 0.1 μl of sample solution into the inner glass capillary and placed the inner glass capillary into the outer glass capillary (**Figure 4D**). Cell-sized W/O microdroplets were generated by Plateau-Rayleigh instability of a jetting-flow of sample solution[17] (**Figure 1B**) and were stable for at least 2 hr[17].

Typical examples of the different sizes of W/O microdroplets generated from the capillary-based centrifugal microfluidic device are shown in **Figure 5**. **Figure 5A-F** shows the digital microscopy images and size distribution histograms (n = 200) of the W/O microdroplets. The W/O microdroplets were generated using an inner capillary with a d_i = 5 (A, B), 10 (C, D), or 20 μm (E, F) orifice diameter while keeping d_o and w constant at 60 μm and 115 μm, respectively. The measurements of the size of the generated W/O microdroplets were acquired by analysis of the microscope image obtained. For the d_i = 5, 10, and 20 μm orifices, the average diameters of the microdroplets were 8.3 μm (standard deviation (SD) 0.9 μm, coefficient of variation (CV) 10.8%), 12.7 μm (SD 1.1 μm, CV 8.6%), and 17.9 μm (SD 1.4 μm, CV 7.8%), respectively. These results indicate that we successfully obtained monodisperse W/O microdroplets by the proposed method. Furthermore, the W/O microdroplet diameters were almost the same as the inner capillary orifice (**Figure 5G**). Thus, the mean size of the W/O microdroplets can easily be tuned over a wide range, typically 5 to 20 μm, using the micro device.

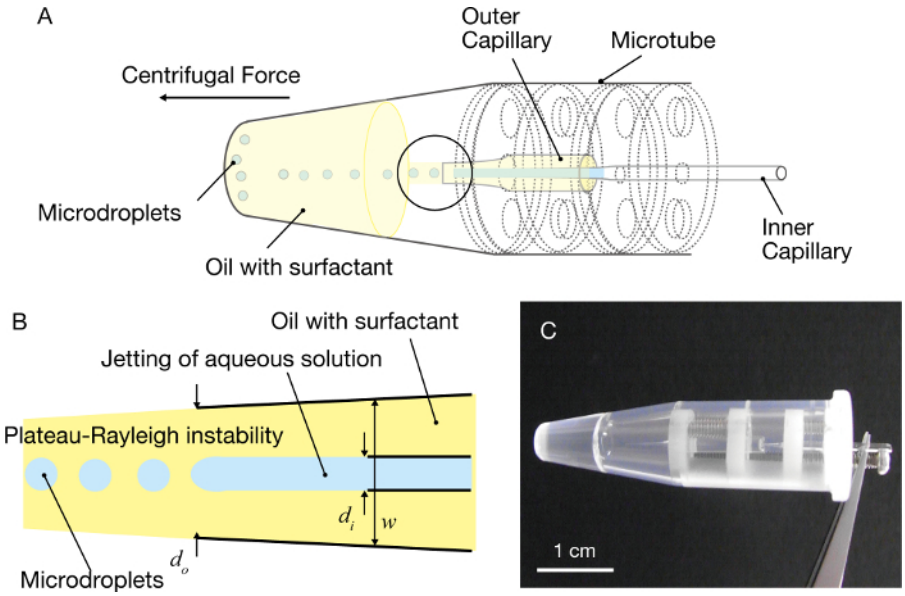

Figure 1. Overview of centrifugal capillary-based axisymmetric co-flowing microfluidic device and formation of W/O microdroplets using the device. (**A**) Illustration of the centrifugal capillary-based axisymmetric co-flowing microfluidic device and process generating W/O microdroplets (within circle), (**B**) W/O microdroplets generated by Plateau-Rayleigh instability of a jetting flow of aqueous solution[17], d_i is orifice diameter of inner glass capillary, d_o is orifice diameter of inner glass capillary, w is internal diameter of the outer capillary, (**C**) Photograph of fabricated device. Please click here to view a larger version of this figure.

