

A microfluidic platform for formation of double-emulsion droplets

Kin-Lok Lao · Jung-Hao Wang · Gwo-Bin Lee

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Abstract A new method for actively controlling the number of internal droplets of water-in-oil-in-water (W/O/W) double-emulsion droplets was demonstrated. A new microfluidic platform for double-emulsion applications has been developed, which integrates T-junction channels, moving-wall structures, and a flow-focusing structure. Inner water-in-oil (W/O) single-emulsion droplets were first formed at a major T-junction. Then the droplets were sub-divided into smaller uniform droplets by passing through a series of secondary T-junctions (branches). The moving-wall structures beside the secondary T-junctions were used to control the number of the sub-divided droplets by selectively blocking the branches. Finally, double-emulsion droplets were formed by using a flow-focusing structure downstream. Experimental data demonstrate that the inner and outer droplets have narrow size distributions with coefficient of variation (CV) of less than 3.5% and 5.7%, respectively. Double-emulsion droplets with 1, 2, 3, and up to 10 inner droplets have been successfully formed using this approach. The size of the inner droplets and outer droplets could be also fine-tuned with this device. The development of this new platform was promising for drug delivery applications involving double emulsions.

Keywords Double emulsion · T-junction · Moving wall · Microfluidics · MEMS

Abbreviations

CCD Charge-coupled device
CV Coefficient of variation

DGI Dodecyl glyceryl itaconate
DI Deionized
EMV Electromagnetic valve
HLB Hydrophilic–lipophilic balance
MEMS Micro-electro-mechanical-systems
O/W Oil-in-water
PDMS Polydimethylsiloxane
 R_1 Volumetric flow rate of inner phase
 R_2 Volumetric flow rate of medium phase
 R_3 Volumetric flow rate of outer phase
RIE Reactive ion-etching
SEM Scanning electron microscope
W/O Water-in-oil
W/O/W Water-in-oil-in-water
O/W/O Oil-in-water-in-oil

1 Introduction

Emulsion droplets have applications in a variety of fields such as food preparation, cosmetics, pharmaceuticals, and chemical engineering industries (Hamouda et al. 1999; Wibowo and Ng 2001). Emulsification involves a process to break one of two immiscible fluids into smaller droplets. The quality of the emulsions highly depends on uniformity, stability, size, and size distribution of the emulsion droplets. Many investigations have been conducted to achieve high-quality emulsions. Traditionally, large-scale blenders (Penfold et al., 2005) and ultrasonic homogenizers (McClements 1999) were commonly used to achieve emulsification. However, it was still challenging to achieve high-quality emulsification in a well-controlled manner since the size distributions of the emulsion droplets could not be well managed using the large-scale equipment.

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Recently, microstructures have been reported to generate uniform emulsion droplets. For instance, a membrane emulsification method by using a liquid pressed through membrane pores to form emulsion droplets, which were then carried away by a continuous phase flowing over the membrane, has been demonstrated (Charcosset et al. 2004). When compared with large-scale stirring or ultrasonic processes, micro-machined devices were capable of enhancing the quality of the emulsification since they could generate more uniform droplets in liquids (Atencia and Beebe 2005). They also have a greater potential to allow for more sophisticated particle designs such as colloidosomes, core-shell polymer particles, three-dimensional colloidal assemblies, and polymerosomes via appropriate operations (Lorenceanu et al. 2005).

Recently, many attempts have been made to generate emulsion droplets in microfluidic devices. Single emulsions, which involve a process for breaking two immiscible fluids into smaller droplets, were extensively investigated in recent years using these devices. Water-in-oil (W/O) or oil-in-water (O/W) emulsion droplets have been successfully demonstrated. These devices were based on two popular approaches, specifically, a T-junction channel method (Xu and Nakajima 2004; He et al. 2005; Van der Graaf et al. 2005; Dendukuri et al. 2005; Xu et al. 2006a, b; Zhou et al. 2006) and a flow-focusing method (Garstecki et al. 2005; Lewis et al. 2005; Tan et al. 2006; Sugiura et al. 2004). One of the most common microfluidic devices for generating emulsion droplets is the “T-junction” channel. The T-shaped channel geometry can force two streams of immiscible liquids to merge such that liquid droplets can be dispersedly generated (Tan et al. 2004; Link et al. 2004). In addition, dispersed liquid droplets may also be created by another flow-focusing approach by using the Rayleigh break-up, where two streams of the same liquid (sheath flows) squeeze a stream of a second immiscible liquid and the combined flow is then forced through a small orifice (Garstecki et al. 2005) or enters a wide channel (Xu and Nakajima 2004). The pressure and viscous forces exerted by the outer fluid ultimately force the inner fluid to break into droplets. The use of two sheath-flow junctions in series allows for the formation of double-emulsion droplets.

In general, these devices were commonly fabricated in elastomeric materials by using a soft lithography process due to their easy availability (Whitesides and Stroock 2001). In addition to microfluidic devices fabricated in elastomeric materials, microfluidic devices could also be fabricated in silicon by using photolithography and wet-etching processes (Kawakatsu et al. 1997) or in stainless steel by using a dicing process (Tong et al. 2001). Other types of microchannels without terraces (Sugiura et al. 2000) or with partition walls between microchannels

(Nakagawa et al. 2004) have also been used for emulsification. Particularly, a deep reactive ion etching (RIE) process was used to fabricate “through-type” microchannels with vertical sidewalls (Kobayashi et al. 2005a). However, it was reported that it was difficult to formulate monodisperse emulsions when using dispersed-phase liquids with a low viscosity for these devices. To overcome this problem, an array of “asymmetric” through-holes, vertically fabricated on a silicon plate, was recently developed (Kobayashi et al. 2005b).

