Designer emulsions using microfluidics

We describe new developments for the controlled fabrication of monodisperse emulsions using microfluidics. We use glass capillary devices to generate single, double, and higher order emulsions with exceptional precision. These emulsions can serve as ideal templates for generating well-defined particles and functional vesicles. Polydimethylsiloxane microfluidic devices are also used to generate picoliter-scale water-in-oil emulsions at rates as high as 10 000 drops per second. These emulsions have great potential as individual microvessels in high-throughput screening applications, where each drop serves to encapsulate single cells, genes, or reactants.

Rhutesh K. Shah[a], Ho Cheung Shum[a], Amy C. Rowat[a], Daeyeon Lee[a], Jeremy J. Agresti[a], Andrew S. Utada[a], Liang-Yin Chu[a,b], Jin-Woong Kim[a,c], Alberto Fernandez-Nieves[a,d], Carlos J. Martinez[a,e], and David A. Weitz[a,f]*

[a]*School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA*

[b]*School of Chemical Engineering, Sichuan University, Chengdu, Sichuan, 610065, China*

[c]*Amore-Pacific R&D Center, 314-1, Bora-dong, Giheung-gu, Yongin-si, Gyeonggi-Do, 446-729, Korea*

[d]*School of Physics, Georgia Institute of Technology, Atlanta, GA 30332, USA*

[e]*School of Materials Engineering, Purdue University, West Lafayette, IN 47907, USA*

[f]*Department of Physics, Harvard University, Cambridge, MA 02138, USA*

**E-mail: weitz@seas.harvard.edu*

An emulsion is a mixture of two immiscible liquids, where one liquid is dispersed in the form of small drops in another liquid that forms a continuous phase[1–4]. Common types of emulsions include oil-in-water (o/w), such as milk, and water in oil (w/o), like butter. They are extremely important for a variety of applications such as macromolecular delivery[5–8], oil recovery[9,10], food processing[11,12], and hazardous material handling[13]. The presence of a native or added surfactant is necessary for the long-term stability of emulsions: the surfactant molecules migrate to the liquid-liquid interface and inhibit droplet coalescence[1,2].

Most conventional methods for making emulsions involve drop breakup using shear or impact stresses generated by manual or mechanical agitation. However, such stresses are not uniform across the system, nor are they finely controlled. Emulsions formed in these ways thus consist of drops that are highly polydisperse in size. Various emulsification methods like membrane extrusion[14,15], viscoelastic

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shear[16,17], microchannel emulsification[18], and microthread generation[19] have been developed and optimized to gain better control over the size and polydispersity of the drops; however, greater control over drop size distribution still remains an important goal.

Microfluidic devices offer an alternate and versatile route to produce emulsions. In contrast to bulk emulsification methods, an emulsion in a microfluidic device is made by precisely fabricating one drop at a time. This process results in a highly monodisperse emulsion. One of the most attractive features of microfluidic techniques is that they enable the fabrication of double, triple, and even higher order emulsions, where the size and number of the encapsulated droplets can be manipulated with unprecedented accuracy[20,21].

We highlight some of the recent developments from our laboratory in fabricating such controlled emulsions using microfluidic devices. While we focus on emulsion formation using capillary microfluidic devices, we also briefly describe emulsion formation in more conventional microfluidic devices made using lithographic techniques. Furthermore, we demonstrate how these emulsions are useful for a variety of applications (Fig. 1). These include fabrication of monodisperse vesicles, liquid crystal shells, and particles with different internal structures.

Controlled single emulsions in microfluidic devices

The principle of drop formation in microfluidic devices can be explained using a water faucet as an example. If we turn on a faucet at a low flow rate, water drips out one drop at a time. The drop size is a result of the balance between the surface forces of the dangling drop and gravity, and therefore depends on the surface tension of the fluid and the size of the faucet. Since both the surface tension and the faucet size are constant, all the drops emerging from a dripping faucet exhibit a narrow size distribution. However, if we increase the flow rate through the faucet, a water stream, or a jet is formed. Although the jet eventually breaks up into drops too, these drops have a larger range of sizes[22–25].

The same principle can be employed in microfluidic channels that have sizes on the order of tens of micrometers. The main difference between drop formation from a faucet and in microfluidic devices is that in the former case drops are formed in air, whereas in the latter case drops are formed in another immiscible liquid. We first describe how this principle is implemented in a capillary microfluidic device and then describe its implementation in more conventional polydimethylsiloxane (PDMS) devices.

