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Reactions in double emulsions by flow-controlled coalescence of encapsulated drops

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We demonstrate a microfluidic method to first generate double emulsion droplets containing two different inner drops, and to then control the internal coalescence of the encapsulated drops. The advantages of the core-coalescence method are illustrated by fabricating high viscosity particles and by demonstrating the dissolution of cell membranes.

Droplet-based microfluidic systems provide many new methods to perform reactions on a small scale.¹ Applications of these methods include chemical and biochemical screening, synthesis of particles, assays for cells, and drug discovery and delivery,^{2–6} which are usually based on single emulsions. For example, two miscible fluids are encapsulated into one droplet,⁷ which allows the two distinct phases to mix and react immediately upon contact. In addition, when it is desirable to initiate the reaction at a certain time, two individual drops containing different chemical compositions can be controlled to coalesce in microfluidic channels. The approaches for controlled coalescence include the use of extra heating⁸ or of electric fields,^{2,3} as well as flow-controlled methods, which do not require additional applied fields. The flow-controlled drop coalescence (or drop fusion) has been explained by “soft collisions”⁹ or a “separation-driven”^{10,11} mechanism. For example, the flow-controlled coalescence of a pair of drops¹² with two different reactants has been applied for the generation of hydrogel particles,^{13,14} and it was observed that undesirable multiple coalescence sometimes occurs when the two drops have different properties.^{13,14} Thus, if the pair of drops to be coalesced are encapsulated in a larger drop, which forms a double emulsion droplet with a pair of inner drops, the undesirable multiple coalescence will be avoided. Here, we identify flow conditions to trigger the internal coalescence in double emulsion droplets, which provides a potential

approach for the realization of an individual chemical reactor with initially separated reactants in a protected environment.

One possible distinguishing feature of double emulsions offers the possibility of storing chemical reagents inside encapsulated drops, which avoids cross-contamination with other materials in the continuous phase.^{15,16} For example, a water-in-oil-in-water (W/O/W) double emulsion is used instead of water-in-oil (W/O) single emulsion in fluorescence-activated cell sorting (FACS),¹⁷ where the aqueous drops together with a fluorescent marker can be well isolated from those drops without a fluorescent marker by the double emulsion structure. Moreover, the external aqueous phase of a W/O/W emulsion is a possible solution to prevent complete desiccation of the aqueous drops in W/O emulsions in the microbatch crystallization of proteins.^{18,19} Although there have been several reports of the successful encapsulation of two different drops inside one double emulsion droplet,⁶ the subsequent internal coalescence of the inner drops (the cores) has not been studied.

In this work, we demonstrate a hydrodynamic method to control the coalescence of two different aqueous cores in a double emulsion droplet using a microcapillary device. The results are rationalized by recognizing a flow regime for the internal coalescence, which is caused by the “separation” movements of the cores inside the shell. Then we demonstrate two applications of this technology: (1) fabrication of monodisperse hydrogel particles and (2) dissolution of cell membranes, which is a key step in cell assays.

We design a microcapillary device to generate dual-core double emulsion droplets, which contain two different inner aqueous drops (labeled cores A and B) inside an oil shell, as shown in Fig. 1. Then, we study the coalescence of the two aqueous cores as a result of the flow in the microfluidic device. The microcapillary device consists of three capillary tubes: the injection tube, the collection tube and the outer tube. The injection tube has four parallel cylindrical capillaries (VitroCom, Inc.), which are connected to four inlets, respectively. Two of the inlets are for the same fluid of the shell, and the other two, placed diametrically opposite from each other, are for the core fluids A and B, respectively. This placement ensures that the core fluids can be individually encapsulated by the shell fluid. The injection tube is tapered using a pipette puller, so that all four capillaries have an inner diameter of 50 μm at their tips. The tips are made hydrophobic by treatment with octadecyltrichlorosilane (Sigma-Aldrich). The collection tube is a cylindrical capillary. After being tapered, it has an inner diameter of 200 μm at the tip. The two tapered tubes are fitted into

