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Microfluidic production of multiple emulsions and functional microcapsules

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Recent advances in microfluidics have enabled the controlled production of multiple-emulsion drops with onion-like topology. The multiple-emulsion drops possess an intrinsic core–shell geometry, which makes them useful as templates to create microcapsules with a solid membrane. High flexibility in the selection of materials and hierarchical order, achieved by microfluidic technologies, has provided versatility in the membrane properties and microcapsule functions. The microcapsules are now designed not just for storage and release of encapsulants but for sensing microenvironments, developing structural colours, and many other uses. This article reviews the current state of the art in the microfluidic-based production of multiple-emulsion drops and functional microcapsules. The three main sections of this paper discuss distinct microfluidic techniques developed for the generation of multiple emulsions, four representative methods used for solid membrane formation, and various applications of functional microcapsules. Finally, we outline the current limitations and future perspectives of microfluidics and microcapsules.

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1. Introduction

Emulsions, fine dispersions of droplets of one liquid in another immiscible liquid, have been utilized in a wide range of industries, including cosmetics, pharmaceuticals, and foods. The emulsion is used as-prepared without further solidification or subjected to further processing to produce various colloidal and granular materials.[1–4] The emulsion drops can contain smaller drops inside them to form drops-in-drops dispersed in a third continuous phase, which are called double-emulsion drops. In addition, drops-in-drops-in-drop or even higher orders of the hierarchy can be prepared; we here refer to drops composed of the single compartment as single-emulsion drops and double-emulsion drops or additional levels of hierarchy as multiple-emulsion drops. Multiple-emulsion drops have served as useful templates to produce microcapsules due to their core–shell geometry.[5,6] Nevertheless, a conventional bulk process of sequential

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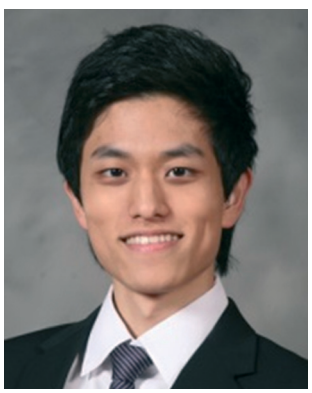

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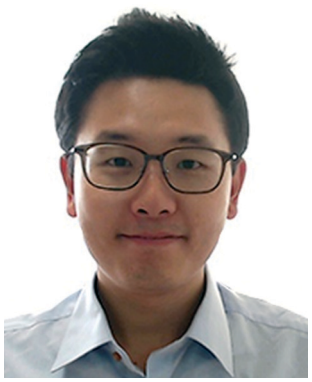
mixing of liquids with carefully-selected surfactants achieves only limited control over order, size, and composition of multiple-emulsion drops, restricting their uses. Recent advances in microfluidics have enabled the production of multiple-emulsion drops in an unprecedentedly controlled manner, albeit production throughput remains relatively low in comparison with a bulk process.[7–9] The order of multiple-emulsion drops is dictated by the number of flows whereas the numbers and sizes of all the inner drops confined in the outermost drop are precisely controlled by manipulating flow rates. Microfluidic emulsification techniques have promoted the development of novel carriers for the controlled release of encapsulants and inspired the introduction of various functions to microcapsules, opening up new applications. Elaborate designs of microcapsules are on the other hand very difficult to achieve with a bulk process.

This review article summarizes and discusses key contributions to this field of microfluidic production of multiple emulsions and solid-shelled microcapsules and their applications. We first discuss the hierarchical levels of multiple-emulsion drops, available sets of immiscible fluids, and the microfluidic techniques for preparing multiple-emulsion drops. We then discuss methods for converting the liquid shells of emulsion drops to solid membranes of microcapsules and study achievable functions of the membranes. In these two sections, we have classified previous works into several categories and discuss strengths and weaknesses in each category rather than describing technical details. The third section deals with applications of microcapsules, designed using multiple-emulsion drops, which range from drug delivery vehicles to cell carriers, colouration granules, microcapsule-type sensors, and many others. A few key contributions are described in detail. Finally, we outline the current challenges and outlook on the microfluidic technology for producing functional microcapsules.



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2. Multiple-emulsion drops

2.1 Order and combination of phases

A single emulsion is composed of two immiscible liquid phases: one phase is dispersed in the other, continuous phase. If the continuous phase is a gas, minute liquid compartments are called aerosol, and if the dispersed phase is a gas, the gas compartments are called bubbles. The double emulsion has a drops-in-drop configuration, where the inner and outer drops are immiscible; the inner drops and the continuous phase are not necessarily immiscible because the inner drops are completely isolated from the continuous phase by the outer drops. The inner drops can contain additional immiscible drops to give a three-level hierarchy of drops-in-drops-in-drops, which are called triple-emulsion drops. In the same manner, the level of the hierarchy can be four (quadruple), five (quintuple) and even larger, as illustrated in Fig. 1a. We here refer to emulsion drops with a level of hierarchy of two or above as multiple-emulsion drops.

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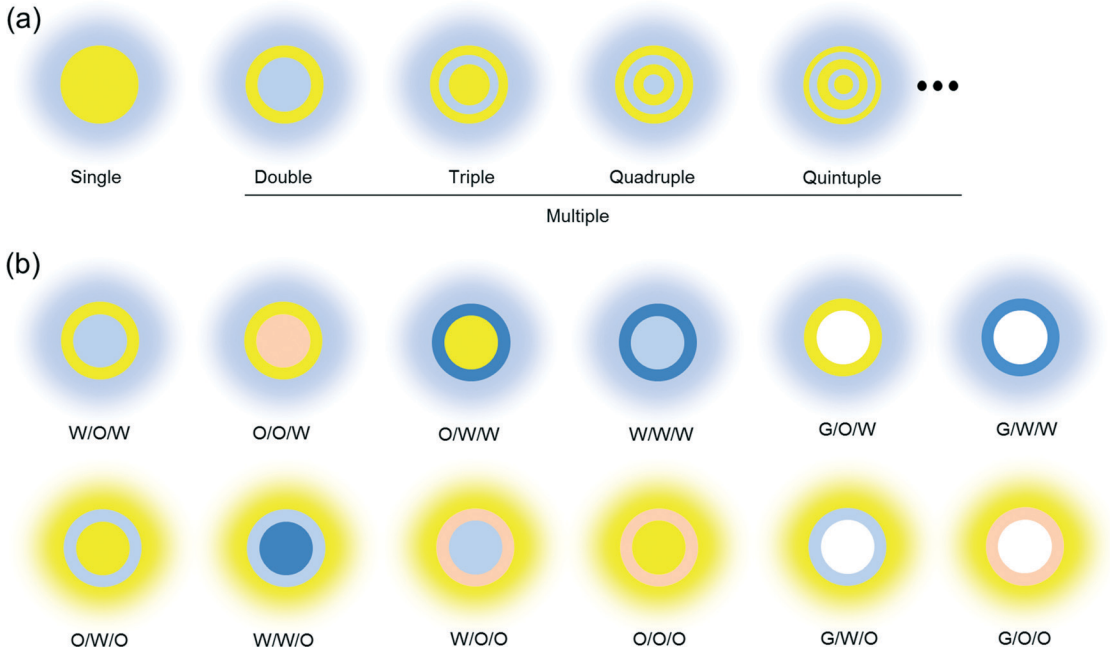

Fig. 1 (a) Levels of hierarchy in emulsion drops: double-emulsion drops or higher orders are classified as multiple-emulsion drops. (b) A set of all composition combinations physically allowable in double-emulsion drops: combinations with a continuous water phase are shown in a top row and those with an oil continuous phase are in a bottom row, where W, O, and G represent water, oil, and gas, respectively. There are a mutually immiscible set of oils and set of aqueous solutions.

In multiple emulsions, neighbouring phases should be immiscible to form separated compartments. Water (W) and oil (O) are immiscible in general and can compose multiple-emulsion drops by being alternatively positioned. For example, two possible configurations for double emulsions with alternating phases are water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O), here referring to the phases in the sequence inner drop/outer drop/continuous phase. There are many sets of oils that are immiscible in each other.[10] For example, fluorocarbon and hydrocarbon are immiscible at room temperature. Two different aqueous solutions are sometimes immiscible when they contain polymers with low affinity to one another.[11,12] For example, aqueous solutions of poly(ethylene glycol) (PEG) and dextran are immiscible when the concentrations of the solutions are high. Therefore, the available set of liquid phases to compose double-emulsion drops is not simply 2 but 8; W/O, O/O, O/W, and W/W are available for inner and outer drops in a water continuous phase and O/W, W/W, W/O, and O/O are available in an oil continuous phase. Moreover, a gas (G) bubble can be contained inside, which gives four additional combinations: G/O/W, G/W/W, G/W/O, and G/O/O. The gas bubble is almost impossible to be added as the middle layer due to the extremely low stability of the structure. Therefore, a total 12 combinations of phases—3 (inner) × 2 (outer) × 2 (continuous)—are available for double-emulsion drops, as illustrated in Fig. 1b. For triple-, quadruple-, and quintuple-emulsion drops, the numbers of available combinations are 24, 48, and 96, respectively.

In principle, all the combinations of phases can be prepared if they satisfy the condition of low miscibility between neighbouring phases. However, not all of them have been reported. That is because some of them are difficult to stabilize, less useful, or under study. The phases should be carefully selected when designing microcapsules by considering all the aspects of post-processing for membrane formation, membrane functionality, and application. The most popular combination for double-emulsion drops is W/O/W. With W/O/W, solid-shelled microcapsules dispersed in water can be directly prepared by simply converting the oil layer to a solid shell, and 100% efficiency of encapsulation of the water core is achievable. Recently, W/W/W drops have been studied for encapsulation of delicate biomaterials because they do not involve any oil phases that are potentially toxic.[12] G/O/W drops have been used to produce gas-filled microparticles.[13–15]

Double-emulsion drops can be thermodynamically stable against phase inversion or internal coalescence if the spreading parameter for the middle phase is larger than 0; they are however still unstable against coalescence between drops at the same level. For example, an O1/O2/W double-emulsion drop is stable if the interfacial tension between O1/W is larger than the sum of those between O2/W and O1/O2; that is, the spreading parameter of O2, $S_{O2} = \gamma_{O1/W} - (\gamma_{O2/W} + \gamma_{O1/O2})$, is larger than 0, where γ is interfacial tension.[16–19] If an O2/O1/W double-emulsion drop is somehow formed, phases are eventually inversed to form stable O1/O2/W drops. Paired drops of O1 and O2 spontaneously evolve to O1/O2/W double-emulsion drops to minimize the total interfacial energy. In general, the tension of the G/W interface is much larger than that of G/O and O/W interfaces, which renders G/O/W stable.[13] When multiple emulsion drops involve miscible fluids in different

levels, drops coalesce, instead of phase inversion, reducing the order by one. For example, W/O/W double-emulsion drops are transformed to O/W single-emulsion drops upon the coalescence of inner drop to continuous phase.