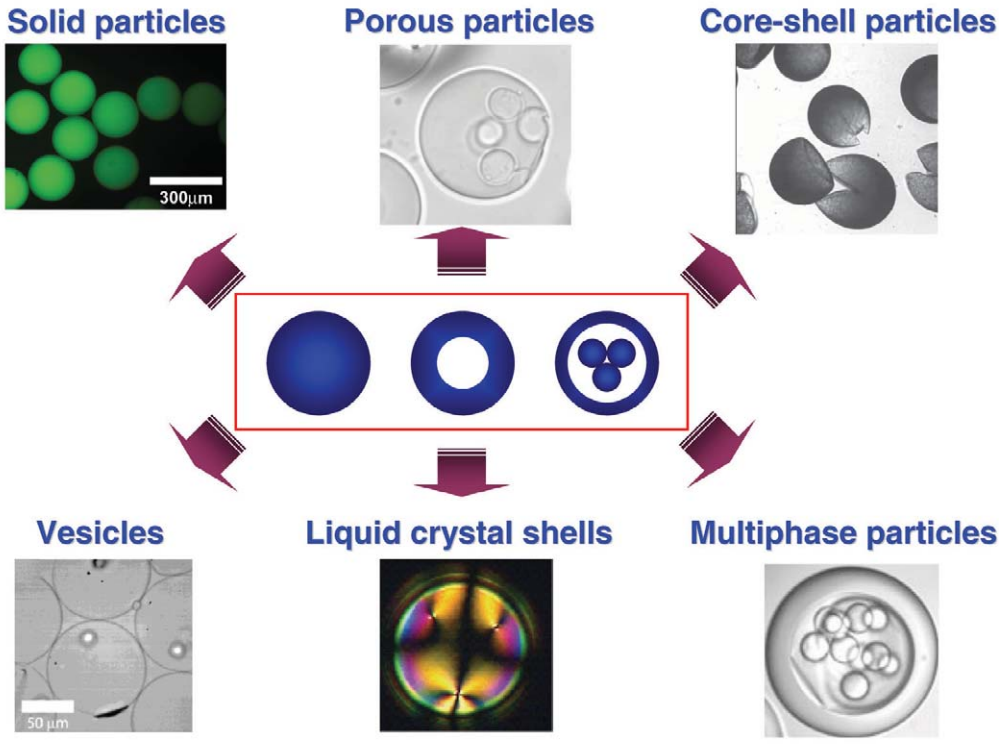

Fig. 1 Overview of some of the applications for controlled emulsions made using microfluidics. (Solid particles: reproduced with permission from[54]. © 2007 Wiley-VCH. Porous particles: reproduced with permission from[53]. © 2007 Wiley-VCH. Core-shell particles: reproduced with permission from[26]. © 2007 Materials Research Society. Vesicles: reprinted with permission from[52]. © 2006 American Chemical Society. Liquid crystal shells: reprinted with permission from[55]. © 2007 American Physical Society. Multiphase particles: reproduced with permission from[21]. © 2007 Wiley-VCH.)

Capillary microfluidic devices

Capillary microfluidic devices consist of coaxial assemblies of glass capillaries on glass slides. One of the inherent advantages of these devices is that their wettability can be easily and precisely controlled by a surface reaction with an appropriate surface modifier. For example, a quick treatment of octadecyltrimethoxysilane will make the glass surface hydrophobic, whereas a treatment of 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane will make the surface hydrophilic. Also, these devices offer the distinct capability of creating truly three-dimensional flows, which is critical for the applications described here.

We begin with a circular glass capillary with an outer diameter of 1–2 mm. This capillary is heated and pulled using a pipette puller to create a tapered geometry that culminates in a fine orifice. This is our 'faucet'. The precisely pulled circular capillary is carefully slid into a square glass capillary to form a simple microfluidic device. Coaxial alignment of the two capillaries is ensured by choosing the capillaries such that the outer diameter of the circular capillary is the same as the inner dimensions of the square capillary. One fluid flows inside the circular capillary while the other fluid flows through the square capillary in the same direction, resulting in a coaxial flow of the two fluids (Fig. 2a). The geometry of this setup is known as a co-flow geometry. When the fluids flow at low rates, individual monodisperse drops are formed periodically at the tip of the capillary orifice, in a process termed dripping (Fig. 2b and 2d)[26,27]. If we increase the flow rate of either fluid beyond a certain critical limit, the result is a jet, a long stream of the inner fluid with drops forming downstream (Fig. 2c). Generally these drops have a somewhat broader size distribution because the point at which a drop separates from the jet can vary.

An alternate geometry for drop formation in capillary devices is the flow-focusing geometry[20,28]. In contrast to co-flow capillary devices, the two fluids are introduced from the two ends of the same square capillary in opposite directions. The inner fluid is hydrodynamically flow focused by the outer fluid through the narrow orifice of the tapered round capillary, as shown in Fig. 3. Drop formation occurs immediately as the inner fluid enters the circular orifice under dripping conditions, whereas drop formation occurs further downstream under jetting conditions[26,27]. A major advantage of this method is that it allows us to make monodisperse drops with sizes smaller than that of the orifice. This feature is useful for making small droplets (~1–5 μm in diameter), especially those from a particulate suspension, where the particles may clog the orifice in the co-flow geometry[29]. The use of a capillary with a larger orifice minimizes the probability of such tip clogging by the suspended particles or any entrapped debris.

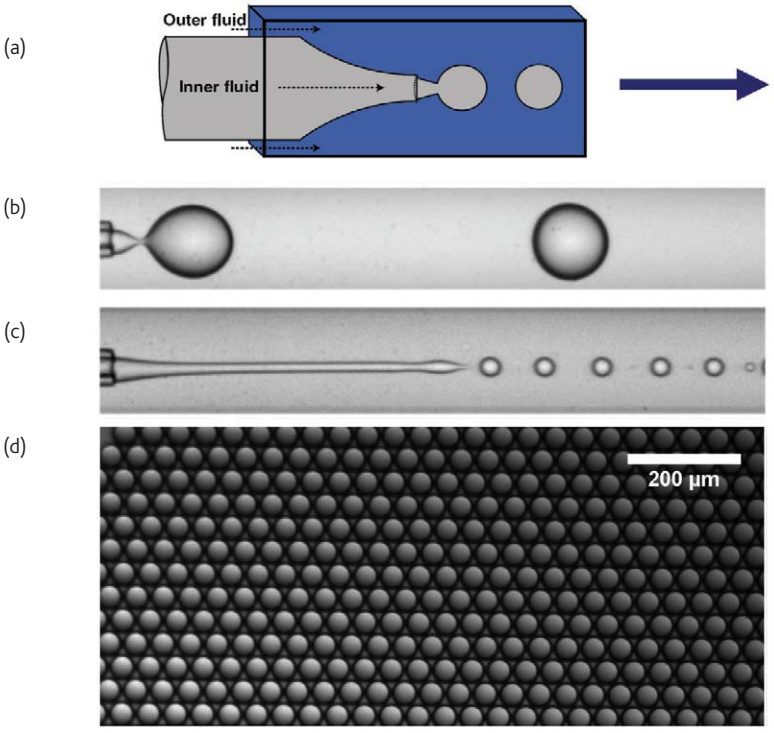

Fig. 2 Single emulsions in a co-flow microfluidic device. (a) Schematic of a co-flow microcapillary device for making droplets. Arrows indicate the flow direction of fluids and drops. (b) Image of drop formation at low flow rates (dripping regime). (c) Image of a narrowing jet generated by increasing the flow rate of the continuous fluid above a threshold value while keeping the flow rate of the dispersed phase constant. (d) Monodisperse droplets formed using a microcapillary device. [Part (a) reproduced with permission from[26]. © 2007 Materials Research Society; parts (b) and (c) reprinted with permission from[27]. © 2007 American Physical Society.]