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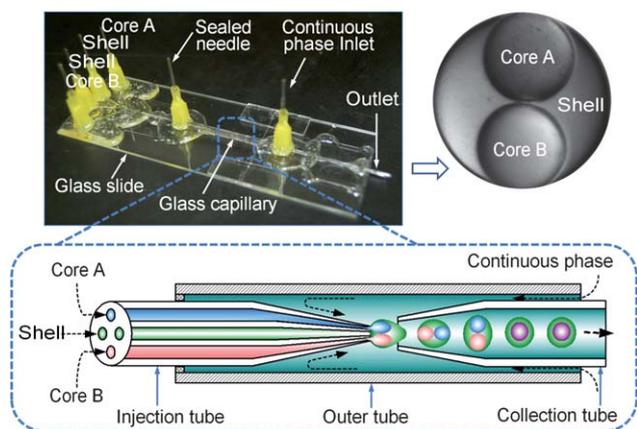


Fig. 1 The glass microfluidic device designed to make double emulsions where each droplet contains two inner cores A and B. The schematic within the dashed boundary shows the flow process of the emulsions through the pair of nozzles, and the dashed arrows on the schematic show the flow directions of the different phases.

the opposite ends of the outer tube, which is a square capillary with an inner dimension of 1.75 mm.

In our experiments, cores A and B are aqueous phases, which are injected into two capillaries of the injection tube with a total flow rate Q_i . The shell fluid is polydimethylsiloxane (PDMS) oil (Sigma-Aldrich) containing 2 wt% surfactant (Dow Corning 749), which is a blend of approximately 50 vol% high molecular weight trimethylsiloxysilicate resin and 50 vol% volatile, low viscosity cyclopentasiloxane. The shell fluid is injected into the other two capillaries in the injection tube with a total flow rate Q_s . Finally, an aqueous solution of water with 10 wt% polyvinyl alcohol ($M_w = 13\,000$ – $23\,000$, Sigma-Aldrich) forms the continuous phase, which enters the device through the interstices between the outer tube and the collection tube on one end at a flow rate Q_c , while the other end of the outer tube is sealed. When all the fluids meet at the junction, water-in-oil-in-water drops with both cores A and B inside one drop are formed, as shown schematically in Fig. 1. The diameters of the cores and the drops are controlled by adjusting the flow rates ratio of the different phases. A high-speed camera (Phantom V9.0) attached to a microscope is used to image the process of the formation of the dual-core double emulsion drops and the subsequent coalescence of the cores.

First, we investigate coalescence of two water cores inside individual double emulsion droplets. Both cores A and B are deionized water containing 0.25 wt% surfactant (Tween 20, Sigma-Aldrich). In order to understand the core-coalescence phenomenon, the effect of the flow conditions on the morphology of the cores and the drop shapes are investigated. As the flow rate ratio is changed among the different fluids, we obtain three different types of double emulsion drops, depending on whether or not the encapsulated drops coalesce. The three different results, including dual-core drops, pre-mixed drops and core-coalesced drops, are identified in a phase diagram for our experimental results, reporting the ratio of the flow rates *versus* morphology in Fig. 2. Each different outcome is indicated with different symbols. The most common result is the dual-core drops where two cores are encapsulated inside one shell, but they do not coalesce at any time, as shown by “●” in Fig. 2(a) and (b).

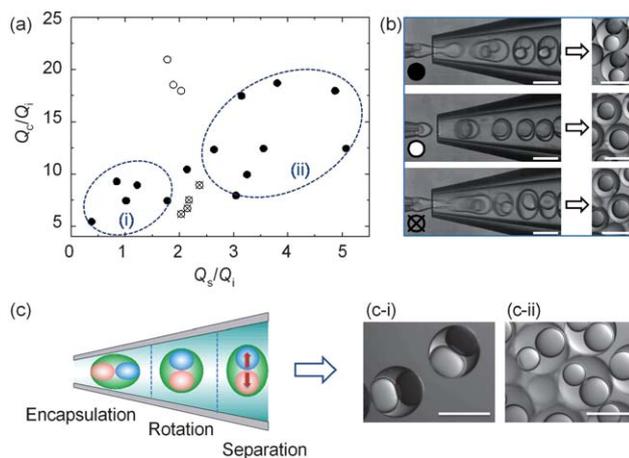


Fig. 2 The morphology of the double emulsion with two core fluids of water. (a) Coalescence of two water cores inside the shell controlled by the flow rate ratios. (●) Dual-core drops; (○) pre-mixed drops; (⊗) core-coalesced drops. (b) Images of three types of the experimental results corresponding to the data in Fig. 2(a). (c) A schematic of the process of coalescence of the cores inside the drop induced by “separation-driven” motions in the divergent tube. (c-i) When the flow rate ratio is within area (i) in (a), the two cores are squeezed by the thin shell and no coalescence occurs. One of the cores is dyed to show up dark. (c-ii) When the flow rate ratio is within area (ii) in (a), the shell is too thick to deform sufficiently to cause coalescence. The scale bars on the images are 200 microns.