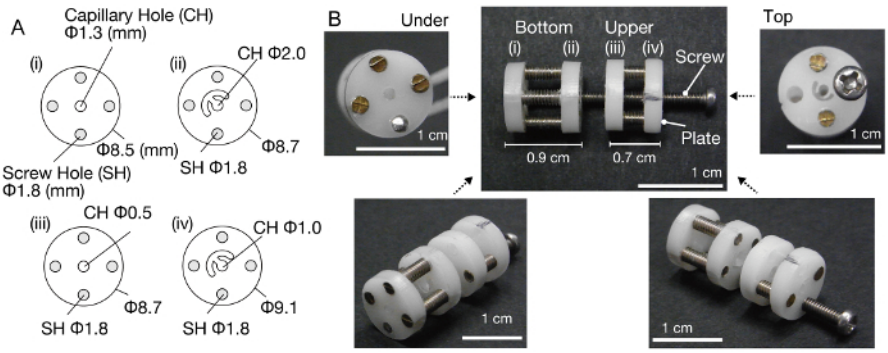

Figure 2. Setup of capillary holder. (**A**) Design of the capillary holder made of polyacetal plastic: The unit of diameters in the holder is mm. (**B**) Photographs of the holder consist of an upper part and a bottom part. Please click here to view a larger version of this figure.