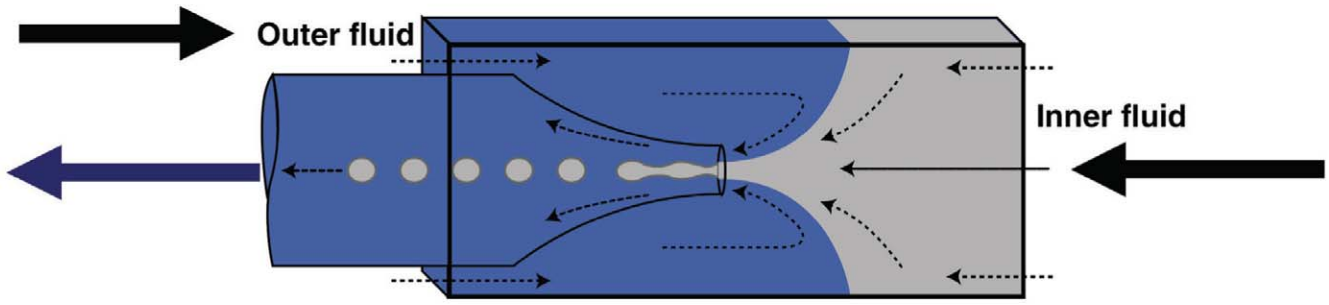

Fig. 3 Schematic of a flow-focusing microcapillary device for making droplets. (Reproduced with permission from[26]. © 2007 Materials Research Society.)

Microfluidic devices made using lithographic techniques

Traditional microfluidic devices fabricated using some variant of semiconductor technology can also be effectively employed for making single emulsions. One important method entails patterning channels in a silicone elastomer of PDMS through the use of soft lithography[30,31]. By integrating a flow-focusing configuration into the microchannels of a PDMS device, these devices can be used for making monodisperse emulsions in a controlled fashion (Fig. 4)[32]. The most attractive feature of this method is the potential ease of mass-producing these devices. Once a mask is designed, a large number of devices can be made by this stamping technique. In addition, lithography allows for the fabrication of highly complex flow channels for upstream adjustments of the fluid streams and downstream manipulation of the droplets, allowing multiple functions to be performed in one device. For example, drops can be split in a controlled manner to form smaller droplets with a monodisperse or bidisperse size distribution[33]. Alternately, droplets can be selectively fused by electrocoalescence to form large drops[34]. These features are useful in many chemical and biochemical applications such as enzyme assays, enzyme inhibitor assays, and protein translation assays in microfluidic systems.

Despite these advantages, PDMS is incompatible with most common organic solvents[35], which limits the use of these devices for material synthesis applications. Efforts are currently underway to coat the PDMS channels with relatively inert materials. However, these technologies are still in their infancy.

Controlled multiple emulsions in microfluidic devices

Multiple emulsions are hierarchical systems in which dispersed drops contain smaller droplets inside. They can be used for the controlled

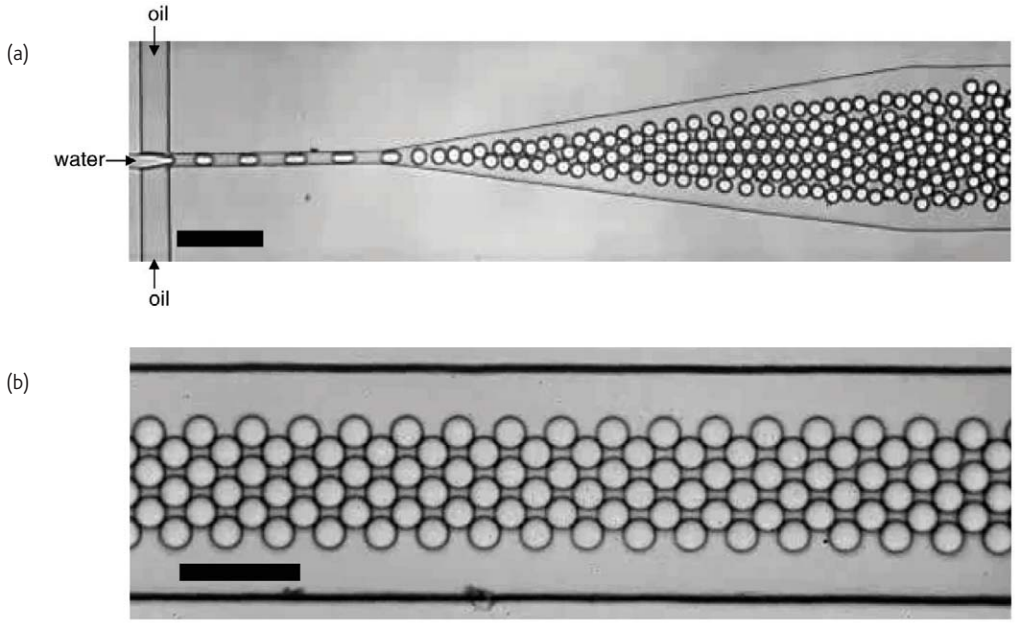

Fig. 4 Formation of single emulsions in a PDMS device: (a) droplet formation by flow focusing; and (b) an array of monodisperse droplets imaged downstream in the device. Scale bars are 50 μm. The drops are made at 10 kHz.