To retard coalescence between drops in the same and different levels or phase inversion, surfactants (surface-active agents) are used to stabilize interfaces between two immiscible fluids. For example, W/O/W double-emulsion drops rupture as soon as they are prepared in the absence of surfactants. The use of appropriate surfactants prolongs the lifetime of double-emulsion drops to the order of seconds, hours, or even days. Surfactants usually have hydrophilic and hydrophobic parts, which align at the interface to minimize undesirable exposure of both parts. This alignment of amphiphiles at the interface reduces interfacial tension and simultaneously forms a physical barrier against coalescence. In practice, surfactants are selected by following the Bancroft rule: the phase in which a surfactant is more soluble constitutes the continuous phase.[20] For W/O/W double-emulsion drops, therefore, water-soluble surfactants are added in the continuous water phase to stabilize the outer O/W interface, whereas oil-soluble surfactants are added to stabilize the inner W/O interface, as illustrated in Fig. 2a. In general, the water-soluble surfactant has a relatively large hydrophilic part —hydrophilic–lipophilic balance (HLB) larger than 7—, favouring an O/W interface, whereas oil-soluble surfactant has a relatively large hydrophobic part, favouring a W/O interface; the physical origin of the Bancroft rule is uncertain.[20] The same rule is applied for the stabilization of O/W/O and higher order emulsions.

The surface-active agents do not necessarily have two distinct parts. Polymers or colloids composed of a single component can stabilize the fluid–fluid interfaces through spontaneous adsorption and barrier formation. For the spontaneous adsorption, the single-component surfactant should have a small energy cost for the disfavoured exposure so that the adsorption reduces the total interfacial energy by removing the area of the free fluid–fluid interface;[21] otherwise, the single component is dispersed in favoured phase without partial exposure to disfavoured phase. Many single-component surfactants do not necessarily obey the Bancroft rule; the phase in which a surfactant is less soluble can constitute the continuous phase as illustrated in Fig. 2b, which is referred to as anti-Bancroft. For example, partially hydrolysed poly(vinyl alcohol) (PVA) is more soluble in water than oil and can stabilize both O/W and W/O interfaces.[22] Therefore, PVA can be included in both the inner and continuous phases of W/O/W double-emulsion drops to stabilize them in the absence of any surfactant in the middle oil phase; PVA in the inner drop follows the anti-Bancroft type, whereas PVA in the continuous phase follows the Bancroft type.

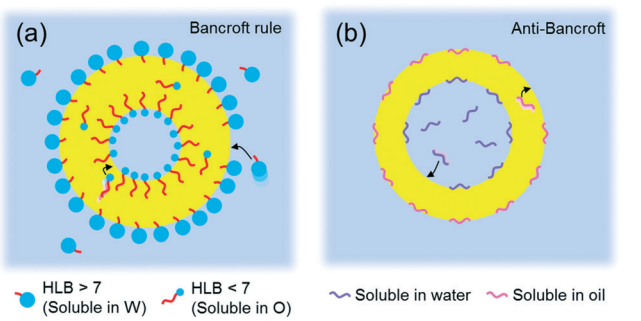

Fig. 2 (a) Schematic illustration showing stabilization of emulsion interfaces by adsorption of surfactants originated from the surroundings. Water-soluble surfactants which have a hydrophilic–lipophilic balance (HLB) larger than 7 stabilize O/W interfaces, whereas oil soluble surfactants with HLB smaller than 7 stabilize W/O interfaces; this rule is applied for typical surfactants and referred to as Bancroft rule. (b) Schematic showing stabilization of emulsion interface by surfactants originated from the dispersed phase; this is anti-Bancroft.

2.2 Production of multiple emulsions in microfluidic devices

2.2.1 Sequential emulsification. Multiple-emulsion drops can be prepared using microfluidic devices with a series of single drop makers. The innermost drops are prepared at the first drop maker, which are then inserted into the second level of drops at the second drop maker and so on.[23,24] Therefore, the level of the hierarchy of multiple emulsions is dictated by the number of the drop makers in series. Each drop maker, prepared by a soft lithography technique, typically has either a cross- or T-shaped junction.[25–28] In general, the flow-focusing effect of the cross-shaped junction provides better control over drop size and formation frequency than the T-shaped junction, and it is therefore more popularly used in the production of multiple-emulsion drops.

To produce drops in a controlled manner, the channel walls should be rendered to have a higher affinity to the continuous phase than to the dispersed phase at each drop maker; this prevents the adhesion or wetting of drops on the wall. For example, the channel surface of W/O drop maker is rendered to be hydrophobic, whereas that of an O/W drop maker is rendered to be hydrophilic. Therefore, a series of drop makers for the production of multiple-emulsion drops should be regioselectively treated.[23] For example, to produce W/O/W/O triple-emulsion drops, W/O drops are generated in the first hydrophobic junction, which are then inserted into O/W drops at the second hydrophilic junction. Finally, the W/O/W double-emulsion drops are inserted into W/O drops at the third hydrophobic junction as illustrated in the top panel of Fig. 3a; the second half of each junction is rendered to have high affinity to the continuous phase. For stable drop formation, the entire channel wall of each junction, except for the one used for injection of the dispersed phase, is needed to have high affinity to the continuous phase.

For regioselective control of channel wettability, photoreactive sol–gel coating, flow confinement method, and selective plasma treatment have been used.[29–31] In a photoreactive sol–gel coating, the entire surface of the channel is first fluorinated and is then locally treated with poly(acrylic acid) (PAAc).[29] For this, the photoinitiator Irgacure 2959 and the

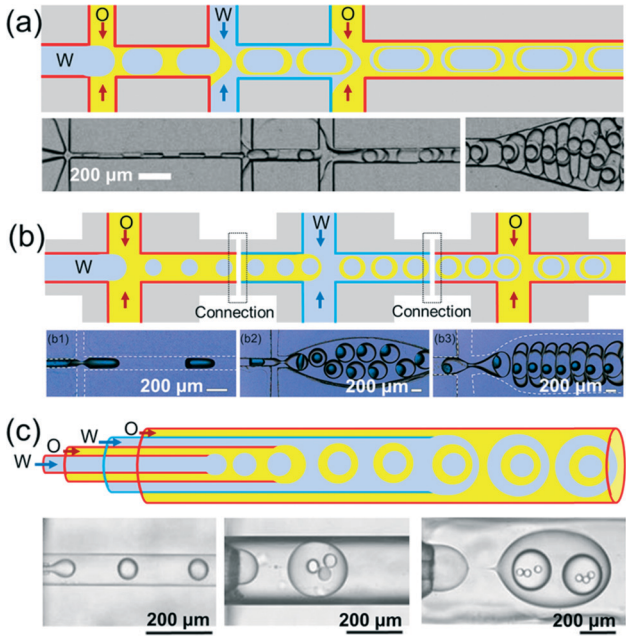

Fig. 3 (a) Set of schematic and optical microscopy image showing the design of a planar microfluidic device with three drop makers in series and production of W/O/W/O triple-emulsion drops through sequential emulsification. (b) The serial connection of three microfluidic devices with single drop maker independently prepared for plug-n-play production of triple-emulsion drops. (c) A microfluidic device composed of four capillaries which are hierarchically inserted to form a series of drop makers for the production of triple-emulsion drops. In the schematics, red and blue colours represent hydrophobic and hydrophilic surfaces, respectively. Reprinted with permission from ref. 32 (a), 35 (b), and 37 (c). Copyright Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (a, c) and Royal Society of Chemistry (b).

silane 3-(triethoxysily)propyl isocyanate are coupled, and then mixed with tetraethylorthosilicate, (heptadecafluoro-1,1,2,2-tetrahydrodecyl)triethoxysilane and other reagents. The microfluidic channel is pre-treated with oxygen plasma and then flushed with the mixture and subjected to 220 °C to cure the coating on the wall. The resulting fluorinated surface is hydrophobic. To modify the surface to hydrophilic at selected area, the hydrophobic channel is filled with a mixture of acrylic acid (AAc) and other reagents, which is then locally irradiated with ultraviolet (UV) light. The photoinitiator in the sol–gel coating produces radials which react with AAc to form PAAc. As a result, the UV-irradiated region is rendered to be hydrophilic, achieving spatially patterned wettability. A flow confinement method does not require local UV exposure.[30] In a similar manner to the previous sol–gel coating, the entire surface of the channel is fluorinated; the photoinitiator is not included for the hydrophobic coating. An ethanolic solution of AAc containing a photoinitiator of Darocur 1173 is used to render the hydrophobic coating hydrophilic. The reactive solution is injected through inlets for hydrophilic treatment, whereas an inert solution is injected though other inlets to maintain the hydrophobic surface. The two solutions are brought into a contact at a junction, and they then flow out by forming a layered stream. Bulk UV irradiation on the device selectively modifies the wall where the reactive solution flows, while the wall remains intact where inert solution flows. Selective plasma treatment provides the simplest way to regioselectively control the wettability.[31] The elastomer commonly used as material for microfluidic devices, a poly(dimethylsiloxane) (PDMS), is intrinsically hydrophobic. Oxygen plasma treatment temporarily renders the surface hydrophilic. By blocking some of the channel ports during oxygen plasma treatment, channel wettability can be locally modulated. For this, inlet ports that need to be hydrophilic are open to ionized oxygen species whereas other inlet ports are closed by scotch tape. Although this method yields indistinct boundaries between original hydrophobic and plasma-treated hydrophilic surfaces and only provides non-permanent treatment, the resulting pattern can be used for multiple-emulsion production.

In all three methods it is difficult to make a distinct boundary between hydrophilic and hydrophobic regions at the right corners of the injection channel. However, rendering the second half of each junction to have high affinity to the continuous phase still facilitates the stable formation of drops in comparison with no treatment, although the triple line among two phases and the solid wall is not firmly pinned. The formation of W/O/W/O triple-emulsion drops in a PDMS device with three drop makers in series is shown in the bottom panel of Fig. 3a. Although the triple line is not pinned at the edge of a right angle at the second and third junctions, the interface is broken up at every moment of inner-drop insertion, which enables the spontaneous synchronization of drop generation frequency at all three junctions.[32] The insertion of a drop causes an abrupt change in flow resistance, which triggers the breakup of the interface. Therefore, the single inner drop is confined in an outer drop at all levels of hierarchy. Although the inner-drop-triggering mode provides a high stability of multiple-emulsion generation, it is difficult to confine two or more inner drops in each outer drop. Furthermore, elastomers commonly used as device materials such as PDMS are incompatible with organic solvents, thereby restricting their uses.[33] Nevertheless, the planar devices, prepared by soft lithography, can be parallelized to produce multiple-emulsion drops in high throughput, being considered as a unique method for commercial scale production of functional microcapsules.

A planar microfluidic device with similar geometry of channels to elastomer devices has been prepared by assembling pieces of glass slips. The channel is formed by aligning the pieces to have a narrow gap, which is sandwiched between the top and bottom covers;[34] typical channel dimensions are larger than those of lithographically-featured elastomer devices. In the absence of inner-drop triggering, the number of the inner drops confined in single outer drop can be controlled by adjusting the relative generation frequency of inner drops to outer drops; the frequency of drop generation is invariant during steady operation of microfluidic devices. The junctions independently prepared by the assembly can be further connected in series to produce multiple-

emulsion drops; this method is referred to as plug-n-play microfluidics.[35] Each junction is rendered to be either hydrophobic or hydrophilic before the connection. Depending on the target composition of the multiple emulsions, the necessary number and sequence of the hydrophobic and hydrophilic junctions in serial connection are determined. For example, hydrophobic, hydrophilic, and hydrophobic junctions are connected to produce W/O/W/O triple-emulsion drops, as shown in Fig. 3b. Moreover, two junctions with same wettability can be inserted in the series, where the first junction is the drop maker and the second is a continuous phase extractor; selective removal of the continuous phase decreases separation between the inner drops, which is beneficial for control over the number of inner drops and size of the outer drop.

