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NOTE

Generation of monodisperse cell-sized microdroplets using a centrifuge-based axisymmetric co-flowing microfluidic device

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We report an easy-to-use generation method of biologically compatible monodisperse water-in-oil microdroplets using a glass-capillary-based microfluidic device in a tabletop mini-centrifuge. This device does not require complicated microfabrication; furthermore, only a small sample volume is required in experiments. Therefore, we believe that this method will assist biochemical and cell-biological experiments.

[**Key words:** Monodisperse microdroplets; Microfluidic device; Centrifuge; Plateau–Rayleigh instability; Biochemical reactions; Cell encapsulation]

In recent years, cell-sized water-in-oil (W/O) microdroplets have attracted much attention as powerful methods (1–5) that enable miniaturized chemical experiments, saving of chemical samples, saving of experimental time, parallel and high-throughput analyses, quantitative chemical experiments, and so on. These microdroplets have been applied to a wide range of biological studies such as protein synthesis (6); single-molecular DNA PCR (7,8); cell encapsulation (9); and simplified models of living cells called artificial cells (5,6). In general, the powerful advantages of W/O microdroplets are derived by producing them monodispersely using microfluidic technologies.

Three methods have usually been employed for producing monodisperse W/O microdroplets (10): the co-flowing method (11,12), flow-focusing method (13,14), and T-junction method (15). In all these methods, microdroplets are formed in precisely fabricated microfluidic channels, in which immiscible liquid flows composed of the oil phase and aqueous phase are generated and spontaneous breakups of the aqueous flow generate monodisperse W/O microdroplets. However, some difficulties hamper the use of microfluidic channels, such as time-consuming photolithography-based fabrication of microfluidic channels in clean rooms, precise control of the liquid flow in the microfluidic channels using micro-syringe pumps, and requirement of a large amount of biological- or chemical-reaction samples because of dead volumes in syringe pumps and injection tubes. To be able to take advantage of W/O microdroplets, the generation process of monodisperse microdroplets needs to be simplified. Here, we report an easy method for generating monodisperse cell-sized microdroplets. This method requires just a tabletop mini-centrifuge and a capillary-based axisymmetric co-flowing microfluidic device fixed in a sampling microtube, named a centrifuge-based axisymmetric co-flowing microfluidic device. The proposed method does not need complicated fabrication, precise control of liquid, or a large sample volume when it is employed. We demonstrate the generation of monodisperse W/O microdroplets in a size-controlled manner by using the above-mentioned device. We also present cell-free protein synthesis and cell encapsulation in microdroplets and discuss the potential applicability of the proposed method to biological studies.

The centrifuge-based axisymmetric co-flowing microfluidic device (Fig. 1) is composed of two kinds of round capillaries (inner and outer capillaries), a capillary holder, and a sampling microtube. This device was developed on the basis of a centrifuge-based droplet shooting device (16). The inner capillary was inserted into the outer capillary, and both were fixed with the holder in the microtube. The holder consisted of an upper part to hold the inner capillary and a lower part to hold the outer one. The distance L between the upper and lower parts of the holder can be adjusted by screwing one of these parts (Fig. 1B). Thus, distance w (Fig. 1A) can be controlled precisely. The detailed design of the holder is shown in Fig. 1B.

The holders were fabricated by cutting a 2-mm-thick polyacetal plastic plate with a milling machine (MDX-40A, Roland DG). The glass capillaries were fabricated using a glass capillary puller (PC-10, Narishige) and a microforge (MF-900, Narishige), and the

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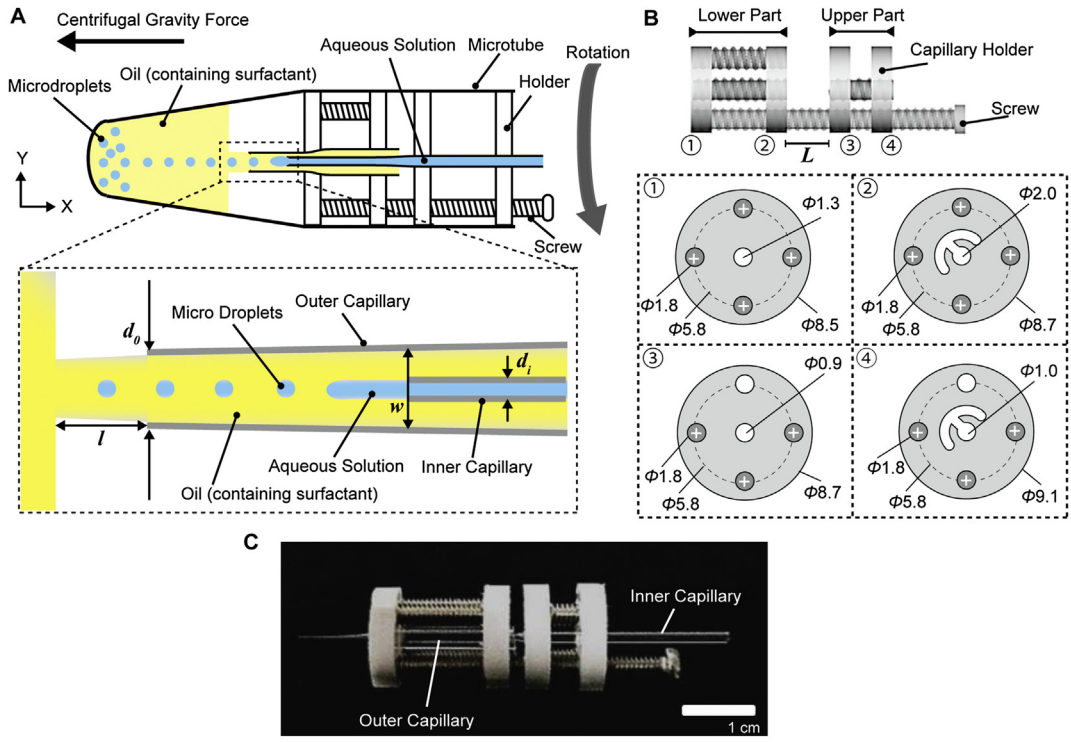