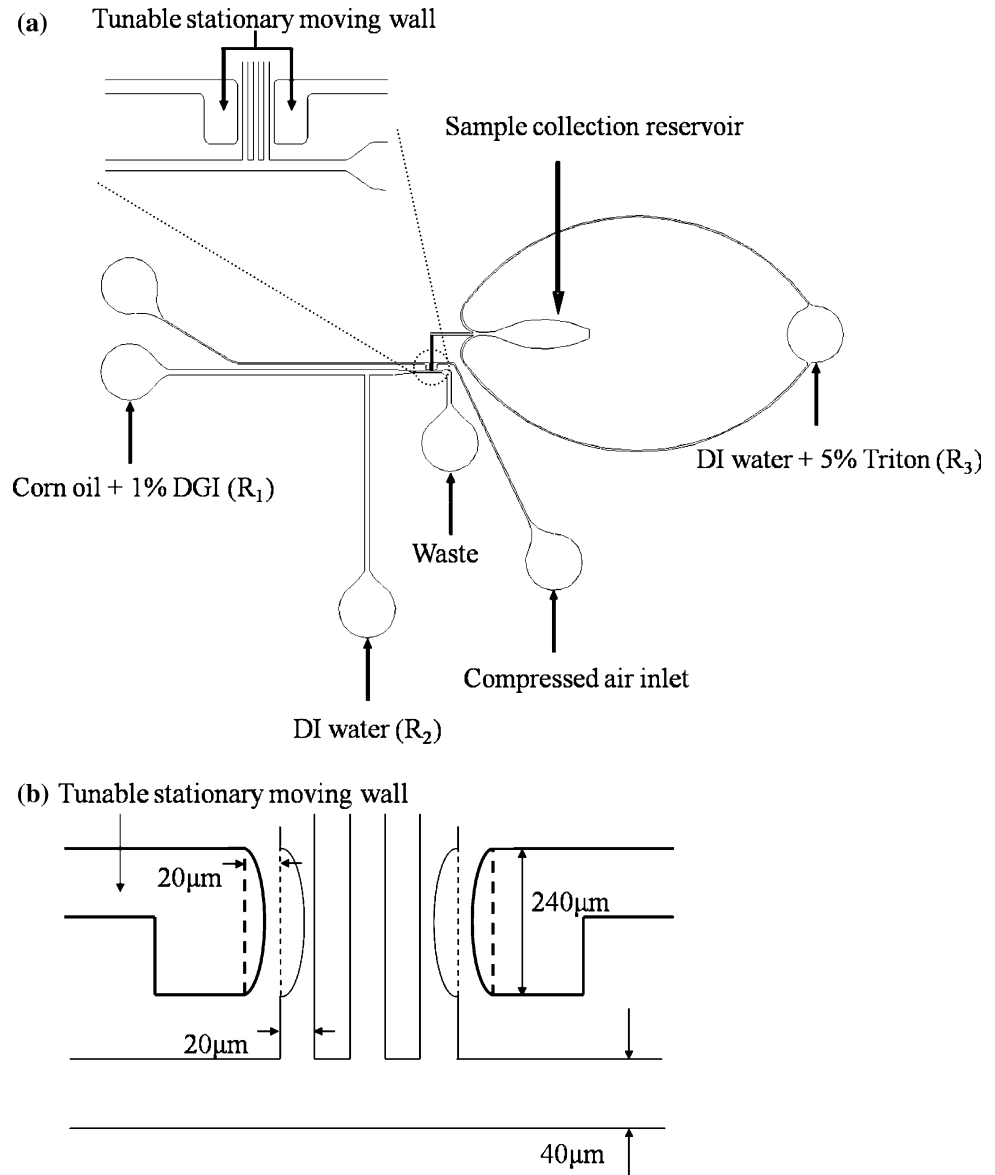
Another approach utilizing active components in microfluidic emulsion devices was demonstrated recently by this research group (Lin et al. 2008). A moving-wall structure was used to chop focused streams such that double-emulsion droplets could be successfully generated. In this study, a new microfluidic device consisting of T-junctions, moving-wall structures, and a flow-focusing structure was developed for generating double emulsions. It has been reported that emulsion droplets formed by using a T-junction with a tunable membrane in a microchannel downstream the T-junction may have a relatively high coefficient of variation (CV) in droplet sizes (Wu et al. 2008). Therefore, a unique two-step droplet-dividing process, which allows the formation of uniform W/O emulsion droplets, was used. A major T-junction was first used to generate inner droplets. Then a series of secondary T-junctions were used to divide one inner droplet into several sub-droplets. This new droplet-dividing method could generate a great quantity of uniform sub-droplets. In addition, a new method for actively controlling the number of internal droplets of water-in-oil-in-water (W/O/W) double-emulsion droplets by using moving-wall structure was demonstrated. Moving-wall structures beside the secondary T-junctions were first used to control the number of the sub-divided droplets by selectively blocking the branches. Finally, a flow-focusing structure downstream was used to form double-emulsion droplets. Therefore, the double-emulsion droplets with 1, 2, 3, and 10 inner droplets were successfully formed using this approach. The size of the inner droplets and outer droplets could be also fine-tuned with this device. This is also the first time for a device with this capability to be reported.

2 Materials and methods

2.1 Design

In this study, a new microfluidic device was used to generate double-emulsion droplets. A unique, two-step, droplet-division process, which allows for the formation of uniform W/O emulsion droplets, was used. The number of the inner droplets enclosed in an outer droplet could be

Fig. 1 Schematic illustration about the working principle behind the microfluidic chip for double emulsions with one, two, or three inner droplets; **a** schematic illustration of the chip, **b** working principle of the moving-wall structures