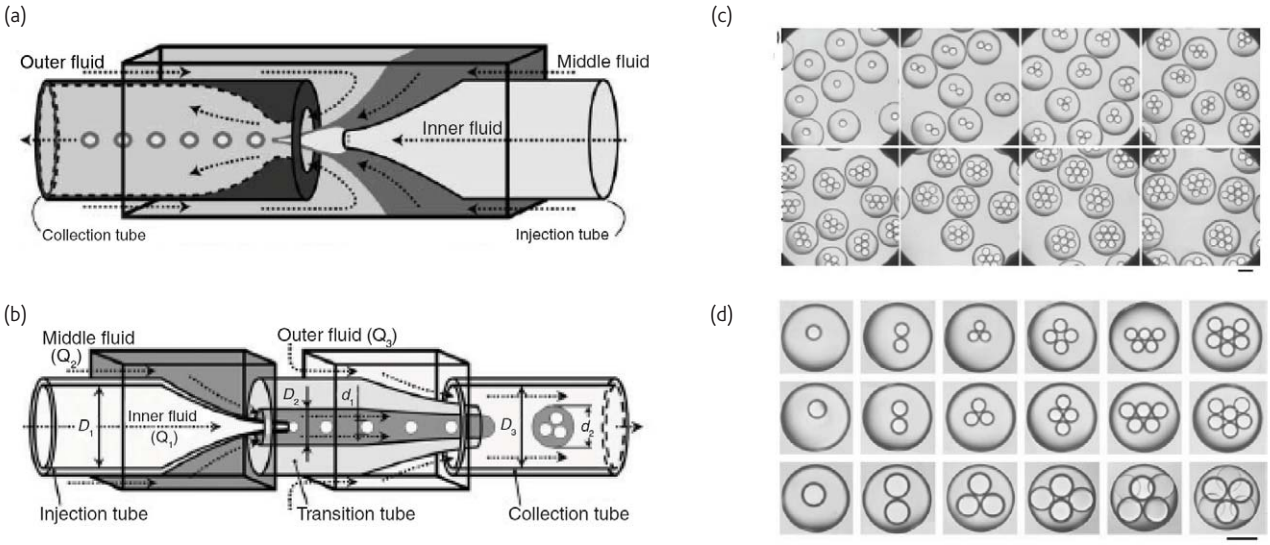

Fig. 5 Fabrication of double emulsions in microfluidic devices. (a) Schematic of a capillary microfluidic device that combines co-flow and flow focusing. (b) Schematic of a device that employs two sequential co-flow emulsion generators. (c) Optical micrographs of monodisperse double emulsions containing a controlled number of monodisperse single emulsions. (d) Optical micrographs of monodisperse double emulsions showing controlled increase in the diameter of the inner droplets in each column, while the number of inner droplets is constant. Scale bars are 200 μm. [Part (a) reproduced with permission from[20]. © 2005 American Association for the Advancement of Science; parts (b)–(d) reproduced with permission from[21]. © 2007 Wiley-VCH.]

encapsulation and release of materials in cosmetics[36,37], drug delivery[6–8], and food applications[11,38,39]. Accurate control of the size and structure of emulsions is often essential for these applications as these features directly affect the loading levels and the release kinetics of the encapsulated substances[40]. Although fabricating such emulsions in a PDMS device is desirable, the process requires highly complex and difficult spatial control of the wettability of the PDMS channels[41]. In contrast, glass capillary microfluidic devices can be more easily adapted to fabricate multiple emulsions; surface wettability in these devices can be easily modified and solvent compatibility issues are practically nonexistent[20].

One design of a glass capillary device for making double emulsions combines both co-flow and flow focusing[20] (Fig. 5a). This device consists of two circular capillaries arranged end-to-end within a square capillary. By ensuring that the inner dimensions of the square capillary are the same as the outside diameters of the round capillaries, we achieve good coaxial alignment. The inner fluid is pumped through the tapered circular capillary while the middle fluid, which is immiscible with the inner and outer fluids, flows through the outer capillary in the same direction. The outermost fluid flows through the outer capillary in the opposite direction and hydrodynamically flow focuses the coaxially flowing stream of the other two fluids, which approach from the opposite end. When the three fluids enter the collection tube, a perfect double emulsion is formed.

A more flexible design employs stepwise emulsification of co-flowing streams to create a double emulsion[21] (Fig. 5b). This device employs two sequential co-flow emulsion generators; it consists of an injection tube that is inserted into a transition tube. The other end of the transition tube is also tapered and is inserted into a third, coaxially aligned cylindrical capillary tube, the collection tube. The innermost fluid flowing through the injection tube is emulsified in the transition tube by coaxial flow of the middle fluid. The single emulsion is subsequently emulsified in the collection tube by coaxial flow of the outermost fluid, which is injected into the outer stream through the square capillary (Fig. 5b). The size and number of both the outer and inner drops can be precisely tuned by adjusting the dimensions of the orifices or the flow rates of the three fluids. Optical micrographs of double emulsions with different numbers and sizes of encased droplets are shown in Figs. 5c and 5d. Such uniformity and control cannot be achieved with traditional bulk emulsification methods for making double emulsions[14,42–44].

The sequential emulsion fabrication process has the advantage that it is highly scalable: higher order emulsions can be made simply by adding more stages. To illustrate this, we have made monodisperse triple emulsions by adding a third co-flow stage as shown in Fig. 6a. As in the case of double emulsions, both the diameter and the number of the individual drops can be efficiently controlled at every level, as shown by the series of triple emulsions with one to seven innermost drops and one to three middle drops in each outer drop (Fig. 6e)[21].

Applications

The ability to generate monodisperse emulsions with precisely designed internal structures has led to the development of novel synthesis and fabrication techniques. In Fig. 1, we gave an overview of some