FIG. 1. Centrifuge-based axisymmetric co-flowing microfluidic device. (A) Schematic illustration of the device and droplet formation in it. The inner capillary is inserted into the outer capillary. The aqueous phase flows in the inner capillary, and the immiscible oil phase flows around the aqueous phase in the outer capillary. These flows are produced by a centrifugal gravity force. d_i is the diameter of the inner capillary orifice; d_o is that of the outer capillary orifice; and w is the internal diameter of the outer capillary, in which the inner capillary orifice is placed. (B) Design of the capillary holder made of polyacetal plastic. The holder consists of an upper part and a lower part. L is the length between the upper part and lower parts. The unit of diameters in the holder is mm. (C) Photograph of fabricated device.

diameters of the inner and outer capillary orifices were adjusted to d_i and d_o, respectively (Fig. 1A).

For synthesis of the microdroplets, 10 μL of hexadecane (Wako Pure Chemical Industries) containing 2% (w/w) Span 80 (Tokyo Chemical Industry) was introduced into an outer glass capillary (1B200-6, World Precision Instruments), and the outer capillary was set in the lower part of the holder. Then, about 0.1 μL of an aqueous solution was introduced into an inner glass capillary (G-1, Narishige) by the capillary phenomenon, and the inner capillary was set in the upper part of the holder (Fig. 1C). After confirmation of insertion of the inner capillary into the outer capillary, the holder was installed in a sampling microtube, the bottom of which was filled with hexadecane containing 2% (w/w) Span 80. Then, the microtube was centrifuged using a tabletop swinging-out-type mini-centrifuge (ATT101, Hitech Co., Ltd.) at a gravity of 1600 $\times g$ (rotation speed: 4200 rpm) for 2–3 s to generate microdroplets. All experiments were carried out at a room temperature.

Fig. 2A shows a microscope image of cell-sized W/O microdroplets generated by the centrifuge-based axisymmetric co-flowing microfluidic device ($d_i = 5$ μm, $d_o = 80$ μm, $w = 115$ μm), as captured by a digital microscope (VHX-2000, Keyence). This figure shows that many W/O microdroplets were generated in a single centrifuge experiment. Fig. 2B shows digital microscopy images and size distribution histograms of the W/O microdroplets generated with this device when the diameter d_i was varied while keeping d_o and w constant at 60 μm and 115 μm, respectively. For $d_i = 5$ μm, microdroplets with an average diameter of 6.6 μm [standard deviation (SD): 1.1 μm, coefficient of variation (C.V.): 17%] were generated, indicating that monodisperse W/O microdroplets were successfully obtained by the proposed method. For $d_i = 10$ and 20 μm, monodisperse W/O microdroplets with an average diameter of 8.6 μm (SD: 1.3 μm, C.V.: 15%) and 13.8 μm (SD: 1.9 μm, C.V.: 14%) were obtained, respectively. Consequently, the diameters of generated droplets were comparable with the diameter of the inner capillary orifice, and their SDs were small. Fig. 2C shows a microscope image and a size distribution of W/O microdroplets generated by a tapping method; the method is a well-used generation method for microdroplets. The microdroplets were generated by 50 times hand-tapping of 50 μL of hexadecane with 2% (w/w) Span 80 including 2 μL of water. The size distribution of microdroplets by the tapping method (average diameter: 10.7 μm, SD: 5.3 μm, C.V.: 50%) was very wider than that by our device. These results show that the diameters of the W/O microdroplets were controlled by changing d_i.

We next investigated the relationship between the diameter of the inner capillary orifice (d_i) and the diameter of the generated microdroplets under varying diameters of the outer capillary orifice (d_o) while keeping w constant at 115 μm (Fig. 2D). We found that the diameters of the generated W/O microdroplets were proportional to d_i and did not depend on d_o. In general, it is known that the diameter of a microdroplet (r) generated by droplet breaking via the Plateau–Rayleigh instability of a jetting flow of the water phase is proportional to the capillary orifice diameter (d_i) (17), that is,

$$r \sim d_i \tag{1}$$

On the other hand, the diameter of a microdroplet generated by dripping of the water phase is proportional to the third root of the capillary orifice diameter (d_i) (16,17), that is,

$$r \sim \sqrt[3]{d_i/G} \tag{2}$$

Thus, from the experimental results (Fig. 2D), we consider that the droplet generation mechanism by this method is droplet breaking by the Plateau–Rayleigh instability of a jetting flow.