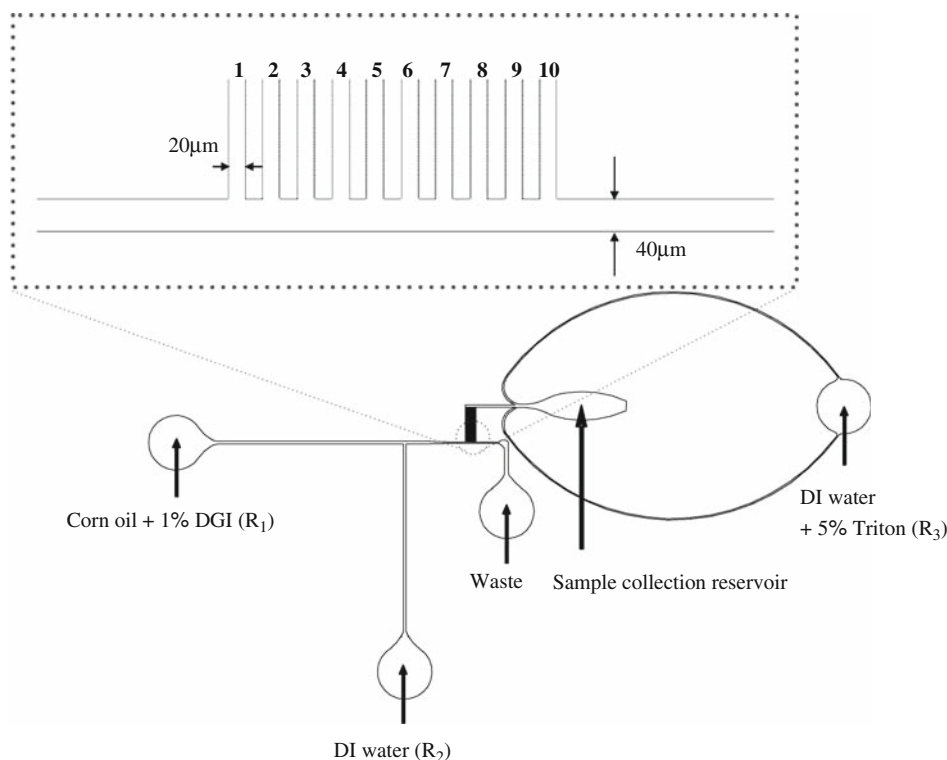


adjusted by using the moving-wall structures. The size of the inner droplets and outer droplets could be also fine-tuned in this device. The microfluidic device consists of three major components including T-junctions, moving-wall structures, and a flow-focusing structure. Figure 1 was a schematic diagram of the operating principle of the microfluidic device. At first, single-emulsion droplets were generated at a major T-junction upstream. Then the single-emulsion droplets pass through a series of secondary T-junctions (branches) to be sub-divided into several uniform sub-droplets. The number of the sub-droplets was equal to the number of branches. Concurrently, the moving-wall structures could be used to block the microchannels in order to actively control the number of the sub-droplets as shown in Fig. 1b. Finally, double-

emulsion droplets were generated when the shear force in the sheath flow breaks the pre-focused stream into droplets. Note that the moving-wall structures were controlled by injecting compressed air to vertically deflect compliant side-walls (Fig. 1b).

In order to form double emulsions with multiple sub-droplets, another similar device with 10 branches was used, as shown in Fig. 2. The purpose of the multiple branches was to generate a great quantity of inner sub-droplets with a high uniformity in diameter. When the inner droplets were formed at the major T-junction, it could be equally divided into 10 sub-droplets. The surplus fluid from the major droplets, after the dividing process, passes through the branches and was finally collected in the waste reservoir. With this approach, double-emulsion droplets with 10

Fig. 2 Schematic illustration of the working principle of the microfluidic chip for double emulsions with 10 inner droplets



size-tunable inner droplets could be generated. A similar approach could be adopted to generate double-emulsion droplets with more inner droplets, which was determined by the number of the branches.