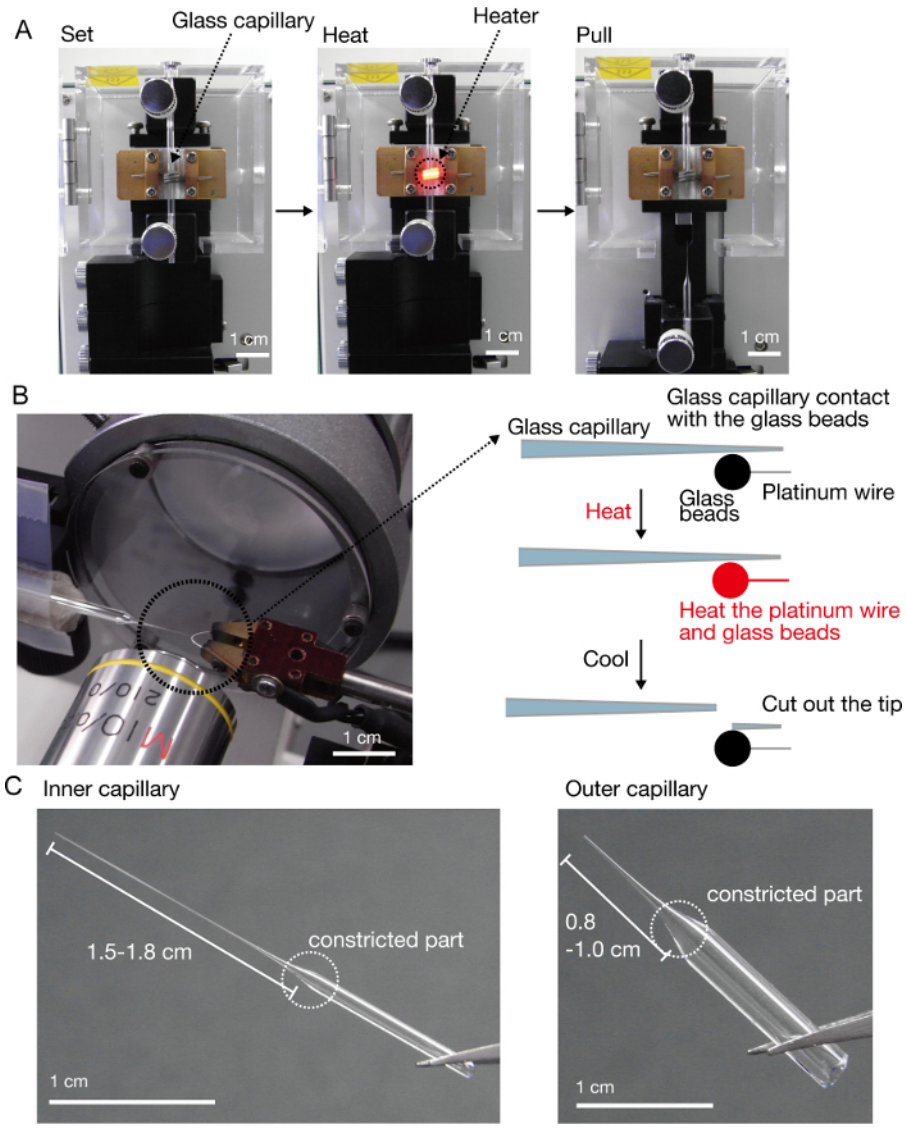

Figure 3. Fabrication of glass capillaries. (**A**) Sharpen the glass capillary using a glass capillary puller, (**B**) The tip of the capillary cut out by microforge (circle of black dots) and scheme of cut out of the tip, (**C**) Photographs of the fabricated inner and outer capillary. Please click here to view a larger version of this figure.

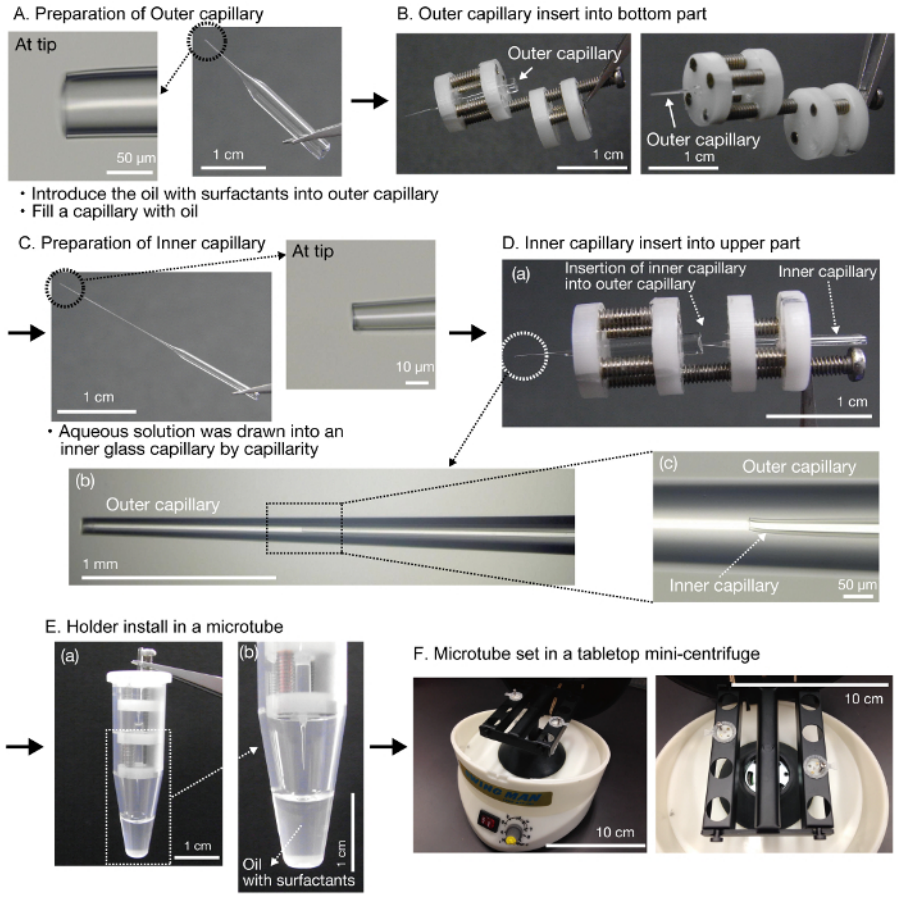

Figure 4. Flow chart of the microfluidic device set-up and generation of W/O microdroplets. (**A**) Preparation of outer capillary. Oil with surfactants introduced into the outer capillary, (**B**) Outer capillary set into the bottom part of the holder, (**C**) Preparation of the inner capillary: Aqueous solution drawn into an inner capillary by capillarity, (**D**) Inner capillary set into the upper part of the holder (a). Checking that the inner capillary was in the outer capillary using a digital microscope (b, c), (**E**) Holder with inner and outer capillaries installed in the sample tube (a). Checking that the outer capillary was kept away from the air-oil interface, (**F**) Finally, sample microtube was centrifuged by a tabletop swinging-out-type mini-centrifuge. Please click here to view a larger version of this figure.