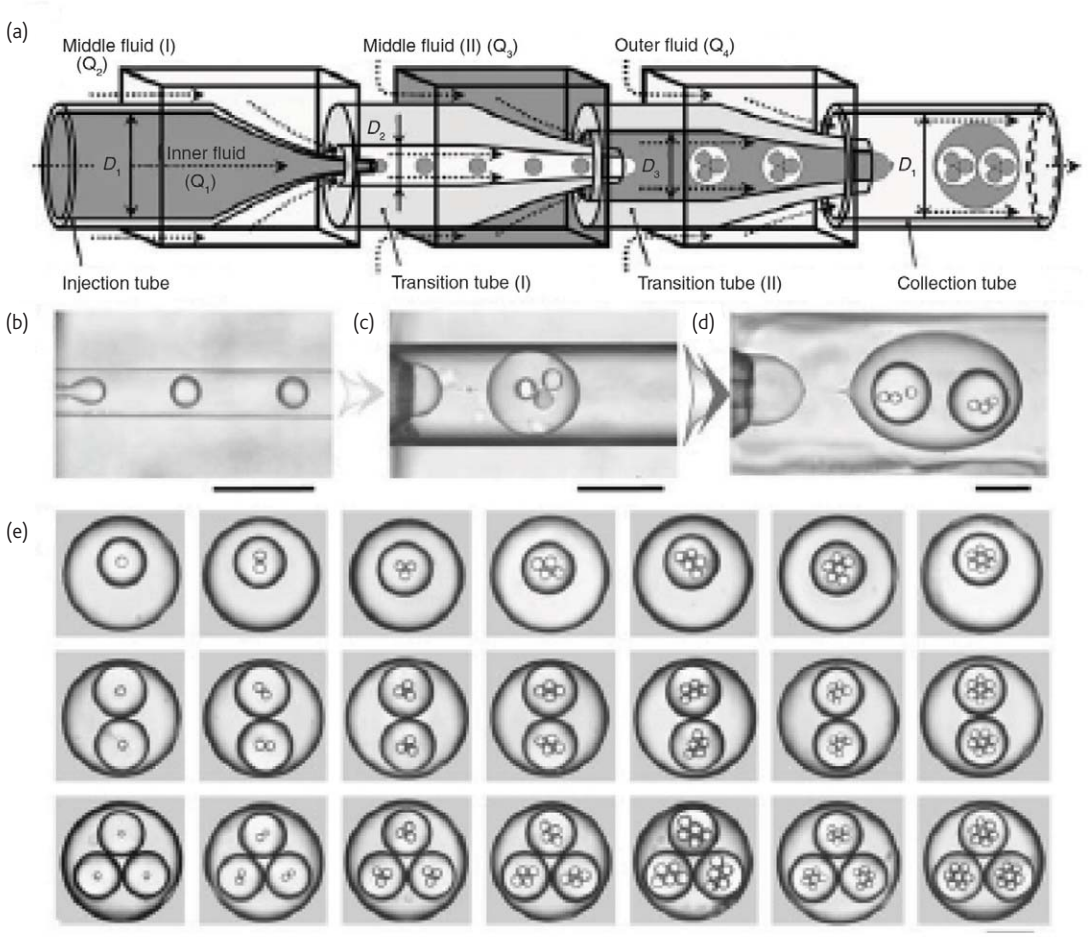

Fig. 6 Fabrication of triple emulsions in a microfluidic device. (a) Schematic of an extended capillary microfluidic device for generating monodisperse triple emulsions. (b)–(d) Optical micrographs taken with a high-speed camera displaying the (b) first, (c) second, and (d) third emulsification stages. (e) Optical micrographs of triple emulsions that contain a controlled number of inner and middle droplets. (Reproduced with permission from[21]. © 2007 Wiley-VCH.)

applications for controlled emulsions made using microfluidics. In the following sections, we provide details of how these emulsions can be tailored to address the requirements of these specific applications.

Fabrication of functional vesicles

Vesicles, in the classical sense, are bilayers of amphiphilic molecules that fully enclose a fixed volume of fluid. They are ideal for encapsulating and releasing drugs or other macromolecules in a controlled fashion, since their mechanical properties, permeability, and selectivity can be tuned by varying their wall thickness or chemical composition. The encapsulated material in such vesicles can also be burst-released at a desired time and location simply by destabilizing the bilayer structure.

Most conventional techniques for vesicle preparation entail rehydration of dried films of amphiphilic molecules[45–47]. The rehydration process causes the molecules to assemble into layers that fuse to form vesicles or other ordered structures such as micelles or worm-like micelles. However, the resultant structures are highly polydisperse. Encapsulation of active materials with these methods is done either concurrently with the vesicle fabrication process, by dissolving the materials in the solution used for rehydration, or post-fabrication, by osmotically driving the materials into the vesicles. In either case, the encapsulation efficiency is low (generally less than 35%)[48–50]. The high polydispersity and low encapsulation efficiency limit the widespread use of these structures for encapsulation and controlled-release applications.

We have developed an alternate strategy to fabricate monodisperse vesicles with high encapsulation efficiency by employing highly monodisperse double emulsions as templates. For example, we make water-in-oil-in-water (w/o/w) emulsions using an organic solvent that contains amphiphilic molecules as the middle fluid and an aqueous solution of the material to be encapsulated as the inner fluid. The amphiphilic molecules stabilize the two oil-water interfaces, one between the inner droplet and the outer shell and the other one between the outer shell and the continuous phase. Subsequent evaporation of the oil phase results in a uniform population of stable