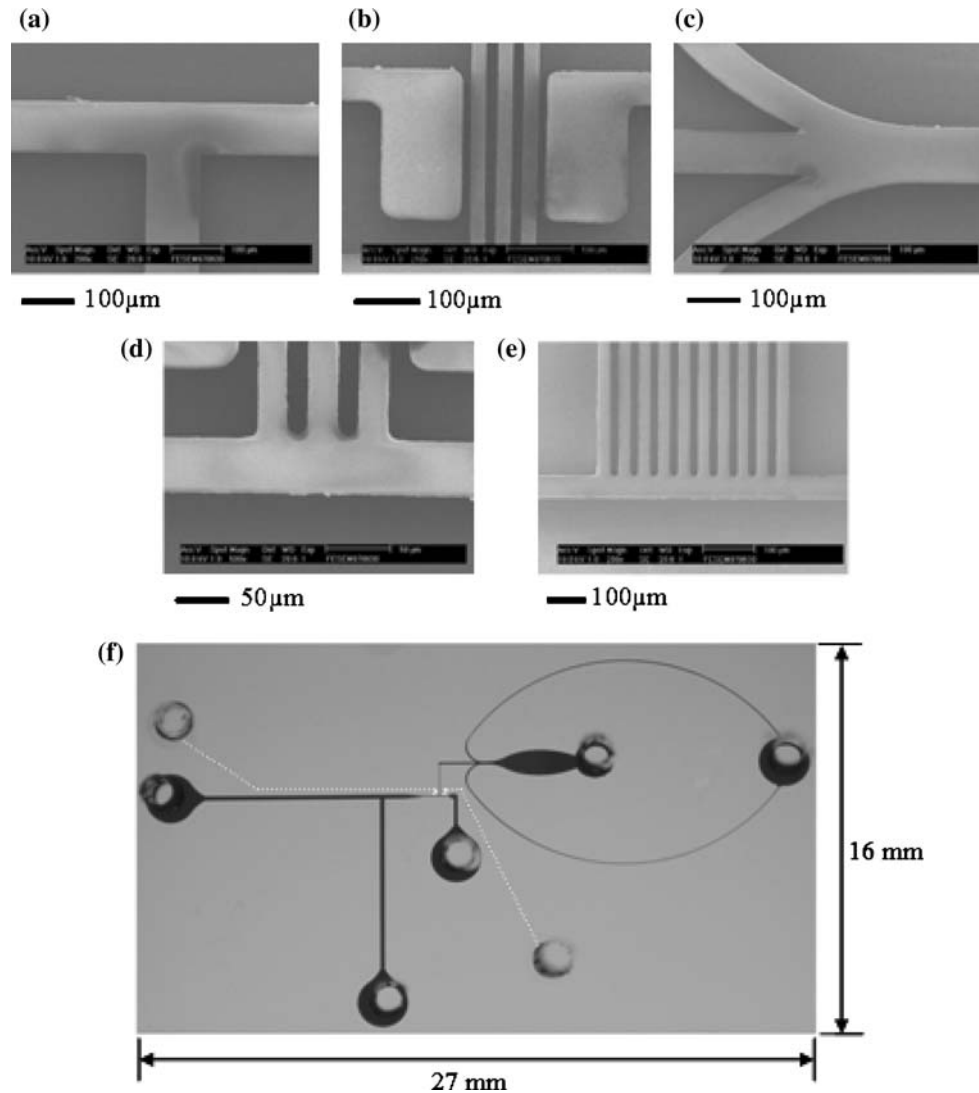
2.2 Fabrication process

The microfluidic devices were fabricated using micro-electro-mechanical-systems (MEMS) technology to form the elastic polydimethylsiloxane (PDMS) moving-wall structures, the major T-junction, the flow-focusing structure, and a series of secondary T-junctions (branches). For the formation of the PDMS-based microfluidic structures, master molds were first formed onto a silicon substrate by using a layer of SU-8 negative thick photoresist (SU-8-50, MicroChem, Newton, MA, USA). The SU-8 master mold could be easily defined and formed utilizing standard lithography and two-stage baking processes (Lin et al. 2008). Briefly, SU-8 negative thick photoresist with a thickness of 100 μm was spin coated on a silicon wafer and was soft baked at 100°C for 35 min. After the soft-baking process, a standard lithography process with an exposure dose of 520 mJ/cm^2 was performed. The exposed SU-8 film was next baked at 65°C for 1 min, and then at 95°C for 10 min to help crosslink-exposed monomers. The exposed SU-8 film was then developed at room temperature and dried with compressed air to produce finished SU-8 templates.

After fabricating the SU-8 master molds, the silicone elastomer and elastomer curing agent (Sylgard 184A and 184B, Sil-More Industrial Ltd, USA) were mixed in a 10:1 ratio and then poured onto the SU-8 microstructure mold. After the degassing process, it was cured at a temperature of 80°C for at least 4 h. The inverse structures of the SU-8 master molds were then transferred onto the PDMS structures after a de-molding process. Finally, the PDMS plate and PDMS structure layers were bonded together using an oxygen plasma treatment to form the completed device.

Scanning electron microscope (SEM) images of the major T-junction, the moving-wall structures, the flow-focusing structure, and branches for the SU-8 molds and the completed microfluidic device were shown in Fig. 3. The depth of these structures was 100 μm . The tunable stationary moving-wall structures were 240 μm in length (Fig. 1b). The pneumatic moving-wall structures were composed of two pneumatic side-chambers with a width of 150 μm and a length of 240 μm , respectively. The interval between the branches and the pneumatic side-chamber was 20 μm , which was used to form the moving-wall structure. The width of the main channel and the side channel of the major T-junction were each 75 μm . The width of the sheath-flow channels was 40 μm . For the formation of the multiple sub-droplets, the dimensions of the main microchannel and the multiple branches were 40 μm and 20 μm in width, respectively (Fig. 2).

Fig. 3 SEM images of SU-8 templates including **a** major T-junction, **b** moving-wall structures, **c** a flow-focusing structure, **d** a secondary T-junction with three branches and **e** 10 branches for subdividing droplets. **f** Photograph of the completed microfluidic device



2.3 Experimental setup

In order to achieve and observe the formation of double-emulsion droplets with multiple inner droplets, an experimental setup consisting of an optical microscope (TE300, Nikon, New York, USA), a charge-coupled device (CCD, TE/CCD512TKM, Roper Scientific, Princeton, NJ, USA), three syringe pumps (KDS200, KD Scientific Inc., MA, USA), and a compressed air control system was setup. The compressed air control system was composed of a control circuit, a compressed air source (MDR2-1A/11, JUN-AIR Inc., Japan), and two electromagnetic valves (EMVs, S070M-5BG-32, SMC Inc., Taiwan). The three syringe pumps were used to drive three different samples to generate double emulsions. For actively controlling the number of the sub-droplets enclosed in the outer droplets, the moving-wall structures were regulated by EMVs, which were controlled by the control circuit and EMVs. A

CCD was adopted to observe the formation of the emulsion droplets and to record images during the droplet formation process.

2.4 Sample preparation

In order to generate W/O droplets at the major T-junction, a deionized (DI) water solution and corn oil (Sigma Chemical, USA) were used as the dispersed phase and the continuous phase, respectively. Moreover, 1% of DGI with a hydrophilic–lipophilic balance (HLB) value of 3.6 was employed as a surfactant to stabilize the W/O emulsion droplets after the breakup of the dispersed-phase stream at the major T-junction. For the double-emulsion process at the flow-focusing structure, a DI water solution with a surfactant of 5% Triton X100 (HLB=13.5, Sigma Chemical, USA) was then used as the outer phase to generate stable W/O/W double-emulsion droplets.

2.5 Experimental procedures

Three different liquids including DI water, DI water with 5% Triton, and corn oil with 1% dodecyl glyceryl itaconate (DGI, Sigma, USA) were injected into the microfluidic chip by using three separate syringe pumps. The major droplets were first generated at a major T-junction upstream. Then the major droplets pass through a series of branches to be sub-divided into several uniform sub-droplets. The moving-wall structures could be used to block the microchannels in order to actively control the number of the sub-droplets. Then, the sub-droplets were moved to the subsequent flow-focusing structure. Finally, double-emulsion droplets were generated when the shear force in the sheath flow breaks the pre-focused stream into droplets. The diameter of the inner droplets could be fine-tuned by the ratio of the volumetric flow rates of the main channel and the side channel (R_1/R_2) at the major T-junction. In addition, the diameter of the outer droplets could be also fine-tuned by the relative sheath/sample flow rate ratio at the flow-focusing structure.

A computer equipped with CCD was used to observe and record images during the process of experiments. Therefore, the size and uniformity of the major droplets, divided droplets, and double-emulsion droplets were measured and counted from the recorded images.