A second distinct double emulsion involves pre-mixed drops, which refer to the case that the core fluids mix before they are encapsulated and so a double emulsion drop with a single inner drop is formed. This case is indicated by “○” in Fig. 2(a) and (b). As a specific example, when the flow rates are $Q_i : Q_s : Q_c = 350 : 650 : 6000 \mu\text{l h}^{-1}$, the cores A and B coalesce as soon as they flow out of the injection tip; the dynamics of the flow inside the shell is not strong enough to maintain separate cores. This response can be used to generate monodisperse double emulsions when the viscosity of the mixed core fluids is small enough for the shell to pinch off. Otherwise, monodisperse drops cannot be generated.

The third distinct double emulsion is the case of “core-coalesced” drops where the cores coalesce in the collection tube shortly after the formation of the initial drop with two distinct cores. This case is indicated by “⊗” in Fig. 2(a) and (b), where it is restricted to a relatively narrow range of Q_s/Q_i and Q_c/Q_i values. For example, with flow rates of $Q_i : Q_s : Q_c = 450 : 950 : 3050 \mu\text{l h}^{-1}$, the two core fluids are separated by the shell phase, and they form a dual-core drop at the beginning of the collection tube, where the two cores are aligned parallel to the flow direction. As the drop flows along the divergent collection tube, we observe that the cores rotate inside the shell to a perpendicular alignment in the diverging channel and tend to be separated by the internal flow, which is followed by coalescence.

The corresponding coalescence mechanism, shown in the schematics of Fig. 2(c), can be understood by the “separation-driven” models of Bremond *et al.*¹⁰ and Lai *et al.*,¹¹ due to the flow of the shell fluid inside a double emulsion droplet.²⁰ Thus, whether or not the cores within the shell coalesce depends on the thickness of the fluid layer separating them. We find that when the shell is too thin, as in the flow regime (i) in Fig. 2(a), coalescence does not occur; we interpret this as resulting from there being insufficient space for the cores to separate significantly. In this case, the cores are deformed even after

they flow out of the device (Fig. 2(c-i)). In contrast, when the shell is too thick, as in the flow regime (ii) in Fig. 2(a), the deformation of the shell is insufficient to cause separation of the cores, and again no coalescence occurs (Fig. 2(c-ii)). Although the coalescence of the cores only happens within a narrow range of the flow rates, the coalescence regime should be located between the flow regimes (i) and (ii) in Fig. 2(a), and we have demonstrated that the coalescence of the cores can be controlled by adjusting the flow rate ratios of the fluids in the glass capillary device.

The unique features offered by controlled coalescence of the cores allow us to realize specific chemical reactions inside one drop. Here we provide two examples that take advantages of the features of the “core-coalescence” technology: one is the fabrication of high viscosity hydrogel particles, and the other is the dissolution of cell membranes, which is an important step in droplet-based cell assays in microfluidics.

The “core-coalescence” approach can be used to fabricate high viscosity hydrogel particles. Monodisperse capsules of gelling biopolymers with controlled size and internal structure are in great demand,⁴ and many methods to fabricate hydrogel particles have been studied including the coalescence of pairs of aqueous drops in single emulsions^{13,14} and the diffusion-controlled gelation.^{4,5} Here, we utilize the same gelation materials, but directly achieve hydrogel particles using the “core-coalescence” method in double emulsion drops.