A series of drop makers can also be implemented in capillary microfluidic devices. Various dimensions of capillaries are used and some of them can be tapered before use. The glass capillaries can be treated to have the desired surface wettability with silane coupling agents and are then assembled to form a microfluidic device; 2-[methoxy-(polyethyleneoxy)propyl] trimethoxyl silane is typically used for hydrophilic treatment and *n*-octadecyltrimethoxyl silane is used for hydrophobic treatment. The drop maker can be simply composed of a small capillary inserted into a larger one with coaxial alignment.[36] The dispersed phase injected through the small capillary is emulsified at the tip into the continuous phase which is injected through the annulus of the larger capillary. The dispersed phase is therefore fully enclosed by the continuous phase. The dispersed and continuous phases flow in the same direction, and the capillary tip has sharp edges with the very low angle, which enables the pining of triple lines at the edge. Therefore, the drop generation in capillary microfluidic devices is more stable and easier to control than in planar devices. For the production of multiple emulsions through sequential emulsification, the capillaries are hierarchically inserted, as shown in Fig. 3c.[37] Regioselective modification of the surface wettability is preferred in the same manner to planar devices; it is best to treat the inner surface of the small capillary to have a high affinity to the dispersed phase and the outer surface of the small capillary and the inner surface of the large capillary to have a high affinity to the continuous phase. However, to avoid such a troublesome treatment, in practice the entire surface of the small capillary is treated to have a high affinity to the dispersed phase and that of the large capillary is treated to have high affinity to the continuous phase. This simple treatment usually does not cause a wetting problem due to stable pinning of the triple line at the sharp edge of the small capillary tip. To produce W/O/W/O triple-emulsion drops, the capillaries in the hierarchy are treated to be hydrophobic, hydrophobic, hydrophilic, and hydrophobic from the innermost one, as shown in Fig. 3c. Although this treatment is similar to the PDMS device in Fig. 3a, triple lines are firmly pinned at the edges of capillary tips, unlike in PDMS devices. The high stability of the drop generation enables us to easily control the number of inner drops by independently manipulating the flow rates of all the fluids. When all junctions are operated at dripping mode in the absence of inner-drop-triggering, the frequency of drop generation at each junction is determined by the flow rates of the dispersed and continuous phases, which is independently controllable from other junctions.[37] Therefore, the number of inner drops confined in outer drop can be exquisitely adjusted from one to a dozen or even larger. The capillary single-drop makers are independently prepared and are then connected in series to produce multiple-emulsion drops through plug-n-play operation.[38] The capillary microfluidic devices provide high stability of drop generation and high controllability over the size and number of inner drops confined in outer drops. In addition, a glass capillary is compatible with the use of organic solvents which are sometimes inevitable for microcapsule production. However, it is difficult to parallelize the drop makers for high throughput production because the devices are manually prepared; planar devices composed of glass slips are also manually prepared, which is appropriate for proto-typing yet inappropriate for the production of multiple-emulsion drops for industrial applications.

2.2.2 Single-step emulsification. Multiple-emulsion drops can be formed by the simultaneous breakup of a core–sheath stream in a single step without a series of drop makers. To accomplish this, the formation of multiple jets composed of two or more coaxial interfaces is a prerequisite. However, the cylindrical interface is intrinsically unstable due to Rayleigh–Plateau instability, leading to the spontaneous breakup into drops;[39] this makes it difficult to form stable multiple jets. This instability can be suppressed by confining the interface near the surface of the solid wall, while increasing inertial force relative to the surface force.[40] Therefore, jets can be stabilized in a microfluidic channel and even formed in a core–sheath type.[41,42] For example, a triple jet of W/O/W/O can be formed in a series of three junctions, as shown in Fig. 4a. The W/O jet is formed in the hydrophobic first junction, which then flows along the narrow hydrophobic channel. The jet is confined by an additional O/W jet at the second hydrophilic junction, which is further shielded by a W/O jet at the third hydrophobic junction. In all junctions and narrow channels, the jets are stabilized by wall confinement and high inertia. The wall is treated to minimize interfacial energy of the continuous phase in each region; otherwise, phase inversion can occur even with high inertia. All three interfaces of the triple jet are almost simultaneously broken up to form W/O/W/O triple-emulsion drops in the slightly wider downstream channel; the breakup occurs from the innermost droplet to the outermost with very short intervals. The core–sheath flow formed in the microfluidic channel can result in thin sheath streams, which leads to the formation of ultrathin shells in multiple-emulsion drops; the shell thickness can be reduced to as small as 1 μm.[43] Such a thin shell is difficult to produce through sequential emulsification. Although the continuous removal of the shell phase in a specially-designed device enables the formation of thin-shelled

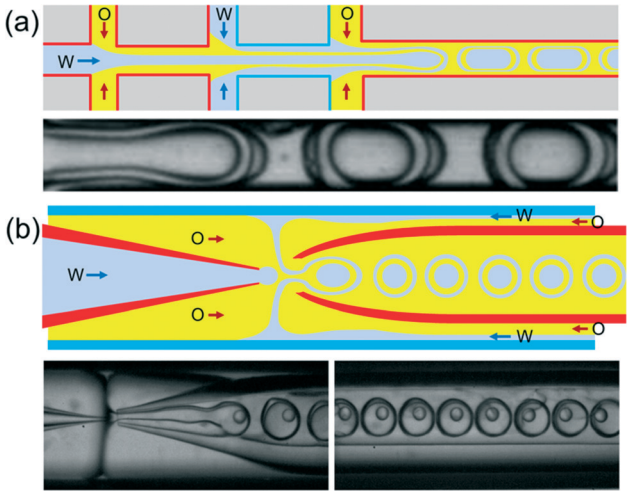

Fig. 4 (a) Set of schematic and optical microscopy image showing the design of a planar microfluidic device with three cross junctions in series to form triple jets and production of W/O/W/O triple-emulsion drops through single-step emulsification. (b) A microfluidic device composed of two tapered capillaries which are coaxially aligned in a square capillary and the production of triple-emulsion drops through single-step emulsification. In the schematics, red and blue colours represent hydrophobic and hydrophilic surfaces, respectively. Reprinted with permission from ref. 42 (a) and 48 (b). Copyright Royal Society of Chemistry (a) and Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (b).

double-emulsion drops, a robust long-term operation is difficult to achieve.[44]

The formation of multiple jets and their single-step emulsification can be easily achieved in a capillary microfluidic device that is composed of two tapered cylindrical capillaries with tip-to-tip alignment confined in the third square capillary. The coaxial alignment of the capillaries facilitates the formation of jets. Moreover, the capillaries compartmentalize the streams of immiscible fluids in the cylindrical geometry, enabling simultaneous injection of multiple fluids into the single orifice. For example, W/O/W double-emulsion drops can be simply formed by injecting the innermost water phase through one of the tapered capillaries, the middle oil phase through the interstices between the tapered capillary and square capillary, and the continuous water phase through the interstices between the other tapered capillary and square capillary.[45,46] The tapered capillary used for the injection of water is treated to be hydrophobic, whereas the other tapered capillary is treated to be hydrophilic before assembly. The inner water and the middle oil flow in the same direction (co-current flow), whereas the continuous water flows in the opposite direction (counter-flow). The W/O and O/W interfaces are coaxially injected into the orifice of the hydrophilic capillary, which are emulsified into W/O/W double-emulsion drops at the widening channel of the tapered capillary; various modes of drop generation have been observed.[47] To produce multiple-emulsion drops with higher order, two immiscible fluids are simultaneously injected through a single channel in the same device geometry. For example, water and oil are injected through the channel used for the continuous phase injection as counter-flows, while maintaining the innermost water and middle oil in the device, as shown in Fig. 4b, where both tapered capillaries are treated to be hydrophobic and the square capillary is treated to be hydrophilic.[48] Even if water and oil are simultaneously injected without alignment, water flows along the hydrophilic square capillary and oil flows along the hydrophobic tapered capillary, spontaneously forming a double-sheath flow in the interstice channel. The innermost water and the middle oil phases are coaxially inserted into the double sheaths at the orifice of the right capillary, forming W/O/W/O triple-emulsion drops through single-step emulsification. A double jet composed of the inner O/W and outer W/O interfaces is broken up by insertion of innermost W/O drops which are produced in a dripping mode. Triple-emulsion drops with O/W/O/W composition can be prepared in the same manner by using oppositely-treated capillary devices. Quadruple-emulsion drops can be prepared by simultaneously injecting oil and water through both interstice channels.

A single jet can be formed when water and oil are simultaneously injected through the narrow capillary channel; the composition is determined by surface nature of the channel wall. The jet can be emulsified into the third fluid which is injected along the outer surface of the narrow capillary. This results in the formation of double-emulsion drops whose shell is very thin when the flow rate of the middle phase is low.[49] In the absence of sufficient inertia and wall confinement, the jet is emulsified into plug-like drops in the channel, which still maintain the core–sheath stream yet discontinuous. When the core–sheath stream is emulsified, double-emulsion drops with an ultra-thin shell are produced. Subsequently, the middle phase between the plug-like drops is emulsified to form single-emulsion drops. Double-emulsion drops with an ultra-thin shell have an average density very close to the density of the core, which is different from that of single oil drops. Therefore, the double-emulsion drops can be easily separated from the mixture by exploiting the density difference; therefore, this discontinuous production is still useful. In fact, the discontinuous production provides higher drop generation stability in a long-term operation of devices because the inner interface is renewed at every moment of plug-drop arrival in the tip of the capillary. Double-emulsion drops with an ultra-thin shell have a relatively long lifetime in comparison with those with thick shells due to high lubrication resistance in the shell.[50,51] This renders them promising as a template for the production of microcapsules. More than two distinct innermost drops can be contained in a single multiple-emulsion drop by parallelizing two or more injection channels, where each channel has core–sheath flow.[52] The multiple cores enclosed by an ultra-thin shell have nonspherical shapes, which show selective coalescence between the innermost drops, while maintaining the order of multiple-emulsion drops; these drops can be used as microreactors. The core–sheath flow can be formed in one of the tapered capillaries in a double-emulsion maker to produce triple-emulsion drops whose inner shell is ultra-thin.[53] The

single-step emulsification provides higher stability of drop generation and enhanced controllability over the sizes of drops at all levels in comparison with sequential-emulsification, albeit it provides poor control over the number of inner drops.