3 Results and discussion

3.1 Formation of single-emulsion droplets

A major T-junction was first used to generate W/O droplets when injecting corn oil (continuous phase) and DI water (dispersed phase) into the main channel and into the side channel, respectively (see Fig. 1a). The relationship between the diameter of the W/O emulsion droplets and the volumetric flow rate ratio of two phases (R_1/R_2) has been investigated in the literature (Okushima et al. 2004; Lin et al. 2008). In brief, the higher the oil/water flow rate ratio (R_1/R_2), the smaller the droplets size that could be generated. However, the size of the resulting W/O droplets may have a relatively large CV value (Wu et al. 2008). Therefore, a series of secondary T-junctions (branches) were designed to divide the major W/O droplets into uniform sub-droplets. It was reported that the hydrodynamic resistance could be an important role when the major droplet was divided. The hydrodynamic resistances basically depend on the width and the depth of the microchannels and also the position of the sub-channels. It is the reason why only 10 branches were designed in this study. With this approach, the CV of the uniformity of the inner droplets can achieve 4–6%, which is still reasonable for

practical applications. For large W/O droplets, it was observed that the excess droplets passes through the branches and were collected in the waste reservoir after sub-dividing droplets, resulting in wasted samples. However, for small W/O droplets, they pass through the branches and could not be equally divided. As mentioned previously, the diameter of the droplets could be adjusted by changing the volumetric flow rate ratio (R_1/R_2). In this study, R_1 (middle phase) and R_2 (inner phase) were fixed to be 50 and 250 $\mu\text{l/h}$, respectively, after experimental optimization. The diameter of the W/O droplets generated at this volumetric flow rate ratio was measured to be 137 μm , which results in the generation of three uniformly divided droplets with a diameter of 40 μm in three branches.

The major W/O droplets could be then sub-divided into three smaller droplets by passing through the branches. The division of the droplet is due to the reduction of the fluidic resistance. Moreover, the shear force generated by the velocity of the major droplet would also break the major droplet to form a sub-dividing droplet. Therefore, the sub-dividing process would be performed when the major droplet passed through a series of T-junctions. However, the volume of the major droplet should be enough to assure the complete sub-division of droplets. After the sub-division process, the excess fluid from the major droplet was collected in the waste reservoir. As shown in Fig. 4a-3, a group of three sub-divided W/O droplets were successfully formed. Apparently, the number of sub-divided droplets was equal to the number of the branches. Therefore, a design with multiple branches could be used to divide the major droplets into a great quantity of sub-droplets if desired. Furthermore, the size of the sub-divided droplets was determined by the width of the branch channel. Therefore, the size distribution of the sub-divided droplets was more uniform than the major droplets. As shown in Fig. 4b-3, the sub-divided droplets have an average size of 30 μm ($R_2/R_1 = 5$) with CV as small as 4.0%, which was much more uniform than the major droplets (about 8.0%) (Wu et al. 2008). Note that the volume of divided three fine droplets was low for some case while the size of the major droplets is too big or the velocity of the major droplet is too high. This may be a disadvantage when internal aqueous phase contains expensive materials including drugs. It can be significantly enhanced if we control the size and velocity of the major droplet such that most of them can be divided in the sub-channels. Another possible way to tackle this issue is to re-cycle the residues from the waste reservoir for the second emulsion generation.

The developed device could actively control the number of inner droplets enclosed in the outer droplets for double emulsions. Moving-wall structures were integrated into the microfluidic chip. By applying compressed air, the moving-wall structures could be deformed vertically to block

Fig. 4 **a** Three different operating modes of the moving-wall structures for generating one, two, and three inner droplets. **b** The histograms of droplet size distributions for the three different operating modes

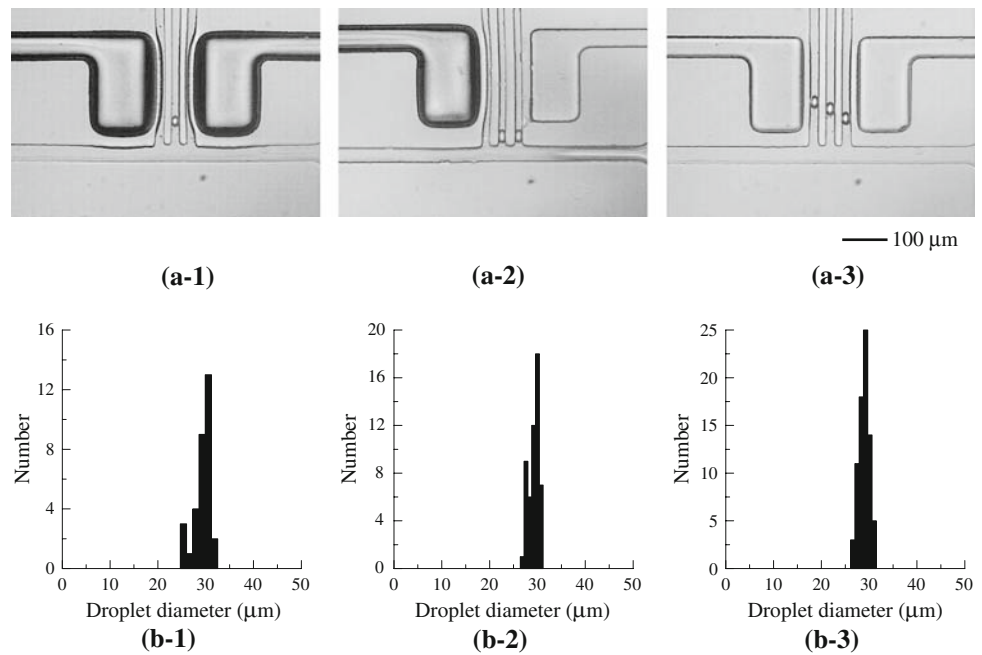
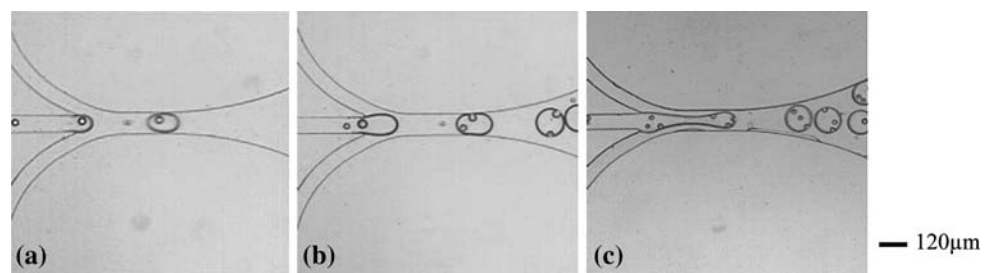


Fig. 5 Processes of W/O/W droplet formation by using a flow-focusing structure. W/O/W double-emulsion droplets with **a** one, **b** two, or **c** three inner droplets were successfully generated



the branch channels. In this study, compressed air with a pressure of 345 kPa was used to completely block the branch channels. As shown in Fig. 4a-1 and a-2, the moving-wall structures could be successfully used to actively control the number of sub-divided droplets. When R_2/R_1 was fixed at 5, the average diameters of the sub-divided droplets for these two cases were both about 30 μm with CVs of 5.7% and 4.9%, respectively (Fig. 4b-1, b-2).