We use the design in Fig. 1 where core A is 0.3 wt% sodium alginate (Sigma-Aldrich) solution and core B is 0.1 wt% CaCl₂ solution. Both of the aqueous solutions contained 0.25 wt% of the surfactant Tween 20 (Sigma-Aldrich). For the flow rates $Q_i : Q_s : Q_c = 350 : 1200 : 6000 \mu\text{l h}^{-1}$, pre-mixed drops are generated. However, the mixture of cores A and B generates a high viscosity gel that cannot be pinched off by the encapsulation phase; the pre-mixed drops are connected to each other by a thin thread of the core fluid (the arrows in Fig. 3(a)). Thus, the pre-mixed method cannot produce mono-disperse hydrogel particles because of the high viscosity of the mixed core fluids, as observed previously by Zhang *et al.*⁴ However, with the flow rates $Q_i : Q_s : Q_c = 450 : 1100 : 3500 \mu\text{l h}^{-1}$, drops are generated with coalescence of core A and B occurring inside the shell phase. Thus, gelation happens after the cores are encapsulated by the shell, and monodisperse hydrogels are fabricated inside the shell (Fig. 3(b)).

The hydrogel particles are encapsulated in oil shells, and the hydrogels can be removed from the double emulsion drops by washing the emulsion with pure water and hexane in turn to remove the aqueous continuous phase and the oil shell, respectively. Without the oil shell, the hydrogel shrinks rapidly when exposed to air; the diameter is reduced 10% in 5 minutes. However, the diameter of the gel particle inside the shell phase is only reduced 2% in 20 minutes, as shown in Fig. 3(c), where small wrinkles appear on the surface of the core. Thus, the condition for core-coalescence of drops cannot only be used to make hydrogel particles, but also provides a means for storage and protection. We have used the microfluidic device to produce up to 4500 double emulsion drops per min, and more than 70% drops have their cores coalesced under the controlled flow rate ratio in a stable double emulsion system. The hydrogel particles generated by the microfluidic method have a polydispersity of 4%, as shown by the optical images in Fig. 3(d), which includes a fitted statistical diameter distribution curve.

This core-coalescence method generates pairs of reagent drops and isolates each pair from the others, which controls the collision probability and avoids the undesirable multiple coalescence between

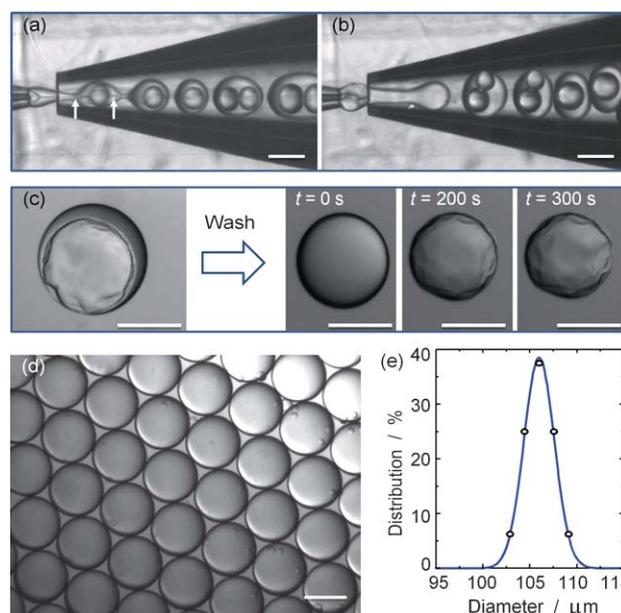


Fig. 3 Generation of hydrogel particles with the core-coalescence method. (a) Pre-mixed drops, where the core fluids coalesce immediately after they flow out of the injection tip, but the mixed fluid is not pinched off by the shell fluid because of the high viscosity. (b) Cores A and B coalesce inside the shell as they pass through the divergent tip of the collection tube. (c) Optical microscopy image of the hydrogel inside the shell exposed for 20 minutes on glass slide. After washing with pure water and hexane, the shell is removed and a hydrogel particle is acquired. The images show the shrinkage of the washed hydrogel particle after 0, 200 and 300 seconds, respectively. (d) Monodisperse hydrogel particles after washing and the statistical diameter distribution based on 32 hydrogel particles. The scale bar on each image is 100 microns.

different pairs, compared with drop coalescence in single emulsions.^{13,14} Moreover, our method may have wider applications than the diffusion-controlled method, since it has no limitation on the solubility of the crosslinker, and the gelation happens as soon as the cores coalesce, without the additional diffusion time (30–60 seconds) of cross-linking agent from the continuous phase.^{4,5} For some reactions requiring a long time, an additional advantage of the method introduced here is that the reaction can be completed inside an enclosed shell independent of the outer flows.