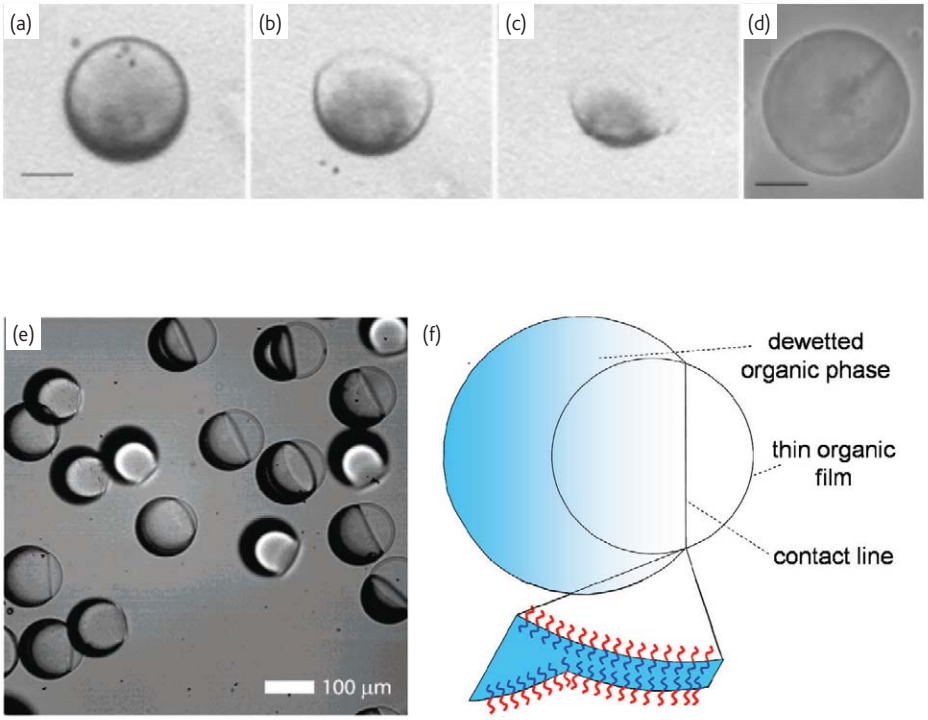

Fig. 7 Polymersomes formed using microfluidic devices. (a–d) Optical micrographs showing the formation of a polymersome from a double emulsion. (a) The middle fluid layer, a volatile organic solvent, is clearly visible. (b, c) Index matching of the inner and outer fluids causes the drop to fade in brightfield microscopy as the solvent evaporates from the middle layer. The images in panels (a–c) were taken 3 mins apart. The scale bar is 40 μm. (d) Polymersome imaged with phase contrast microscopy. The scale bar is 30 μm. (e) Optical micrograph showing dewetting during solvent evaporation from a double emulsion droplet. (f) Schematic of the proposed structure of a double emulsion droplet with partial wetting of the organic phase on a thin layer of solvated block copolymer brushes. (Adapted and reproduced with permission from[51,52]. © 2005, 2006 American Chemical Society, respectively.)

bilayer membrane vesicles. Since we can precisely control the size of the inner droplet, we can encapsulate exactly the same amount of active material in each vesicle. Moreover, since the innermost and outermost fluid streams are completely separated, the encapsulation efficiency can approach 100%, provided all other loss mechanisms are eliminated.

We illustrate this experimental approach by forming monodisperse polymer vesicles, also known as polymersomes, from a diblock copolymer, poly(n-butyl acrylate)-*block*-poly(acrylic acid) (PBA-PAA), using a capillary microfluidic device[51]. PBA is a hydrophobic polymer whereas PAA is a hydrophilic polymer. Hence, when an oil containing PBA-PAA is used as the middle phase to form w/o/w double emulsions, the block copolymer chains migrate to the two oil/water interfaces, and thereby stabilize the double emulsion. Upon evaporation of the organic oil phase, the PBA-PAA diblocks self-organize into a vesicle. Optical micrographs showing the formation of a polymersome from a double emulsion drop are presented in Figs. 7a–7d. As the oil evaporates, the optical contrast is lost. This makes it difficult to observe the drop by bright-field microscopy since both the inner compartment and the outer surrounding solutions are aqueous (Figs. 7a–7c). However, when imaged with phase contrast microscopy, the structure of the resulting polymersome is clearly visible (Fig. 7d). These polymersomes have a solid flexible structure as shown by their collapse in response to an osmotic pressure[51].

Another example of a functional vesicle that we are currently fabricating using the multiple emulsion technique is a liposome, a vesicle with phospholipid bilayers. Phospholipids, which constitute the majority of biological membranes found in nature such as the plasma membrane, have polar head groups and hydrophobic tails and self-assemble into bilayers in an aqueous environment. They provide excellent model systems for studying the physical properties of biological membranes and also have great potential for encapsulation and targeted drug delivery as they can be made fully biocompatible. However, since lipid vesicles are more fragile than polymersomes, their preparation is more delicate.

A key control variable in the fabrication of polymersomes or liposomes using these microfluidic techniques is the concentration of the amphiphilic molecules: if the concentration is lower than the amount required to fully cover the oil-water interfaces, the vesicles will be unstable. It is therefore necessary to work with a slight excess concentration of these molecules. In a recent study[52], we fabricated polymersomes from polystyrene-*block*-poly(ethylene oxide), PS-PEO, using an excess amount of the diblock copolymer. A distinct drop of oil is observed on one side of the shell during the solvent evaporation