3.2 Formation of double-emulsion droplets

A flow-focusing structure was used for the formation of double-emulsion droplets. By using this structure (Fig. 1a), double-emulsion droplets with a tunable number of inner droplets could be generated. Figure 5 shows the formation of W/O/W droplets with different numbers of inner droplets (one to three droplets) by using the developed device. Here, the major droplets were formed using the following operating conditions. R_1 and R_2 were equal to 250 and 50 μl/h, respectively, and R_3 was 100 μl/h to generate double-emulsion droplets. The sizes of the inner droplets

are observed to be relatively uniform. As shown in Fig. 5b, the size of inner droplets in the first outer droplet looks quite different. It is due to the fact that these two inner droplets are at different vertical location and also under a focusing process. Out-of-focusing issue under a microscope and the squeezing of the droplet may be the reasons for this observation. However, after they are encapsulated in the outer droplets and measured at the static state, the variation of the sizes is within 4–6%. Figure 6 shows a histogram of the W/O/W emulsion droplets. The total number of counted droplets was 61. The average diameter and CV of the double-emulsion droplets was 124.48 μm and 2.56%, respectively. The average size of the inner droplets was about 30 μm. The relationship between the external diameter of the W/O/W droplets and the volumetric flow rate of the outer phase (R_3) was shown in Fig. 7 when R_1 and R_2 were still kept at 250 and 50 μl/h, respectively. Note that only one inner droplet was enclosed inside the outer droplet in this case. It could be clearly seen that the higher the volumetric flow rate, the smaller were the diameters of the double-emulsion droplets.

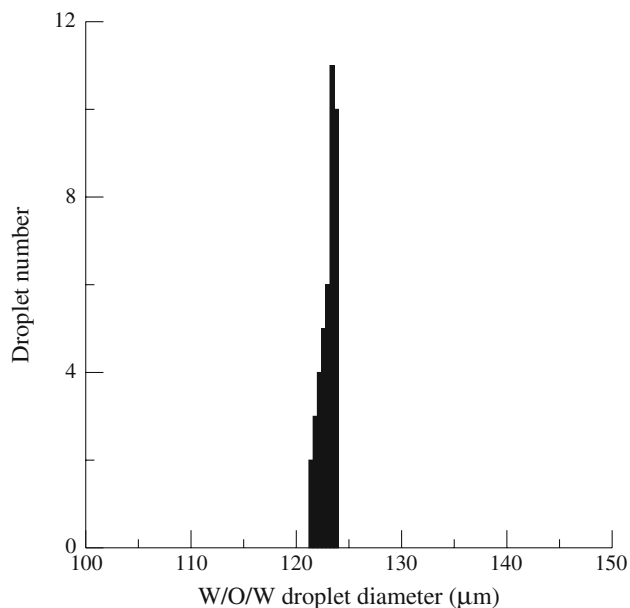


Fig. 6 The size distributions of W/O/W droplets by using the flow-focusing structure

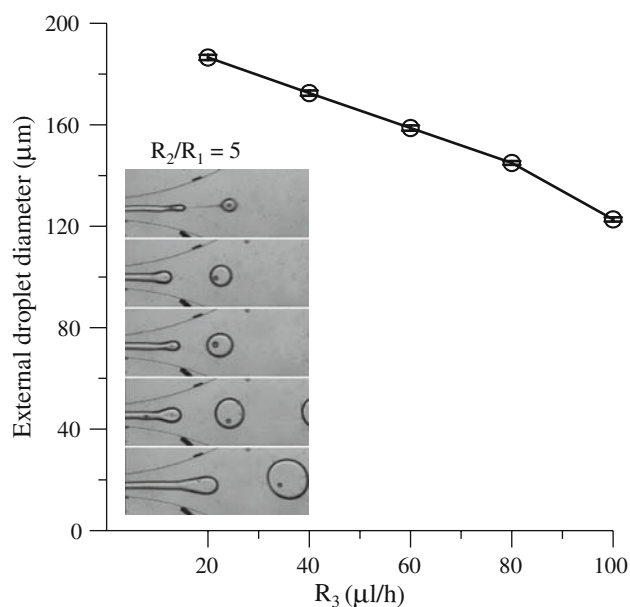


Fig. 7 The relationship between the external droplet diameter and the volumetric flow rate of the outer phase (R_3) for W/O/W droplets when R_1 and R_2 were kept at 250 and 50 $\mu\text{l/h}$, respectively. The double-emulsion droplets at $R_3 = 20, 40, 60, 80,$ and $100 \mu\text{l/h}$ were also shown in this figure

3.3 W/O emulsion droplets with 10 branches

In this study, another design with 10 secondary T-junctions (branches), but without moving-wall structures, was used to form a large quantity of sub-divided droplets. As shown in Fig. 8, a group of 10 sub-divided W/O droplets could be generated every time after the major W/O droplet passes

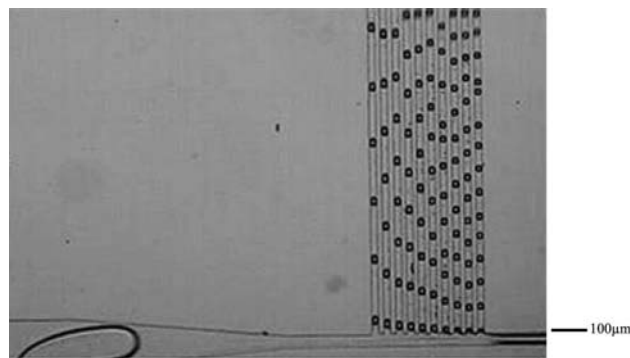


Fig. 8 Sub-divided droplets were formed after the original W/O droplets passed through the 10 branches