2.2.3 Phase separation in emulsion drops. A homogeneous phase composed of several species, including at least two immiscible liquids, can show phase separation when it is subjected to composition changes. A single drop of such homogeneous mixtures can exhibit phase separation upon the composition change by mass transfer with the surroundings through the interface. The phase separation yields at least two immiscible phases, which can form an onion-like configuration.[54–56] At the early stage of the separation, very small domains are formed by spinodal decomposition, which are then coalesced by Ostwald ripening, forming two immiscible phases. The resulting phases are not pure and can further experience phase separation through an additional change of the composition, as shown in Fig. 5a. The number of phase separation events and therefore a level of the hierarchy is dictated by the initial composition of the mixture in the drop; it is experimentally shown that quintuple-emulsion drops can be prepared from single-emulsion drops through phase separation.[56] The resulting multiple-emulsion drops have been used to produce polymer capsules and vesicles.[55,56] It is not necessary for all species to be liquids. For example, an aqueous solution containing PEG and dextran has been emulsified in an aqueous solution of PEG and glycerol to form W/W single-emulsion drops; periodic perturbation of the flow rate has been utilized to break the W/W jet into drops because the interfacial tension is extremely low. The water is extracted from the single-emulsion drops, which enriches the polymers, finally leading to phase separation between PEG-rich and dextran-rich waters. The resulting W/W/W double-emulsion drops have a PEG-rich core and dextran-rich shell.[57,58] These all water double-emulsion drops can serve as biocompatible templates for capsule production.

In the absence of composition changes, two immiscible fluids can also become homogeneous by heating; for example, fluorocarbon oil and hydrocarbon oil are miscible above a certain temperature. Therefore, the homogeneous mixture can be emulsified in a third fluid with no solubility in the two fluids, which is then cooled down below the phase separation temperature to form double-emulsion drops.[10] The shell phase is selected by the surfactant in the surrounding to minimize the interfacial energy. With special surfactants designed to exhibit conformational change induced by light or pH, phase inversion between the core and shell can be controlled *in situ*. Phase separation is the simplest way to produce multiple-emulsion drops with an onion-like configuration. In addition, high throughput production is even possible; parallelization of single-drop makers is relatively easy. Nevertheless, limited sets of materials for the production of these types of multiple-emulsion drops are available, which restricts practical uses.

2.2.4 Other techniques. Double-emulsion drops composed of three immiscible fluids, such as a set of O1, O2, and W, are thermodynamically stable against phase inversion if the spreading parameter of the shell is larger than 0, as we discussed in section 2.1.[16–19] Such drops can be prepared by bringing two drops consisting of the eventual inner and middle phases into physical contact. One drop engulfs the other, spontaneously forming the double-emulsion drops to reduce interfacial energy. The one-by-one contact can be achieved by synchronizing the frequencies of drop generation in two drop makers integrated into a single device, as shown in Fig. 5b.[59,60] Using the same principle, two distinct inner drops can be enclosed by one outer drop. In addition, double-emulsion drops can be enclosed by an additional layer to form triple-emulsion drops.

With a microfluidic device equipped with a tapered capillary, a small drop of continuous phase can be automatically inserted into the single-emulsion drop, thereby forming double-emulsion drops. The single-emulsion drops injected through the tapered capillary are accelerated when the drops are larger than the capillary orifice, inducing high inertia force. The rear interface of the drops is indented by the force, which is broken up to a droplet within the single-emulsion when the inertial force overwhelms the capillary force.[61] Therefore, the inner drops formed by the insertion have the same composition as the continuous phase. When W/O/W double-emulsion drops with a small water core are injected through the tapered capillary, a new small water core is inserted to form double-emulsion drops with two distinct cores, as shown in Fig. 5c. This insertion of the continuous phase is beneficial for sampling the continuous phase.

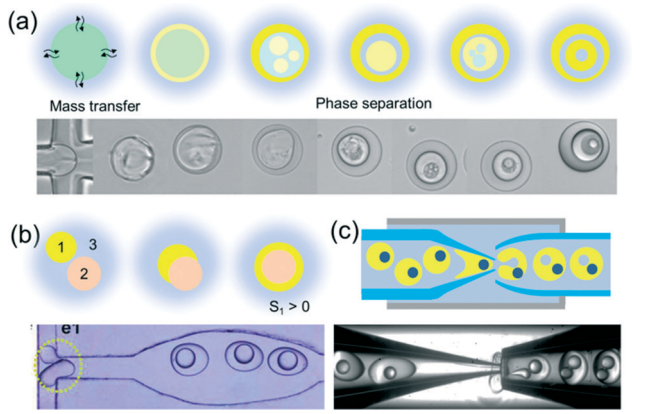

Fig. 5 (a) Set of schematic and a series of optical microscopy images showing the evolution of single-emulsion drop to quadruple-emulsion drops through phase separation caused by mass transfer between the drop and the surrounding. (b) Formation of double-emulsion drops by the spontaneous engulfing of one drop by the other, where two drops are independently prepared, which are then brought into a contact in a single microfluidic device. (c) Insertion of a small drop of the continuous phase into a single-cored double-emulsion drop to form dual-cored double-emulsion drops in a capillary microfluidic device composed of the narrowing channel followed by sudden expansion. Reprinted with permission from ref. 55 (a), 59 (b), and 61 (c). Copyright Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (a), Royal Society of Chemistry (b), and Elsevier (c).

3. Solid shells and functionality

3.1 Methods for shell solidification

Selected layers of multiple-emulsion drops have been converted to solid membranes for the production of stable microcapsules. For the shell solidification, four different methods have been used: polymerization, consolidation, freezing, and dewetting. The methods significantly influence the physical and chemical properties of the solid membranes. Therefore, one of the methods should be carefully selected to design microcapsules with desired functionality for target applications.

3.1.1 Polymerization. Monomers or oligomers in selected layers of multiple-emulsion drops can be polymerized to form solid shells. During the polymerization, monomers are linked by covalent bonds, which yields stable membranes; this is the only method to form chemical bonds during the solidification. The polymerization is triggered by external stimuli; otherwise, the reaction would occur during drop generation in microfluidics, which increases viscosity and disturbs the drop generation. Light triggering is the most convenient way to initiate the polymerization. For this, a small amount of photoinitiator is added in the monomer solution. The photoinitiator produces radicals under UV irradiation, which activate monomers. The activated monomers attack another monomer to form a chain, and the reaction propagates throughout the shell. For heat triggering, the photoinitiator is replaced with a thermal initiator which produces radicals above a certain temperature; thermal triggering is less favoured because high temperatures frequently cause unstable interfaces, leading to coalescence.

Various monomers have been used to produce solid shells. The properties of the shell are predominately determined by the chemical structure of the monomers. For example, monomers of ethoxylated trimethylolpropane triacrylate (ETPTA), triprophylene glycol diacrylate (TPGDA), or 1,6-hexanediol diacrylate (HDDA), immiscible with water, yield rigid shells with high Young's moduli through polymerization.[17,41,50,62–64] The monomers containing either photo- or thermal initiators are used as the oil phase of W/O/W double-emulsion drops or higher orders. The monomers with two or more acrylate groups form crosslinked network in the shell through polymerization as shown in Fig. 6a, providing high chemical resistance; the solid shell formed by polymerization of ETPTA is included in the rightmost panel. A monomer of ethylene glycol phenyl ether acrylate (EGPEA) or an oligomer of silicone rubber precursors form elastic shells with low moduli.[61,62] Although EGPEA has one acrylate group and forms linear chains without crosslinking, the chains can be entangled to provide sufficient mechanical stability. There are many water-soluble monomers which form hydrogels through crosslinking. The aqueous solutions of monomers containing initiators are used as the water phase of O/W/O or higher orders; crosslinkers are sometimes dissolved in the solution if the monomers form only linear chains. An aqueous solution of acrylamide containing

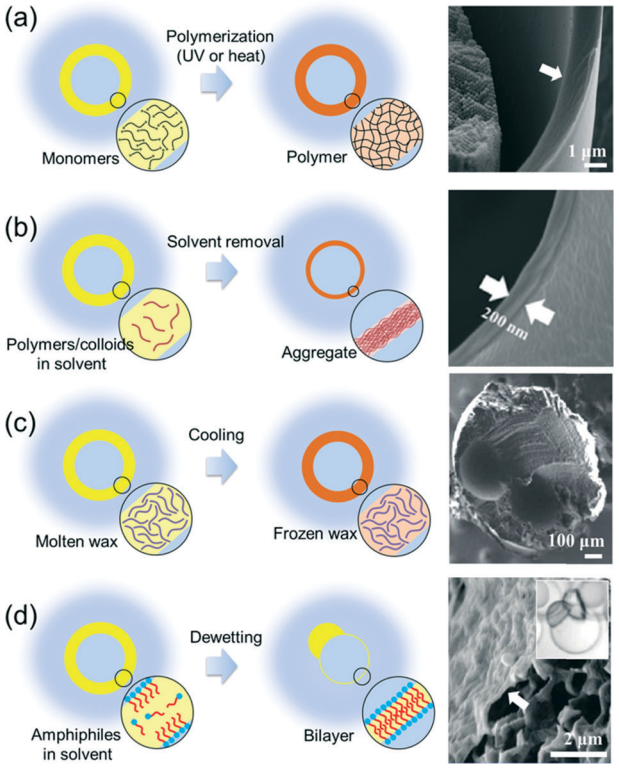

Fig. 6 (a–d) Sets of schematic and scanning electron microscopy (SEM) image showing the process of shell solidification and resulting shells: polymerization (a), consolidation (b), freezing (c), and dewetting (d). The shells are indicated with arrows in SEM images of (a, b, d) and optical microscopy image of double-emulsion drop which undergoes dewetting of the middle oil phase is included in the inset of the right panel of (d). Reprinted with permission from ref. 64 (a), 22 (b), 74 (c), and 81 (d). Copyright Nature Publishing Group (a) and American Chemical Society (b–d).

crosslinker or a mixture of water and poly(ethylene glycol)diacrylate (PEGDA) have been widely used to form a hydrogel shell.[65,66] An aqueous solution of *n*-isopropylacrylamide (NIPAm) containing crosslinker has been used to produce a temperature-responsive shell made of poly(*N*-isopropylacylamide) (PNIPAm).[65,67]

Gel precursors can be crosslinked by forming ionic complexes, instead of covalent bonds. For example, sodium alginate molecules form a gel in the presence of divalent cations such as calcium ions. The ionic gelation in the shells of multiple-emulsion drops yields stable hydrogel shells.[68] However, divalent ions should be introduced to the shell containing sodium alginate after drop production to avoid clogging the microfluidic channel. To achieve this, nanoparticles or molecular complexes, which release divalent cations by triggered ionization, have been incorporated in the shell;[69] chemical cues or UV irradiation have been used to render an environment acidic, which ionizes atoms to divalent ions.

Polymerization makes covalent bonds between molecules in the shell, thereby providing high chemical resistance. In addition, a long lifetime of multiple-emulsion drops is not

prerequisite because most polymerization processes are fast. Moreover, the solidification is simply done by UV irradiation or heating. These features make polymerization the most popular method for shell solidification. Nevertheless, other methods have been used because the membranes formed by the polymerization do not fulfil all the physical and chemical properties required for various applications of microcapsules.

3.1.2 Evaporation-induced consolidation. The solid shells of microcapsules can be prepared by the consolidation of polymers or colloids in volatile middle phases of multiple-emulsion drops. The volatile solvent in the middle phase slowly diffuses into the continuous phase and vaporizes into the surrounding air. This selective removal of solvent from the middle layer concentrates the solid materials and finally consolidates them, forming solid shells as illustrated in Fig. 6b.[70,71] The shell becomes thinner over the course of the consolidation process, while the core maintains its size; osmotic pressure difference between the core and the surroundings can cause size change of the core.[72] The consolidation process is slow relative to a polymerization process, requiring a long lifetime of multiple-emulsion drops; otherwise, internal coalescence reduces the order of multiple-emulsion drops, resulting in undesired products.