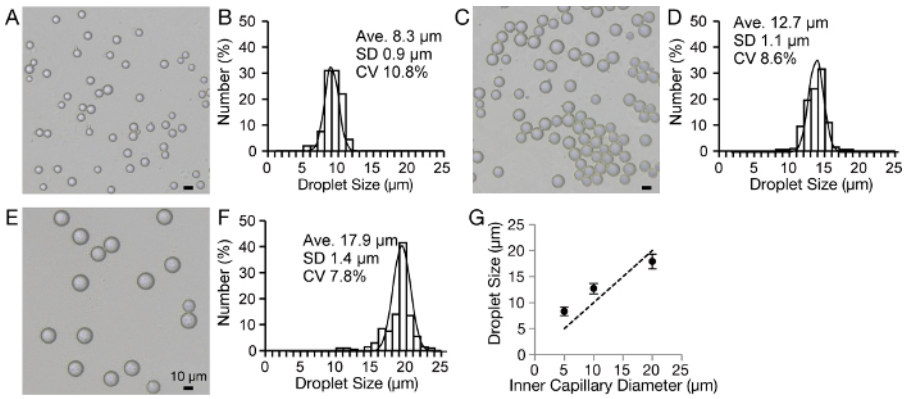

Figure 5. Formation of monodisperse cell-sized W/O microdroplets. Digital microscope images and size distribution histograms (n = 200) of generated microdroplets using capillaries of various diameters, d_i = 5 (**A**, **B**), 10 (**C**, **D**), and 20 µm (**E**, **F**), (**G**) Correlation between the orifice diameter of the glass capillary and the diameter of the generated W/O microdroplets. Error bars show the standard deviations of the diameter of the generated W/O microdroplets. Please click here to view a larger version of this figure.

Discussion

Using this device, the monodisperse W/O microdroplets were generated by Plateau-Rayleigh instability of a jet-flow[17]. Microscopic examination did not reveal the presence of satellite droplets. In the fabrication of the device, three critical steps are essential to successfully generate monodisperse W/O microdroplets. First, to supply a straight flow of oil containing surfactant and aqueous solution, the capillary holes of four discs must be arranged in a concentric pattern. Second, the inner capillary was carefully inserted into the outer capillary because the tip of the

capillary breaks easily if it contacts the upper holder. This operation can be difficult, so we recommend using a magnifying glass. Finally, in order to make a jetting-flow of aqueous solution[17], the position of the inner capillary into the outer capillary must be set to w = 100-150 µm. If the size distribution of W/O microdroplets generated by centrifugal microfluidic device is not monodisperse, check the position of the inner capillary in the outer capillary. To change the position of the inner capillary in the outer capillary, please turn the screw in the upper part of the holder. Thereby, distance w can be controlled precisely.

To make monodisperse W/O microdroplets, there are current limitations. There is difficulty in increasing the centrifugal rate (if needed) because all the experiments in the study were performed at the maximum centrifugal rate of the desktop centrifuge. Additionally, droplet generation is difficult from a variety of different sample solutions, the limitation being dependent on the centrifuge design. Multi-barreled capillaries as the inner capillary may provide the encapsulated microdroplets from various combinations of materials and solutions[18].

The microfluidic device has two primary advantages over conventional microchannel methods: i) easy and robust fabrication, and ii) the requirement of only a small volume (0.1 µl) of the sample solution. First, the fabrication of the capillary-based centrifugal axisymmetric co-flowing microfluidic device is simple and robust. Only thin capillaries, a capillary holder, and a sample microtube are required. The fabrication time is 5-10 min for the device. Fabrication of the device takes less time compared to the fabrication of other microfluidic device. Moreover, the capillary holder is robust and can be reused. Therefore, the only consumables are the glass capillaries and sample microtube in the device, which makes it less expensive than other microfluidic systems. Finally, since the oil and aqueous flows were produced by centrifugal force, there was no wasted volume. For 1-2 sec, the device generates a large number of microdroplets.