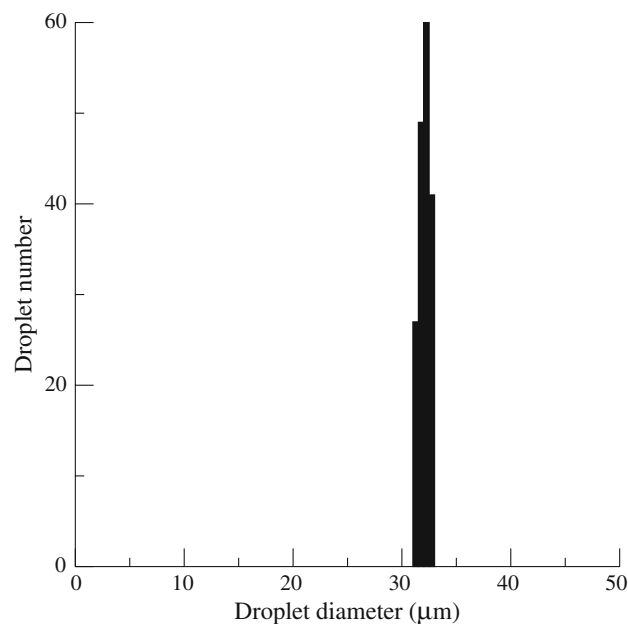


Fig. 9 The size distributions of sub-divided W/O droplets by using a series of secondary T-junction with 10 branches. The average diameter of the sub-divided droplets was measured to be 32.33 μm with a CV of 3.5%

through the 10 branches. In this case, R_1 and R_2 were equal to 50 and 250 $\mu\text{l/h}$, respectively. The size distributions of the W/O droplets by using the 10 branches were shown in Fig. 9. The average diameter of the droplets was measured to be 32.33 μm with a CV of 3.5%. Therefore, the uniformity of sub-divided droplets was maintained even after increasing the number of branches. Similarly, by precisely controlling the volumetric flow rates, 10-in-1 W/O/W double-emulsion droplets could be successfully formed as shown in Fig. 10. In this case, R_1 , R_2 , and R_3 were equal to 50, 250, and 50 $\mu\text{l/h}$, respectively. Note that each outer droplet contains 10 inner droplets. In Fig. 8, it showed that a lot of single-emulsion droplets can be simultaneously generated. The flow rate in this case was much higher than

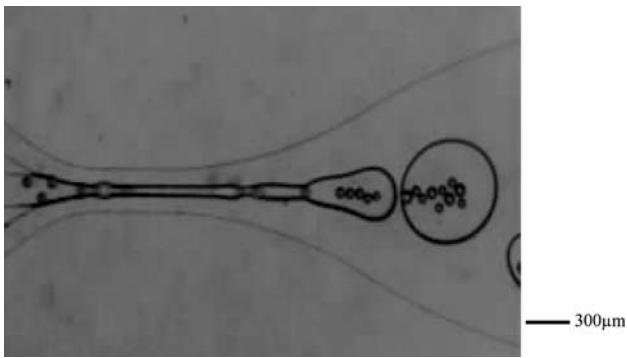


Fig. 10 10-in-1 W/O/W double emulsions were successfully formed. R_1 , R_2 , and R_3 were 50, 250, and 50 $\mu\text{l/h}$, respectively

that for double emulsions. For the double-emulsion application, the flow rate was decreased and only one inner droplet was formed in each sub-channel (see Fig. 11a) to ensure that these 10 droplets can be encapsulated in an outer droplet. These data had been shown in Fig. 10.

Several parameters were found to play important roles on the uniformity and size of the sub-divided droplets. It was found that the diameter of sub-divided droplets decreases slightly along with the branch number during the sub-division process from a major droplet. The size of the sub-divided droplet in the first branch was observed to be slightly larger than those in the subsequent branches.

Therefore, it was a critical issue to maintain the uniformity of the sub-divided droplets when increasing the number of the branches for double-emulsion droplets containing multiple inner droplets. In this case, the number of the branches was then limited to 10 such that the CV in diameter would remain within 3.5%.

The size of the sub-divided droplets was mainly dependant on one operating parameter, specifically, the size of the major droplets. Figure 11 shows a series of images of sub-dividing droplets. As described previously, the size of the major droplets increases with the increasing R_2/R_1 ratio. In this test, R_2 was increased from 210 to 310 $\mu\text{l/h}$ in increments of 20 $\mu\text{l/h}$ while R_1 was fixed at 50 $\mu\text{l/h}$. The diameters of the major droplets were measured to be 126, 132, 137, 147, 151, and 159 μm , respectively. Experimental data show that larger sub-divided droplets were generated from bigger major droplets. Therefore, R_2/R_1 could be used to fine-tune the size of the major droplets and the sub-divided droplets as well. Tunable inner droplets to be enclosed in the outer droplets were now possible. It was found experimentally that R_2/R_1 should not be <4 . Otherwise, the major droplets would be too small to fill up the 10 branches with reasonable uniformity.

The velocity of the major droplets was also investigated. As shown in Fig. 12, major droplets with different

Fig. 11 The size of the sub-divided droplets was determined by the size of the major droplets formed at the major T-junction. In this test, R_2 was adjusted to be **a** 210 $\mu\text{l/h}$, **b** 230 $\mu\text{l/h}$, **c** 250 $\mu\text{l/h}$, **d** 270 $\mu\text{l/h}$, **e** 290 $\mu\text{l/h}$, and **f** 310 $\mu\text{l/h}$, respectively, and R_1 was fixed at 50 $\mu\text{l/h}$. The surplus fluid from the major droplets was collected in the subsequent waster reservoir