To improve the stability of multiple-emulsion drops, molecular surfactants or colloidal nanoparticles are carefully selected for adsorption at the interfaces. In particular, colloidal nanoparticles are strongly anchored, forming a stable physical barrier against the coalescence. For example, colloidal nanoparticles in the volatile oil phase of W/O/W double-emulsion drops stabilize both inner and outer interfaces, maintaining the core–shell geometry until the consolidation is completed, where excess colloidal nanoparticles form a solid shell composed of close-packed colloids.[71] The lifetime of multiple-emulsion drops can be prolonged by making the middle shell ultra-thin, while maintaining the material set of liquids and surfactants. The thin liquid layer exerts high lubrication resistance, which dramatically retards the migration of interfaces, thereby delaying the contact between two interfaces. The stabilization with thin shells allows for a high degree of freedom to select materials, being beneficial for designing microcapsules with the desired properties.[49] Multiple-emulsion drops with an ultra-thin oil shell have been prepared by single-step emulsification of a core–sheath flow, as we discussed in section 2.2.2.

During the consolidation, polymers or colloids are densely packed, which results in high mechanical stability; the shell can be further tailored to increase the stability by post-processing such as annealing or sintering.[73] Although the solid shells have low chemical resistance due to the absence of covalent bonds, chemical-responsive failure of the shell is sometimes beneficial for triggered release of encapsulants.[22] In fact, if the high stability of multiple-emulsion drops is secured, the consolidation process allows the use of unlimited sets of materials, thereby being potentially useful for designing functional microcapsules; any polymers dissolvable or any colloids dispersible in volatile solvents can be used to produce solid shells. The reduction of shell thickness during the consolidation is appealing for the production of ultra-thin solid shells. For example, biodegradable microcapsules with 100 nm-thick poly(lactic acid) (PLA) or poly(lactic-*co*-glycolic acid) (PLGA) shells can be prepared from W/O/W double-emulsion drops of which middle oil layer is a solution of the polymers in toluene;[49] such a thin and biodegradable shell is difficult to prepare with a polymerization process.

3.1.3 Freezing. Hydrocarbon molecules containing more than twenty carbon atoms and various lipid molecules are solid at room temperature and molten above their freezing point typically in the range of 30–50 °C. This molten wax is injected into microfluidic devices to form the liquid shell of multiple-emulsion drops; for this, whole parts of the experimental setup, including the microfluidic devices, should be maintained above the freezing point to avoid clogging of the channels. The liquid shells of hydrocarbons or lipids can be solidified by cooling below the freezing point, as illustrated in Fig. 6c.[74,75] The solid shells thaw upon heating, losing stability against internal coalescence. Therefore, microcapsules with frozen wax shells can release their encapsulants by heat triggering, potentially serving as delivery vehicles. However, the wax freezing usually entails the formation of pores and cracks, through which encapsulants leak; precipitation reactions between chemicals in the core and the surroundings have been used to block the pores in the shell.[76,77] Furthermore, the frozen shells have poor mechanical stability even at room temperature. These drawbacks restrict the use of the freezing process.

3.1.4 Dewetting. In the processes of polymerization, consolidation, and freezing, materials in the shell are conserved. The dewetting process yields microcapsules whose membrane is composed of a molecular bilayer; these microcapsules are called vesicles. Liposomes are vesicles with a bilayer of lipid molecules, which are typically 4–7 nm thick, and polymersomes are vesicles with a bilayer of amphiphilic block-copolymers, which are 10–30 nm thick. Both liposomes and polymersomes have been prepared from multiple-emulsion drops through the dewetting process.[78–80] Typically, a mixture of two different organic solvents containing either lipids or amphiphilic polymers is used as the middle phase of W/O/W double-emulsion drops to produce vesicles: one is a good solvent with high volatility, and the other is a poor solvent with low volatility. Amphiphiles are aligned at both inner and outer interfaces to minimize interfacial energy, where the hydrophobic part of the molecule remains in the oil phase, and the hydrophilic part protrudes into the water phase. The good solvent rapidly diffuses to the continuous phase, thereby increasing the concentration of poor solvent. This reduction of solvent quality leads to an attraction between the hydrophobic parts. Therefore, when two monolayers of amphiphiles at the inner and outer interfaces are brought into a contact, unbalanced forces at the contact point cause the dewetting of oil on the surface of the inner drop, overlapping the two monolayers to form a bilayer as illustrated in Fig. 6d.[78,81] The oil drop is expelled, forming a

bulb; the bulb remains to form a local multilayer or becomes completely separated from the water core, leaving a single bilayer on the entire interface.

Polymersomes with a bilayer membrane have been prepared from double-emulsion drops with thick shells; oil shell thickness has no influence on bilayer thickness. Polymersomes with multiple compartments have been templated from double-emulsion drops containing multiple inner drops.[82,83] Polymersomes-in-polymersomes, or double polymersomes, have been prepared by inserting the polymersomes into an inner drop of the second double-emulsion drops and triple polymersomes have been also prepared by repeating the insertion.[84] Polymersomes with double bilayers have been templated by W/O/W/O/W quadruple-emulsion drops, where polymersomes formed from inner W/O/W bud through outer polymersomes from outer W/O/W in presence of depletion attraction between two bilayers.[85] Liposomes are difficult to prepare from double-emulsion drops with thick shells due to the low stability of lipid-adsorbed interfaces. Double-emulsion drops with ultra-thin shells provide high stability, which maintain the core–shell geometry until the bilayer is formed by the dewetting process.[86]

3.2 Functionality of solid shells

Solid membranes prepared by the methods discussed above can show various functions, depending on the material and structure of the membranes. The membrane is a window for communication between core materials and surroundings. Therefore, the function of membranes needs to be adequately developed for target applications.

3.2.1 Impermeable membranes. The most basic function of solid membranes is an isolation of the core from the surroundings without a material exchange, as shown in Fig. 7a. The isolation is particularly important for maintaining functionality of core materials that are fragile against impurities. For long-term isolation, membrane materials have to be mechanically and chemically stable. At the same time, any interconnected pores in the membranes have to be small enough to prevent the passage of relevant materials. To satisfy these conditions, polymerization and consolidation of polymers are appropriate for membrane formation; frozen wax usually has cracks and dewetting-induced molecular bilayers have low mechanical stability. Densely packed polymers have a low permeability to water and almost no permeability to molecules or ions whose hydrodynamic diameter is larger than approximately 1 nm. For example, the polymer membrane made from triacrylate monomers, ETPTA, has very low permeability to sodium ions; a crystalline colloidal array (CCA) formed by electrostatic repulsion in the core enclosed by the membrane is conserved for at least several months in the ionic environment.[87] Inorganic shells with low permeability can also be prepared by sintering after shells of silica nanoparticle aggregates that are formed from W/O/W double-emulsion

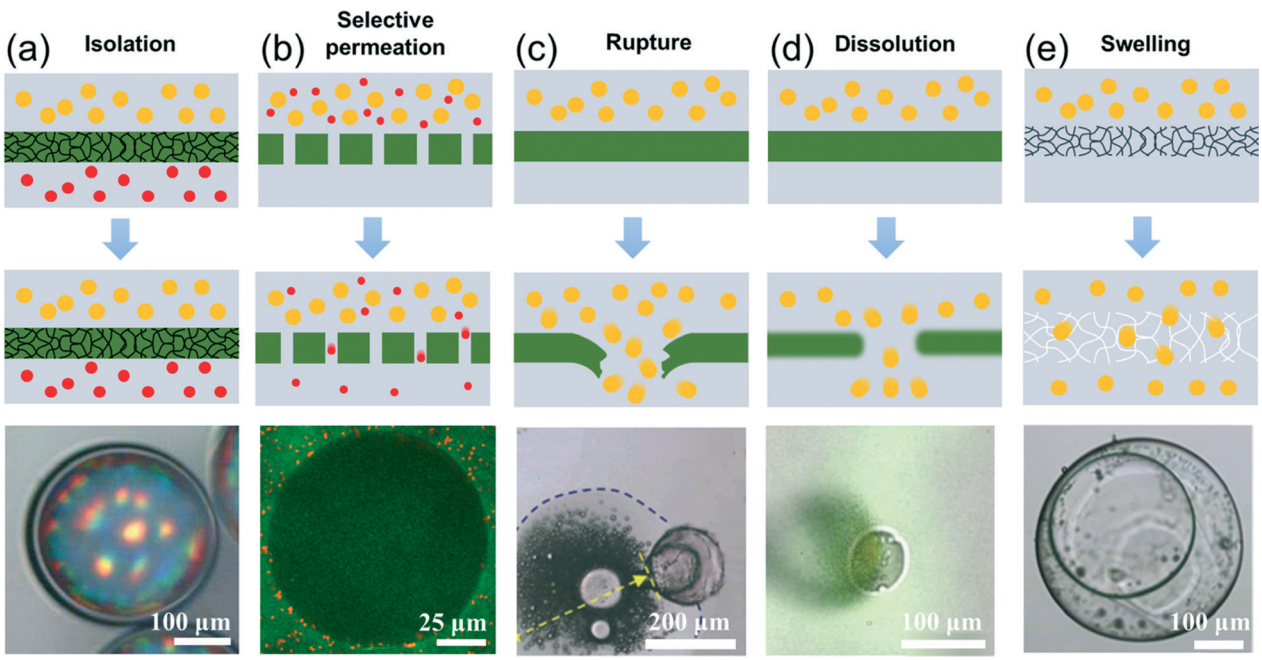

Fig. 7 (a–e) Sets of schematics and microscopy image of microcapsules with functional membranes: (a) isolation, (b) selective permeation, (c) rupturing, (d) dissolution, and (e) swelling. The optical microscopy image in (a) shows the isolation of crystalline colloidal arrays (CCAs) in the core from ionic impurity in the surrounding. The confocal microscopy image in (b) shows selective permeation of 100 nm green particles from the mixture with 1 μm red particles. The optical microscopy image in (c) shows the rupture of poly(*N*-isopropylacylamide) (PNIPAm) membrane at high temperature and the release of encapsultants. The optical microscopy image in (d) shows the dissolution of the membrane at pH 9 and the release of encapsulants. The optical microscopy image in (e) shows the microcapsules whose hydrogel membrane is swollen in the presence of barium ions. Reprinted with permission from ref. 87 (a), 94 (b), 97 (c), 99 (d), and 102 (e). Copyright American Chemical Society (a, b, d), Royal Society of Chemistry (c) and Elsevier (e).

drops; the core can only be filled with gas after this post-process.[73]

3.2.2 Semipermeable membranes. Capsule membranes containing interconnected pores with consistent size regulate material transport through the membrane in a passive manner. The membranes selectively allow transport of materials with a hydrodynamic size smaller than the pores, as shown in Fig. 7b. The semipermeable property of these membranes is useful for various applications. For example, cells in the capsules can be isolated from the immune system, while maintaining a metabolism as small nutrients and wastes are exchanged through the membrane; this is known as immunoisolation.[88] Catalysts in the semipermeable capsule can be isolated from contaminants, while promoting chemical reaction.[89] To provide size-selective permeability, various strategies have been employed in multiple-emulsion templates.