Microdroplets are ideal candidates to carry out high-throughput experiments involving small amounts of sample solution. With this device, it is theoretically possible to generate hundreds of thousands of 10-µm-sized microdroplets per second from 0.1 µl of the sample solution. Thus, the device accommodates work with precious biological samples by minimizing the volume of the samples needed for rapid quantitative analysis. This device can be used to analyze biochemical reactions[6-9] and single-cell enzymatic reactions[10].

Disclosures

No conflicts of interest are declared.

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References

1. Song, H., Chen, D.L., Ismagilov, R.F. Reactions in droplets in microfluidic channels. *Angew. Chem., Int. Ed.* **45** (44), 7336-7356 (2006).
2. Huebner, A., *et al.* Microdroplets: a sea of applications? *Lab Chip.* **8**, 1244-1254 (2008).
3. Taly, V., Kelly, B.T., Griffiths, A.D. Droplets as microreactors for highthroughput biology. *ChemBioChem.* **8** (3), 263-272 (2007).
4. Teh, S.Y., Lin, R., Hung, L.H., Lee, A.P. Droplet microfluidics. *Lab Chip.* **8**, 198-220 (2008).
5. Takinoue, M. Takeuchi, S. Droplet microfluidics for the study of artificial cells. *Anal. Bioanal. Chem.* **400** (6), 1705-1716 (2011).
6. Hase, M., Yamada, A., Hamada, T., Baigl, D., Yoshikawa, K. Manipulation of cell-sized phospholipid-coated microdroplets and their use as biochemical microreactors. *Langmuir.* **23** (2), 348-352 (2007).
7. Zheng, B., Tice, J.D., Roach, L.S. Ismagilov, R.F. A Droplet-Based, Composite PDMS/Glass Capillary Microfluidic System for Evaluating Protein Crystallization Conditions by Microbatch and Vapor-Diffusion Methods with On-Chip X-Ray Diffraction. *Angew. Chem., Int. Ed.* **43** (19), 2508-2511 (2004).
8. Nakano, M., *et al.* A. Single-molecule PCR using water-in-oil emulsion. *J. Biotechnol.* **102** (2), 117-124 (2003).
9. Diehl, F., *et al.* BEAMing: single-molecule PCR on microparticles in water-in-oil emulsions. *Nat. Methods.* **3**, 551-559 (2006).
10. He, M., *et al.* Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets. *Anal. Chem.* **77** (6), 1539-1544 (2005).
11. Baroud, C., Gallaire, F., Dangla, R. Dynamics of microfluidic droplets. *Lab Chip.* **10**, 2032-2045 (2010).
12. Utada, A.S., Nieves, A.F., Stone, H.A., Weitz, D.A. Dripping to jetting transitions in coflowing liquid streams. *Phys. Rev. Lett.* **99** (9), 094502 (2007).
13. Cramer, C., Fischer, P., Windhab, E.J. Drop formation in a co-flowing ambient fluid. *Chem. Eng. Sci.* **59** (15), 3045-3058 (2004).
14. Anna, S.L., Bontoux, N., Stone, H.A. Formation of dispersions using "flow-focusing" in microchannels. *Appl. Phys. Lett.* **82**, 364-366 (2003).
15. Takeuchi, S., Garstecki, P., Weibel, D.B., Whitesides, G.M. An axisymmetric flow-focusing microfluidic device. *Adv. Mater.* **17** (8), 1067-1072 (2005).
16. Thorsen, T., Roberts, R.W., Arnold, F.H., Quake, S.R. Dynamic pattern formation in a vesicle-generating microfluidic device. *Phys. Rev. Lett.* **86** (18), 4163-4166 (2001).
17. Yamashita, H., *et al.* Generation of monodisperse cell-sized microdroplets using a centrifuge-based axisymmetric co-flowing microfluidic device. *J. Biosci. Biotech.* **119** (4), 492-495 (2015).
18. Maeda, K., Onoe, H., Takinoue, M., Takeuchi, S. Controlled synthesis of 3D multi-compartmental particles with centrifuge-based microdroplet formation from a multi-barrelled capillary. *Adv. Mater.* **24** (10), 1340-1346 (2012).