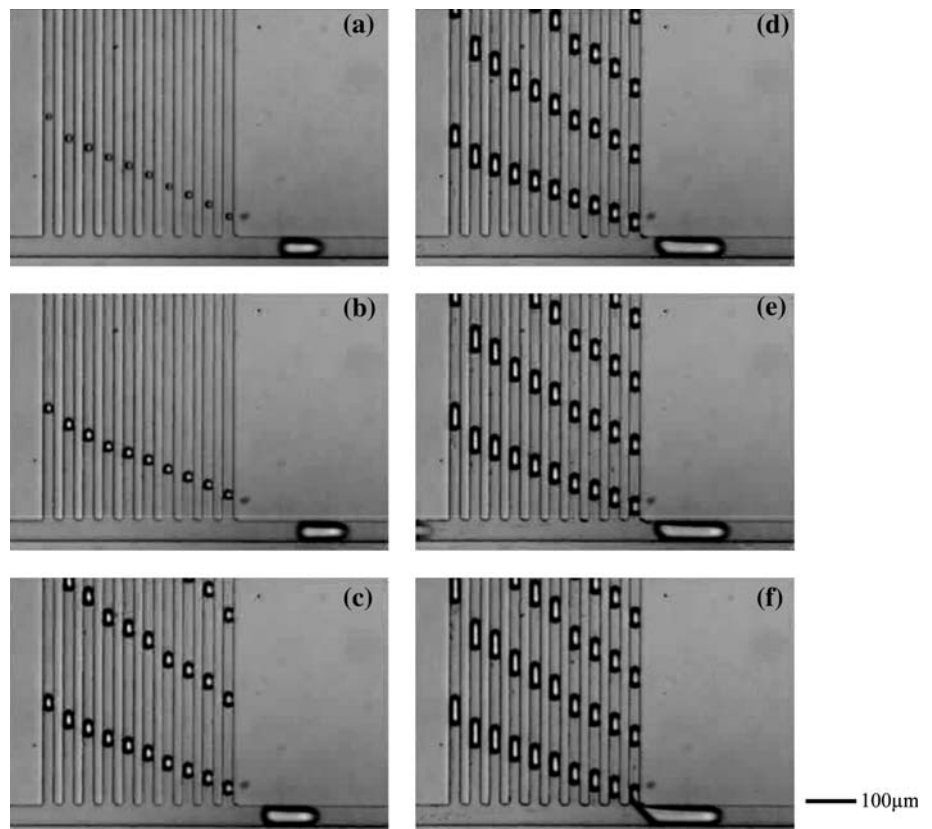
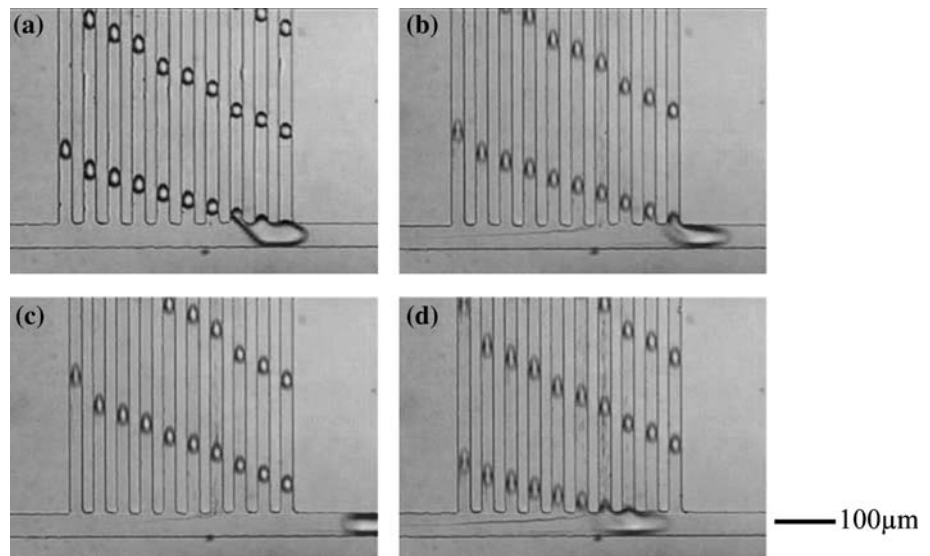


Fig. 12 Droplet division process for major droplets with the same size passing through the branches at different velocities. A fixed value of $R_2/R_1 = 5$ was used; **a** $R_1 = 50 \mu\text{l/h}$ and $R_2 = 250 \mu\text{l/h}$, **b** $R_1 = 100 \mu\text{l/h}$ and $R_2 = 500 \mu\text{l/h}$, **c** $R_1 = 150 \mu\text{l/h}$ and $R_2 = 750 \mu\text{l/h}$, and **d** $R_1 = 200 \mu\text{l/h}$ and $R_2 = 1,000 \mu\text{l/h}$



velocities pass through the 10 branches and the corresponding sub-divided droplets were formed. In this case, a fixed value of $R_2/R_1 (=5)$ was used while the resultant velocity of the major droplets was increased. For example, R_2 was 250, 500, 750, and 1,000 $\mu\text{l/h}$ when R_1 equals to 50, 100, 150, and 200 $\mu\text{l/h}$, respectively. With this approach, the velocity of the major droplets could be adjusted while keeping the size of the major droplets constant. It was observed that the diameters of the sub-divided droplets were almost the same for the four cases. The average diameter of the sub-divided droplets for the case with the highest velocity was 29 μm , which was slightly smaller than the case with the lowest velocity (30 μm). The uniformity of the sub-divided droplets for each case was also within 3.5%. Therefore, the velocity of the major droplets does not affect the uniformity and size of the inner droplets.

4 Conclusions

In this study, a new microfluidic device has been successfully demonstrated to form double-emulsion droplets. The formation of the major droplets was simply performed by using a major T-junction. Uniform inner droplets could be then generated by sub-dividing the major droplets at a series of secondary T-junctions (branches). The size of the inner droplets could be fine-tuned by varying the volumetric flow rate ratio to form various sizes of the major droplets. In addition, the number of inner droplets could be actively adjusted by using moving-wall structures. Finally, double-emulsion droplets have been successfully formed by using a flow-focusing structure. The diameter of the double-emulsion droplets were also fine-tuned by varying the sheath/sample flow rate ratio. Therefore, this developed

microfluidic device may be a promising platform for double-emulsion applications such as generating drug carriers for intra-vascular therapies.

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