The simplest way to make interconnected nanopores with consistent size in capsule membrane is the consolidation of nanoparticles in the shell of W/O/W double-emulsion template;[71,90,91] the resulting microcapsules are referred to as colloidosomes. The colloidosomes show size-selective permeability as the dimensions of the interstitial volume is consistent across the shell. The dimensions of the interstitial volume and cut-off of permeation are dictated by the size of nanoparticles, thereby being controllable. The colloidosomes can be rendered to be magneto-responsive by incorporating magnetic nanoparticles in the colloidal shell.[91] Although colloidosomes have well-defined pores and clear cut-off, the membrane composed of physically consolidated nanoparticles provides low mechanical stability.

To improve mechanical stability, pores are templated by small emulsion droplets or colloids embedded in solid membranes. For example, W/O/W double-emulsion drops whose oil shell is a polymer solution containing tiny water droplets form microcapsules with the porous polymer membrane.[92] The pores templated by droplets are poorly interconnected, thereby achieving a limited control over the permeability. Nevertheless, the membrane is mechanically stable. Colloidal particles embedded in the polymer matrix are not able to form interconnected pores in a similar manner to droplet templating. However, single colloids can be exposed to both inner and outer phases when the shell thickness is smaller than the colloid diameter.[93] In such a case, a monolithic polymer membrane is perforated by spherical pores, providing well-defined cut-off of permeation as well as high mechanical stability. Nevertheless, pore size is limited by shell thickness, which makes it difficult to prepare nanopores.

To simultaneously achieve high mechanical stability and high controllability over the permeability, phase separation between polymers is employed within the shell of double-emulsion drops. Two immiscible polymers form a homogeneous solution at low concentration, while they are separated at high concentration. Therefore, the homogeneous solution of polymers confined in the shell of W/O/W double-emulsion drops shows phase separation as polymers are consolidated by solvent diffusion.[94] The degree of phase separation is influenced by the interaction parameter between two polymers and the degree of polymerization. Therefore, the size of pores formed by selective removal of one polymer can be controlled. For example, the combination of PLA and polystyrene (PS) with a relatively large interaction parameter results in large pores of a few hundred nanometres, whereas a combination of PLA and poly(methylmethacrylate) (PMMA) with a small interaction parameter results in small pores of a few nanometres. To selectively dissolve one polymer from the consolidated membrane, the use of organic solvents is a prerequisite, which potentially damages delicate encapsulants. To obviate the use of organic solvents, polymerization-induced phase separation is also employed.[95] Instead of the polymer mixture, a homogeneous mixture of liquid monomer and inert oil is the used for shell formation. Upon polymerization, the degree of polymerization increases, which reduces miscibility of the growing polymer chain to the inert oil, thereby leading to phase separation. Therefore, a porous membrane can be obtained by removing the inert oil through washing with nontoxic solvents or water. Average pore diameter and cut-off of permeation can be controlled by adjusting interaction parameter between the monomer and inert oil or adjusting volume fraction of the inert oil. The semipermeable microcapsules prepared by polymerization-induced phase separation are used as catalyst carriers. The catalytic Pt nanoparticles encapsulated in the capsules promote the hydrogenation of 4-nitrophenol as both reactant and product are allowed to diffuse through the pores in the membrane.[96] At the same time, the membrane protects the catalyst from the contaminants in surrounding and enables the reuse of the catalyst.

3.2.3 Stimuli-responsive membrane. Solid membranes can be designed to release encapsulants in response to external stimuli. This functionality is useful for the controlled release of active materials. To achieve this, membrane permeability to encapsulants should be dramatically increased in the presence of the desired stimuli. Three different types of responses have been used to increase the permeability, which are the rupture, dissolution, and swelling of membranes.

Solid membranes rupture when they are subjected to stress larger than their yield point. Upon the rupture, encapsulants in the core rapidly diffuse out through a large opening, as shown in Fig. 7c. To induce this rupture, the membrane materials need to be delicate or the mechanical stress exerted on the membrane needs to be high. A delicate hydrogel can be used as shell material. For example, microcapsules composed of an oil core and a PNIPAm shell rupture in water upon heating. PNIPAm has a lower critical solution temperature (LCST) around 32 °C. Therefore, the gel shell shrinks as the temperature increases and internal pressure is built up. At a certain point, the thinnest part of the shell is not able to endure the pressure, leading to shell rupture and the release of the oil; if the oil core contains tiny water drops, the molecules in the tiny drops can be released into the surrounding water.[97] A membrane made of highly crosslinked

polymers is mechanically stable. Therefore, high pressure should be exerted to cause a rupture. If the microcapsules have metal nanostructures with high photothermal activity in their shell, the membrane can be ruptured under irradiation of laser.[93] The irradiation locally heats the microcapsules through the conversion of irradiation to thermal energy, which leads the vaporization of water in the core. This builds a very high internal pressure, which results in the rupture of solid membranes.

Rupture of the solid membrane is difficult to control and not popularly used for controlled release. A more facile strategy for shell rupture is melting the solid membrane. Emulsions with liquefied shells are susceptible to internal coalescence as we discussed in section 2.1. Therefore, heating can simply cause rupture of the shell and release of encapsulants. For example, microcapsules composed of a water core and a frozen lipid shell become unstable when they are heated above the melting point of the shell. The water core enclosed by the liquefied shell coalesces to the surrounding water, thereby releasing the encapsulants.[75] Polymers with a low glass transition temperature can be used for the thermoresponsive rupture. For example, a membrane made of PLGA ruptures at high temperature; the rupturing dynamics are different from that of a normal double-emulsion drop with a liquid shell due to the viscoelastic properties of the polymer melt. The temperature can be raised by near-infrared (NIR) irradiation if the membrane contains gold nanorods.[98] This light-induced rupture provides a facile way for the triggered release of encapsulants.

Solid membranes prepared by the consolidation of polymers can be dissolved or degraded under certain conditions of the surrounding fluids, as shown in Fig. 7d. If the membranes are composed of polymer chains with either cationic or anionic groups, they dissolve in basic or acid solution, respectively.[99] Therefore, such microcapsules can be used for the pH-responsive release of encapsulants, which is potentially important for the organ-specific release of active ingredients; for example, microcapsules can protect bioactive components in the stomach, but release them in the intestine. The membrane composed of biodegradable polymers degrade over time and finally release the encapsulants. For example, PLGA, PLA, and polycaprolactone (PCL) degrade as ester groups in their backbone are hydrolyzed.[49] Polysaccharide polymer, chitosan hydrogel, degrades as an amino group is protonated in acidic condition.[100] The small molecules from the degradation are soluble in water. The rate of degradation depends on the polymer, pH, and temperature.

Membranes composed of polymers that are crosslinked by covalent bonds maintain membrane integrity without dissolution in a good solvent. Instead, the polymer networks are swollen by the solvent. The swollen polymers, such as hydrogels in water, have large mesh, through which molecules smaller than the mesh can diffuse. By contrast, when the polymer network is collapsed by deswelling, permeability is dramatically reduced. Therefore, membranes whose degree of swelling is controllable with stimuli are useful for the controlled release of encapsulants, as shown in Fig. 7e. For example, hydrogel shells made of PNIPAm is permeable to dextran with a molecular weight of 4000 g mol^{-1} at a temperature lower than LCST, whereas impermeable at a temperature higher than its LCST.[101] Therefore, the microcapsules can encapsulate molecules when the temperature is increased above the LCST and release them as the temperature is decreased below the LCST. In a similar manner, pH-responsive hydrogels such as poly(2-hydroxymethylmethacrylate-*co*-acrylic acid) (p(HEMA-*co*-AAc)) can be used for encapsulation and release at a certain pH. The stimuli are not limited to environmental conditions such as pH and temperature. Specific molecules can be used as stimuli for hydrogel responses. For example, a PNIPAm hydrogel copolymerized with prepolymers containing crown ether group swells in the presence of the heavy metal ions barium and lead.[102] The PNIPAm hydrogel copolymerized with 3-acrylamidophenylboronic acid (AAPBA) shows swelling-deswelling behaviour depending on the concentration of glucose at physiological temperature.[103]

4. Applications of functional microcapsules

4.1 Microcarriers for controlled release

Microcapsules prepared from multiple-emulsion drops can provide advanced release functions through exquisite control of the materials and structures of cores and membranes, as we discussed in section 3.3. Moreover, high efficiency of encapsulation and large payloads can be achieved by direct injection of encapsulants into the cores of multiple-emulsion drops, and a consistent release response is expected of the high uniformity of microcapsules in size and composition. These superior characteristics over bulk encapsulation make microcapsules useful for the controlled release of actives such as drugs, nutrients, fragrances, and cosmetics.

4.1.1 Sustained release. Sustained release of drugs is sometimes important for the long-term treatment of diseases. To fulfil this long-term release, cores and membranes of microcapsules have been carefully modified. For example, the water core of polymersomes is filled with a hydrogel. The hydrogel mechanically supports the bilayer membrane, thereby increasing its stability. At the same time, the hydrogel serves as a diffusion barrier. Therefore, polymersomes subjected to weak osmotic stress slowly release their hydrophilic encapsulants through the small pores in the loosened yet integrated bilayer membrane; the rate is reduced approximately 6 times in comparison with gel-free polymersomes.[104] The hydrophobic molecules can be loaded in thermally-hydrocarbonized porous silicon microparticles, which are further encapsulated in the core of liposomes using double-emulsion drops. The additional lipid bilayer barrier retards the release of the hydrophobic drugs adsorbed on the microparticles; it takes approximately 6 hours for the complete release of the model hydrophobic drug, piroxicam, from the

liposomes, whereas it takes 2 hours from the microparticles in the absence of liposomes.[105]

The period of release can be increased to tens of days or even longer by employing biodegradable membranes or hybrid membranes. Microcapsules composed of a water core and a biodegradable membrane can release both hydrophilic and hydrophobic drugs. The hydrophobic drugs can be loaded in the membrane by consolidating biodegradable polymers and the drug in a thin shell of W/O/W double-emulsion drops. The hydrophilic drugs can be loaded into the water core at high concentration. As ester groups in the backbone of biodegradable polymers, such as PLA, PLGA, and PCL, are hydrolysed, small polymer fragments are formed and dissolved in water, degrading the membrane. Therefore, hydrophobic drugs in the membrane are continuously released during the degradation. The hydrophilic drugs in the core are released as the thinnest part of the membrane is opened by the degradation, as shown in Fig. 8a.[49,106] If microcapsules are perfectly monodisperse and subjected to the same conditions, they will release the encapsulants at the same time. However, there is a small variation in thickness homogeneity of membranes capsule-by-capsule even if they are prepared from monodisperse emulsion drops. Therefore, hydrophilic drugs are released in multiple shots at different moments from hundreds of microcapsules over a long period of time. Microcapsules with a 100 nm-thick PLA membrane slowly release a small hydrophilic dye for the first 30 days without the initial burst.[49] The release is a little bit accelerated afterward, which lasts for 70 days, as shown in Fig. 8a. The release of hydrophilic encapsulants can be significantly retarded by forming hybrid layers. Microcapsules prepared from W/O/O/W triple-emulsion drops can possess an inner oil layer and an outer polymer membrane which isolate the water core from the surrounding. Only 2% of the small molecules in the core are released in a month when the microcapsules are free from osmotic stress.[107] The microcapsules from W/O/W and W/O/O/W emulsion drops achieve a limited loading of hydrophobic materials.