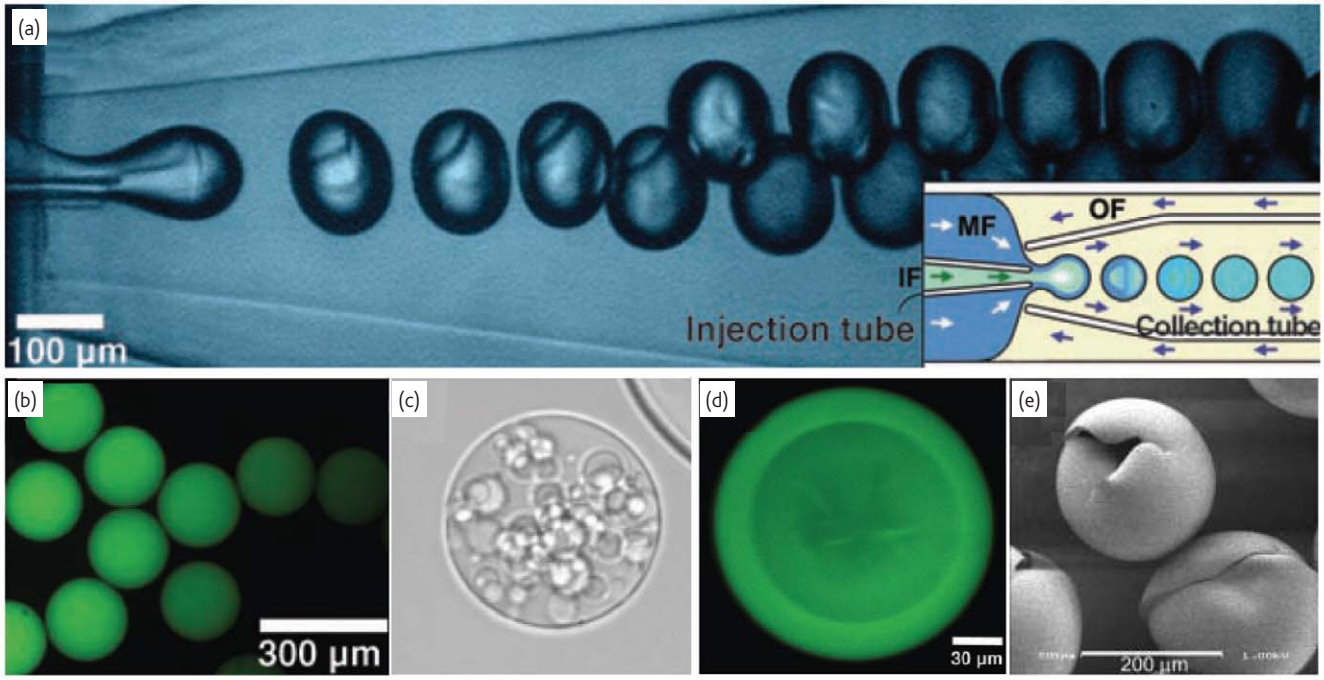

Fig. 8 Monodisperse particle synthesis in microfluidic devices. (a) Formation of premicrogel drops in a capillary device. The inset shows the device geometry. The outer fluid (OF) is silicon oil. The middle fluid (MF) is an aqueous solution containing NIPAM monomer, a cross-linker, a reaction accelerator, and other functionalizing chemicals. The inner fluid (IF) is an aqueous solution containing the reaction initiator. (b) Fluorescence image of fluorescein isothiocyanate (FITC)-labeled PNIPAm microgels prepared using this device. (c) PNIPAM microgel with spherical voids formed by dissolving embedded polystyrene beads. (d) Fluorescence microscope image of an FITC-labeled microgel shell prepared from a premicrogel double emulsion that contained a single silicon oil droplet. (e) Scanning electron micrographs of mechanically crushed rigid shells of a cross-linked epoxy made from double emulsions. [Parts (a–d) adapted and reproduced with permission from[53,54]. © 2007 Wiley-VCH; part (e) reproduced with permission from[20]. © 2005 American Association for the Advancement of Science.]

(Fig. 7e). This is attributed to the dewetting transition caused by the excess diblock copolymer, which creates a depletion interaction and leads to the partial wetting of the organic phase on the copolymer bilayer (Fig. 7f). Eventually, the oil phase completely evaporates leaving a small region of thicker polymer on the corresponding side of the polymersome. This inhomogeneity is minimized by restricting the amount of excess diblock polymers[52].

Microparticle fabrication

The ability to create uniform drops using capillary microfluidic devices can be exploited to fabricate monodisperse spherical microparticles. We have demonstrated this by fabricating monodisperse poly(N-isopropylacrylamide) (PNIPAm) microgels by an *in situ* polymerization reaction in a capillary microfluidic device[53,54]. PNIPAm microgels are thermoresponsive cross-linked polymeric particles that are formed by polymerizing NIPAm monomer and a cross-linker. The polymerization reaction is chemically initiated and is generally performed at elevated temperatures (~70°C). However, the use of an accelerator allows this reaction to occur instantaneously at room temperature. We have generated monodisperse PNIPAm microgels by taking advantage of these fast reaction kinetics together with the ability of microfluidic devices to isolate fluids right until drop formation. Using a double emulsion capillary device, we flowed an aqueous solution of NIPAm monomer, cross-linker, and accelerator as the middle fluid; the inner phase contains the reaction initiator dissolved in water, and the outer phase consists of silicon oil. Monodisperse drops containing the middle and the inner fluids are formed in the collection tube as shown in Fig. 8a. When the accelerator comes in contact with the initiator in the drops, it starts a redox reaction that polymerizes the monomers, resulting in the formation of monodisperse microgels (Fig. 8b). In this device, the injection tube (the microcapillary containing the inner fluid) is inserted slightly into the collection tube. This allows the drops to form before the accelerator meets the initiator, thus eliminating the possibility of plugging of the collection tube orifice by untimely polymerization of the monomers.

This method can be adapted to make microgels with different internal structures. As an example, we have fabricated monodisperse microgels with embedded voids using a two-step process[53]. In the first step, solid microbeads are embedded in the microgels by adding these beads to the aqueous monomer mixture. In the second step, these embedded solid beads are dissolved by immersing the microgels in a suitable solvent, thus forming microgels with voids of the same size as the beads (Fig. 8c). Such microgels with voids respond faster to changes in temperature compared with their voidless counterparts[53].

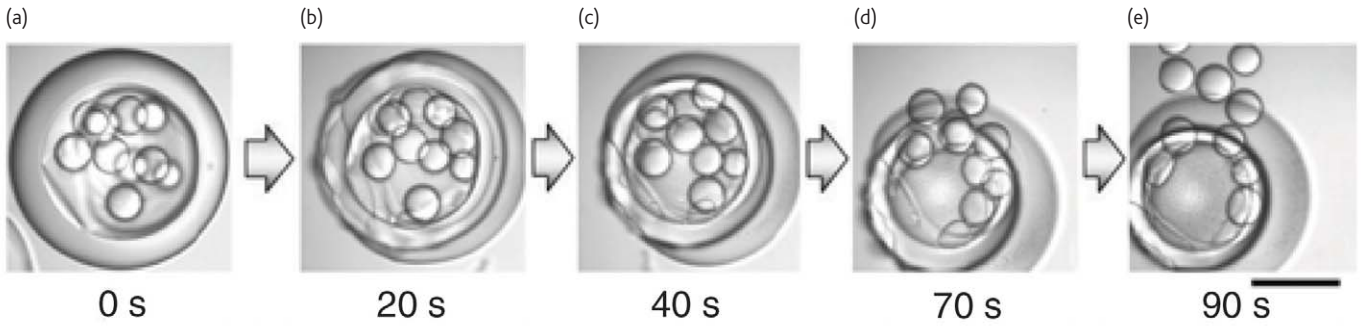

Fig. 9 Thermosensitive hydrogel microcapsule for the burst-release of encapsulated materials. This capsule was fabricated from a triple emulsion. (a) Optical micrograph of a microcapsule that consists of a shell of a temperature-sensitive hydrogel that encapsulates an oil core that contains several water droplets. (b–e) A time series of optical micrographs displaying the microcapsule behavior upon rapidly increasing the temperature from 25–50°C. The time series begins once the temperature reaches 50°C. Upon heating, the thermosensitive hydrogel shell rapidly shrinks and expels water; however, since the inner oil core is incompressible, the hydrogel shell breaks and instantaneously releases the encapsulated oil and innermost water droplets into the continuous oil phase. The scale bar is 200 μm. (Reproduced with permission from[21]. © 2007 Wiley-VCH.)