The coalescence of two aqueous drops for the dissolution of cell membranes. The advantages of droplet-based microfluidics include high throughputs and small sample volumes, while the encapsulation of organic liquids by an immiscible oil phase provides isolation of droplets, which tends to eliminate the risk of cross-contamination. However, it is still a challenge to combine cells and specific reagents inside droplets. The approach for such integration using dielectrophoresis^{2,3} is limited because it cannot be applied to aqueous droplets and organic-containing suspensions.²¹

Here we use the core-coalescence method to demonstrate how to merge drops containing cells and a chemical that dissolves the cell membrane, which is a necessary step for bio-applications, such as polymerase chain reaction (PCR) and protein quantification. In the experiments, core A is 1 vol% Chinese Hamster Ovary (CHO) cells in cell culture medium containing 10 vol% fetal bovine serum (FBS) and 90 vol% Dulbecco's Modified Eagle Medium (DMEM). Cells are labeled with 5 $\mu\text{g ml}^{-1}$ fluorescent dye of calcein green AM

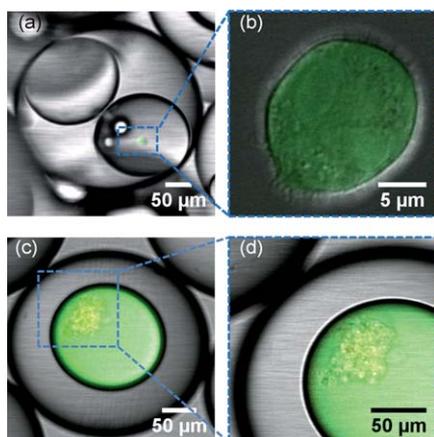


Fig. 4 Dissolution of the cell membranes. (a) Dual-core drops generated at the flow rate ratios $Q_i : Q_s : Q_c = 350 : 900 : 4500 \mu\text{l h}^{-1}$. A cell containing fluorescent dye is encapsulated in one core. (b) Larger magnification image of the cell in (a). (c) Core-coalesced drops generated at the flow rate ratios $Q_i : Q_s : Q_c = 350 : 1200 : 5000 \mu\text{l h}^{-1}$. After the membranes are dissolved, the fluorescent dye fills the entire core but is confined. (d) The remaining ghost cells can be seen with larger magnification.

(Invitrogen).²² Core B is 1 vol% Triton X-100 in deionized water, which is used to dissolve cell membranes. By controlling the flow rates $Q_i : Q_s : Q_c = 350 : 900 : 4500 \mu\text{l h}^{-1}$, dual-core drops are fabricated. Since the two cores are not coalesced, the cell membrane does not dissolve and the fluorescent dyes are confined inside the cell, as shown by the small fluorescent dot in the cell-containing core in Fig. 4(a), and the labeled cell is shown in Fig. 4(b). As we change the flow rates to $Q_i : Q_s : Q_c = 350 : 1200 : 5000 \mu\text{l h}^{-1}$, core-coalescence of drops occurs. The reagent in core B mixes with core A that contains the labelled cells and dissolves the membrane of the cells. After the breakup of the cell membrane, the fluorescent dyes are released and fill the entire coalesced core, which is encapsulated by the oil shell, as shown in Fig. 4(c) and (d).

We believe that the approach we have demonstrated here is promising because it is suitable for the coalescence of aqueous drops, and it is a purely flow-controlled method, which avoids the application of external thermal or electrical fields. Additionally, our approach is a one-step technology to realize cell encapsulation and droplet merging, which avoids the process of reinjection and other additional steps for handling drops and cells.

In summary, we present an approach for flow-controlled coalescence of different cores inside double emulsions in a microfluidic device. To illustrate possible applications, we provide demonstrations of the formation of nearly monodisperse hydrogel particles and the dissolution of cell membranes, which is a necessary step in many cell assays. The double emulsion not only acts as a “chemical reactor” that

encapsulates high viscosity fluids or even solid particles, but also provides isolation between the reaction products and the environment, which may protect the products from being contaminated by the environment, or may prevent harmful reaction products from being released to the environment. Furthermore, this coalescence of two aqueous cores provides a safe and efficient way of mixing organic materials such as cells with certain reagents that are dissolved in a water phase, which may have potential applications for cell assays and screening.

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