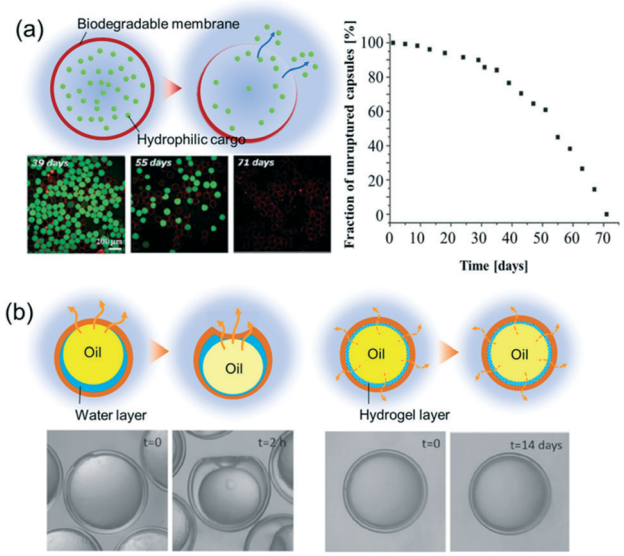

Fig. 8 (a) Sustained release of encapsulants from microcapsules composed of a water core and a biodegradable membrane. Schematic illustration (top left panel), and a series of confocal microscopy images (bottom left), and a time-dependent fraction of unruptured capsules (right) are shown. (b) Comparison of release rate between microcapsules with a water layer (left) and a hydrogel layer (right). The water layer allows direct contact between the oil core and the hydrophobic membrane, whereas the hydrogel layer prevents the contact and significantly retards the release. Reprinted with permission from ref. 49 (a) and 109 (b). Copyright Royal Society of Chemistry (a) and Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (b).

A large payload of hydrophobic cargo can be achieved by using O/W/O/W triple emulsion drops, where the oil core is used for cargo storage and the oil shell is used for membrane formation; either multiple small cores or a single large core can be used. The release of the cargo from the core can be highly slowed down by forming a thin hydrogel layer in the middle water shell.[108,109] In the absence of the hydrogel layer, the oil core can directly contact the hydrophobic membrane through which the hydrophobic cargo diffuses out. The hydrogel layer can prevent direct contact between the oil core and hydrophobic membrane. Therefore, hydrophobic cargo first has to diffuse through the hydrogel layer in order to be released from the microcapsules. In general, hydrophobic molecules have low solubility in the water phase, thereby resulting in sustained release from the microcapsules with a hydrogel layer. When α-pinene is encapsulated in microcapsules with a hydrogel layer made of PEGDA as a hydrophobic model fragrance, the release is highly suppressed in comparison with microcapsules without the hydrogel layer, as shown in Fig. 8b. The microcapsules with the hydrogel layer maintain their spherical shape until 14 days, while the microcapsules without the hydrogel layer buckle due to shrinkage of core volume within 2 hours.[109]

4.1.2 Triggered release. Microcapsules whose membrane is responsive to controllable stimuli, such as light and acoustic wave, are useful for the on-demand release of encapsulants. The triggering by the external stimuli enables the release of drugs at the proper time and location, thereby enhancing the efficacy of drugs. The membranes responsible for the triggered release should be designed to have stimuli-dependent permeability.

Light is the most facile stimulus for triggering a response. In particular, NIR has relatively long penetration depth on tissue, thereby being useful for the *in vivo* release of drugs. Light-responsive membranes have usually been prepared by incorporating metal nanoparticles in thermoresponsive membranes. The metal nanoparticles can convert radiation to thermal energy through the plasmonic photothermal effect. Therefore, light irradiation increases the temperature of microcapsules, which triggers the release of encapsulants through thermoresponsive membranes. For example, microcapsules with an ultra-thin PLGA membrane containing gold nanorods release hydrophilic encapsulants from their cores upon irradiation with NIR, as shown in Fig. 9a; gold nanorods have a high efficiency of photothermal effects in the

NIR region. The irradiation increases the temperature above glass transition temperature of PLGA, thereby partially liquefying the membranes. The hydrophilic cargo leaks through the opening of the molten viscoelastic shell.[98] Polymersomes whose bilayer membrane contains PNIPAm-*b*-PLGA diblock copolymers and gold nanoparticles rupture upon irradiation with visible light. The elevation of the temperature above the LCST of PNIPAm causes the PNIPAm blocks to collapse, which destabilizes the bilayer as the hydrophilic–hydrophobic balance is disrupted.[110] Therefore, the release of hydrophilic encapsulants can be triggered by the irradiation.

The thermoresponsive membrane can be designed to have a permeability that is reversibly controlled by temperature, while maintaining its overall size and shape without rupture. When such membranes contain photothermal nanoparticles, the permeability can be reversibly tuned by irradiation of light. The reversible tuning enables the repetitive loading and release of actives. For example, gold nanorod-laden PNIPAm membranes have a high permeability at a temperature lower than its LCST, as the hydrogel is highly swollen. When the microcapsules are irradiated by NIR, the temperature increases above LCST, resulting in deswelling of the membranes. The collapsed hydrogel network has a small mesh size and therefore low permeability. Therefore, the microcapsules allow an influx of molecules from the surroundings at a temperature lower than the LCST, which are then encapsulated using NIR irradiation. The hydrophilic molecules in the core can be released to the fresh surroundings when the NIR is turned off; the microcapsules cool down below LCST, which increases the permeability of the membrane.[101] An alternative approach to creating membranes with tunable permeability is to disperse gold nanorod-laden PNIPAm particles in the polymeric membrane. Below the LCST, PNIPAm particles are swollen by water, which fills the all the pores of the membrane. Therefore, molecules larger than the mesh size of the PNIPAm network are encapsulated in the core of microparticles. Upon irradiation with NIR light, the particles shrink, thereby open the pores. Therefore, the encapsulated molecules are released through the macropores formed in the membranes.[111]

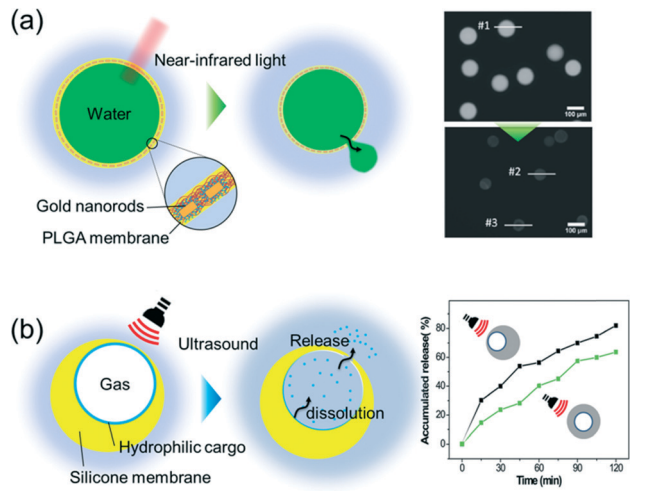

Fig. 9 (a) Schematic (left) and fluorescence microscopy images (right) showing the near-infrared light-triggered release of aqueous core, where poly(lactic-*co*-glycolic acid) (PLGA) membrane contains gold nanorods. (b) Schematic showing ultrasound-triggered release of hydrophilic cargo from gas-filled microcapsule (left) and time-dependent accumulated release from the microcapsules with eccentric (black squares) and concentric (green squares) gas cores (right). Reprinted with permission from ref. 98 (a) and 112 (b). Copyright Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (a) and Royal Society of Chemistry (b).

Microcapsules can be rendered to be ultrasound-responsive by inserting a gas compartment in the core. The gas-cored microcapsules can be prepared easily by drying water from the core of microcapsules prepared by W/O/W double-emulsion templates. During the drying, hydrophilic cargo in the water core is deposited on the inner wall of the gas-filled core. The gas-filled microcapsules dispersed in water rupture when they are subjected to ultrasound; ultrasound exerts a compressive force on the polymer membrane. Therefore, water rapidly invades the core and the dried cargo dissolves in the water.[112] The sensitivity of the membrane can be further enhanced by eccentrically positioning the core, as shown in Fig. 9b; the thin membrane of an eccentric microcapsule has low mechanical stability, thereby being sensitive to ultrasound. Gas can be directly inserted into microcapsules using G/O/W/O triple-emulsion drops, where the oil shell is used for loading of hydrophobic cargo and the water shell is used for formation of hydrogel membrane. Upon ultrasound application, the microcapsules rupture, thereby releasing the cargo into the surrounding oil.[113]

4.1.3 Smart release. Microcapsules with a so-called "smart release" function sense environmental conditions and release encapsulants only when subjected to the desired conditions. Such a smart function is convenient because continuous monitoring of the environmental condition and external triggering are not required. Various physiological conditions have been used as cues responsible for the release, which include temperature, pH, and the concentration of specific molecules and ions. The materials and structure of microcapsules have been carefully selected to provide smart release of desired encapsulants as a result of target cues.

Microcapsules with thermoresponsive membranes have been used to cause encapsulant release at a certain temperature range. For example, microcapsules composed of a water core and a frozen wax membrane rupture when the temperature is higher than the melting point, thereby releasing hydrophilic encapsulants from the core. The wax membrane can contain hydrophobic drugs, of which the release can be accelerated at molten state. Microcapsules prepared using a wax with a melting point immediately below body temperature can release the hydrophilic anti-cancer drug doxorubicin from their core and the hydrophobic drug paclitaxel from their wax membrane when they are injected into the body, thereby being potentially useful for cancer treatments.[75] An alternative design for a thermoresponsive microcapsule can be composed of an oil core and a PNIPAm membrane. The

oil can be irreversibly ejected as the membrane shrinks and ruptures above the LCST.[97] The membrane rupture provides a single shot release. If the thermoresponse of the membrane is reversible, the microcapsules can be used repeatedly. For example, microcapsules with a water core and a PNIPAm membrane allow diffusion of molecules at a temperature below the LCST, while preventing the diffusion at a temperature above the LCST.[101] Porous membranes whose pores are filled with PNIPAm particles can be used to tune the permeability reversibly through the temperature.[114] As we discussed in section 4.1.2, such thermoresponsive membranes can also be used for light-triggered release by incorporating photothermal metal nanoparticles.