The procedure for making solid microgels can also be modified to make microgel capsules with a uniform core-shell structure[54]. This method entails the formation of o/w/o double emulsions using the aqueous mixture of the NIPAm monomer, cross-linker, and initiator as the middle phase and oil containing the reaction accelerator as the inner phase. Once the drops are formed, the accelerator diffuses from the internal oil phase into the surrounding monomer mixture, initiates the polymerization reaction, and results in the formation of microgel capsules with a core-shell structure (Fig. 8d). In another study, we have made solid polymer shells by photopolymerizing an ultraviolet (UV)-curable monomer that comprises the middle phase of w/o/w emulsions[20,26]. The solid core-shell morphology of the particles is confirmed by electron microscopy of the crushed particles (Fig. 8e).

The multiple emulsion technique can also be employed to produce novel particles with complex architectures. To illustrate this potential, we have used a w/o/w/o triple emulsion structure to fabricate thermoresponsive microgel capsules that encapsulate both aqueous and oil phases[21]. We used a surfactant-containing oil as the outer fluid and water as the innermost fluid. The middle fluid (II) is an aqueous solution of the monomer, cross-linker, and initiator, while the inner middle fluid (I) is an oil with a reaction accelerator. When the accelerator in the middle fluid (I) diffuses into the outer aqueous shell, the monomer in the shell is polymerized to form a solid capsule. The fabricated microcapsule consists of a shell of thermosensitive hydrogel encapsulating an oil core that contains several water droplets (Fig. 9a). Upon heating from 25°C to 50°C, the thermosensitive hydrogel shell rapidly shrinks and expels water. However, since the inner oil core is incompressible, the hydrogel shell breaks and instantaneously releases the innermost water droplets into the continuous oil phase (Figs. 9b–9e). Thus, the structure protects the innermost water droplets in the hydrogel shell until their temperature-induced release[21] and, hence, could be very useful for the controlled release of active substances.

Shells of liquid crystals

The double emulsion technique can also be used to create shells of liquid crystals. To achieve liquid crystal shells, the liquid crystal is mixed with an appropriate solvent to reduce its viscosity and make it isotropic. This mixture is the middle phase between the aqueous inner and outer phases. After the double emulsions are formed, the solvent evaporates leaving shells of liquid crystal (Fig. 1). The thickness and size of the shells can be regulated by varying the fluid flow rates. Our fundamental studies of liquid crystals confined in spherical shells reveal that the type and number of defect structures in these crystals change with the thickness of the shell. For very thin shells, a rich variety of previously undiscovered defects have been observed[55].

Drops and gels for biological applications

In addition to fabricating novel materials, the ability to fabricate microfluidic emulsions with precise control over size and composition has great potential for studies of biology in droplets. Here we describe the use of both single and double emulsions for biological applications.

Using PDMS microfluidic devices, we can make monodisperse water droplets in oil at high production rates that can approach 1–10 kHz[56]. The picoliter-scale volumes of these drops enable high-throughput screens to be performed with million-fold reductions in volume and reagent costs compared with conventional screening techniques. Moreover, these picoliter volumes are comparable to those of single cells, such that single nucleic acids, proteins, and whole cells are at functionally relevant concentrations. For example, one gene in a drop of 15 μm is at a concentration of a few picomolar. We have developed a set of tools to fuse drops[34], incubate them, detect encapsulated reaction products[57], and manipulate drop position[56,58]. All of these manipulations can be performed in PDMS microfluidic devices, enabling the drops to be used as individual reaction vessels for high-throughput chemical reactions and other screening applications.

We can also use emulsions as a template for the synthesis of biocompatible solid gel particles that provide a scaffold for biological material such as cells. We demonstrate this by fabricating biocompatible cross-linked alginate microgels from w/o/w double emulsions: the inner phase is an aqueous alginate solution, the middle phase is silicon oil, and the outer phase is an aqueous solution containing the cross-linker, Ca[59]. Double emulsions are formed and collected, but they do not remain stable; when the inner phase breaks through the middle oil phase into the outer phase, the divalent calcium ions come in contact with the alginate and cause the chains to cross-link, resulting in hydrogel particle formation. By incorporating yeast cells into the inner phase, cells become embedded in the hydrogel matrix. The resulting hydrogel particles can be separated from the oil phase, transferred to different solutions, and stored, making them versatile for numerous applications. For example, these gels provide a three-dimensional matrix for immobilizing cells, and can thus facilitate cell transplantation, while allowing for diffusion of nutrients and metabolites to and from the cells[60,61].

Conclusion

We have presented novel, facile, and scalable techniques for producing monodisperse emulsions in microfluidic devices. Our techniques allow us to fabricate emulsions whose structural features can be controlled with unprecedented accuracy. These emulsions are useful for a variety of applications ranging from microparticle fabrication to vesicle formation, and chemical synthesis to high-throughput screening of single cells.

To further expand the application portfolio of these emulsions, it will be necessary to engineer pathways to scale up their production. This can be accomplished by operating microcapillary devices in parallel or, alternatively, with devices microfabricated from PDMS. With such parallelization, production rates on the order of 1 kg/day are feasible, a rate that is sufficient for commercial production of high value-added ingredients, such as those used in cosmetics or pharmaceuticals. Ultimately, it is conceivable that even higher production rates may be achieved from further scale up through even greater parallelization. mt

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