Smart release at a certain pH range is beneficial for site-specific therapy where the pH is different from normal conditions, including the stomach, colon, and tumour sites. To achieve pH-responsive release, various methods and materials have been used to compose microcapsules. For example, membranes are prepared from W/O/W double-emulsion drops by consolidating an anionic diblock copolymer consisting of AAc and methyl methacrylate, and pH-insensitive polymers in the oil shell to release hydrophilic cargo from the core at basic conditions; the anionic polymer is insoluble at acidic and neutral conditions and soluble at basic conditions.[99] In a similar manner, the cationic triblock copolymer poly(*n*-butyl methacrylate-(2-dimethylaminoethyl)-methacrylate-methyl methacrylate) is used to form a membrane which dissolves at acidic conditions. The base-responsive microcapsules are useful for drug delivery in the colon, and the acid-responsive ones are useful for delivery in the stomach. The acid-responsive microcapsules can also be prepared from O/W/O double-emulsion drops by gelating chitosan.[100] The chitosan in the water shell is gelated at neutral conditions by terephthalaldehyde originating from the oil core. The resulting microcapsules are finally dispersed in water, while maintaining their oil core. The oil cores are released only at acidic conditions at which point the gel membrane is degraded. To achieve the acid-responsive release of one drug and sustained release of the other from single microcapsules, the oil cores are laden with drug-loaded PLGA nanoparticles.[115] In an acidic mixture of water and ethanol, the chitosan gels are degraded, and the oil core is released to the surrounding. The drug dissolved in the oil is immediately released upon the degradation of the gel and the drug loaded in the PLGA nanoparticles is slowly released as the PLGA degrades afterwards. A pH-responsive ultra-thin membrane can be prepared by interfacial complexation between oppositely charged polymers.[116] If a water core with pH 3.7 and an oil shell of W/O/W double-emulsion drops contains PAAc and branched poly(ethylenimine) respectively, two polymers will form a complex at the inner water–oil interface through electrostatic attraction. The membrane of polymer complexes enclosing the water core is spontaneously separated from the oil drop, forming a stable microcapsule at pH 2. The membrane is disassembled at pH 5 or above, thereby releasing hydrophilic encapsulants from the water core.

Aforementioned methods use the dissolution of membrane by pH conditions, thereby requiring the sacrifice of the microcapsule for the release of encapsulants. Therefore, it allows only a single shot of release. The membranes made of pH-responsive hydrogels provide a reversible change of permeability depending on the pH, thereby allowing repeated uses. For example, microcapsules with a cationic hydrogel membrane of poly(*N*,*N*-dimethylaminoethyl methacrylate) are prepared by photocrosslinking the gel precursors in the water shell of O/W/O double-emulsion drops.[117] When the core and surrounding medium are replaced with water, the microcapsules show a reversible change in their volume depending on the pH. The membrane is swollen and permeable to a certain size of molecules at acidic conditions, while deswollen and impermeable to the same molecules at basic conditions. Therefore, molecules supplied by the surroundings at acidic conditions can be encapsulated in the core by increasing the pH, which can then be released once again at acidic conditions. pH-responsive gel membranes can be prepared by gelating chitosan with glutaraldehyde. The degree of gel swelling increases as the pH decreases from neutral conditions, thereby providing pH-dependent permeability; chitosan gelated by glutaraldehyde maintains its network at moderately acidic conditions, instead of being dissolved. In addition, a pH-responsive membrane can be further modified to be thermoresponsive by dispersing PNIPAm particles in the membrane.[118] The PNIPAm particles serve as thermoresponsive microvalves. As the temperature increases above the LCST, the particles shrink, thereby opening the valves. Therefore, permeability or rate of release can be highly increased by decreasing the pH and increasing the temperature at the same time. The microcapsules with pH-dependent permeability can be prepared by incorporating pH-responsive polymers in the interstices of colloidosomes.[119] The colloidosomes are prepared by consolidating aminosilane-modified silica nanoparticles in the oil shell of W/O/W double-emulsion drops. The aminosilane group is functionalized with an initiator, from which the pH-responsive polymer poly(2-(dimethylamino)ethyl methacrylate) is grown. The resulting organic–inorganic hybrid membrane is swollen at acidic conditions, thereby being more permeable than the deswollen membrane at basic conditions.

The hydrogel membrane can be modified with receptors to be responsive to specific molecules or ions. Therefore, microcapsules with a membrane made of the modified hydrogels can release encapsulants into the medium containing target molecules above a certain threshold concentration; microcapsules with such gel membranes are prepared from O/W/O double-emulsion drops. For example, a PNIPAm gel can be modified with AAPBA to make a glucose-responsive membrane.[103] In the presence of glucose, the AAPBA in the gel forms a complex with glucose, which increases the internal osmotic pressure, thereby leading to swelling, as shown in Fig. 10a. To maximize the concentration-dependent volume change at body temperature, PNIPAm gel is further modified with AAc, which increases its LCST to body temperature. The

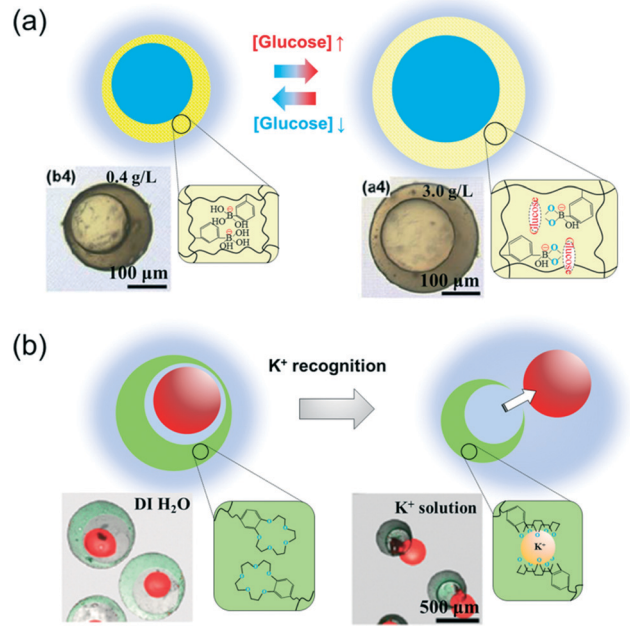

Fig. 10 (a) Smart microcapsules of which permeability is reversibly tuned by the concentration of glucose. The PNIPAm gel membrane is modified with 3-acrylamido phenylboronic acid (AAPBA) which captures glucose. Swelling ratio and permeability of membrane increase so does the concentration of glucose. (b) Smart microcapsules that release their oil core selectively in the presence of potassium ions at high concentration. Potassium ions are captured by two crown ether groups in PNIPAm gel, which leads to gel shrinkage and membrane rupture, resulting in the release of oil. Reprinted with permission from ref. 103 and 121 (b). Copyright Royal Society of Chemistry (a, b).

microcapsules become highly swollen when the concentration of glucose is increased from 0.4 to 3.0 g L^{-1} at 37 °C, at which point hydrophilic encapsulants are released through the swollen membrane. This glucose-sensitive release is useful for diabetes therapies. In a similar manner, different sets of targets and receptors have been used. For example, the heavy metal ions barium and lead and the crown ether 18-crown-6 form stable host–guest complex. Therefore, a PNIPAm gel modified with pendants of 18-crown-6 is selectively swollen in the presence of these ions; the volume change is significant in the temperature range of 34–46 °C.[102] It is well known that 2-naphthalenesulfonic acid and beta-cyclodextrin form a host–guest complex. Using PNIPAm gels modified with beta-cyclodextrin, 2-naphthalenesulfonic acid-responsive microcapsules can be prepared.[120]

Recognition of potassium ions and subsequent smart release is useful for the treatment of abnormal cells with reversed concentrations of sodium and potassium ions.[121] To achieve this, 15-crown-5 is incorporated in a PNIPAm gel as a receptor for potassium ions. Two 15-crown-5 groups and one potassium ion form a 2 : 1 complex. Therefore, the gel matrix shrinks in the presence of potassium ions as two 15-crown-5 groups in the network sandwich one potassium ion, as shown in Fig. 10b. When the microcapsules contain an oil core, the oil is ejected from the microcapsules into the solution of potassium ions as the gel membrane squeezes the core and finally ruptures.

4.2 Encapsulation of cells

Microgels provide biocompatible 3D microenvironments to living cells and allow for the fast diffusion of small molecules such as oxygen and nutrients. Therefore, cells have been encapsulated in microgels to use them as scaffolds for tissue engineering and implantable carriers for cell therapy. Microfluidic techniques have employed W/O emulsion drops as templates to make monodisperse microgels. However, a long-term exposure of cells to oil reduces the viability of cells. To minimize the oil contact, W/O/W double-emulsion templates have been recently used. The inner core is used for the formation of a cell-laden hydrogel, and the middle oil phase is used as a sacrificial layer for temporary isolation of the core from the surrounding water environment. As soon as the cores containing cells are gelated by photocrosslinking gel precursors such as gelatin methacrylate (GelMA), the oil layer is ruptured to expose the microgels to a biocompatible water environment, as shown in Fig. 11a.[122] The residence time of microgels within the oil layer is reduced to a few tens of minutes, which results in the high viability of encapsulated cells in comparison with encapsulation based on single-emulsion templates.

Irradiation with UV for photocrosslinking is sometimes fatal to delicate cells. To avoid the use of UV, dual-cored double-emulsion drops with an ultra-thin middle layer are used, where two distinct cores contain sodium alginate and divalent calcium ions, respectively. The dual-cored double-emulsion drops with ultra-thin middle layers take on a nonspherical shape, which exerts strong capillary forces. Therefore, the oil continuously drains out from the layer between the two cores, finally leading to core-to-core coalescence; although the oil in the outermost layer also drains out, the drainage rate is relatively low due to the high lubrication resistance. The coalescence results in the mixing of sodium alginate and calcium ions in the fused core of the double-emulsion drops, thereby forming a hydrogel through ionic gelation, as shown in Fig. 11b.[123] The microgels are then released to the surrounding water phase by rupturing the oil layer to minimize oil contact. This method does not require any UV irradiation or chemical cues for gelation, thereby achieving high biocompatibility.

4.3 Photonic ink capsules

Double-emulsion drops have been used as templates for encapsulating fragile colloidal photonic structures with stable solid membranes. Colloidal arrays exhibit structural colours through diffraction of a selected wavelength of light.[124] The structural colour is usually iridescent and never fades as long as the structure persists, unlike conventional chemical pigments, providing novel applications in aesthetic coatings and reflection-mode displays. For these uses, the colloidal arrays should be stabilized in a granular format. Double-emulsion

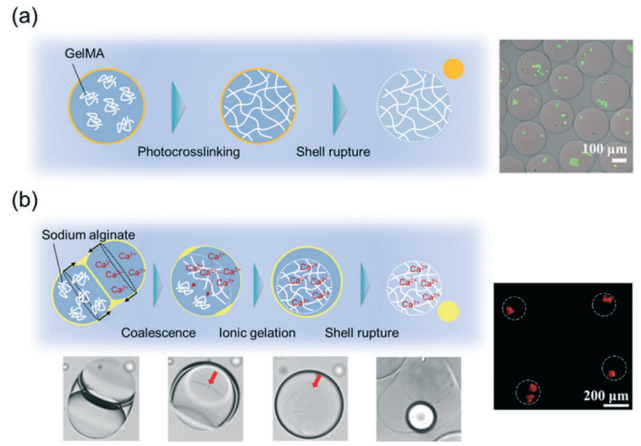

Fig. 11 (a) Schematic showing the production of microgels using single-cored double-emulsion drops (left) and fluorescence microscopy image of mammalian cell-laden microgels (right). The microgels are prepared by photocrosslinking gelatin methacrylate (GelMA) in the core, which are then released into the continuous water phase. (b) Schematic and a series of optical microscopy images showing the production of microgels using dual-cored double-emulsion drops (left) and fluorescence microscopy image of microalgae cell-laden microgels (right). The selective coalescence between two distinct cores leads to ionic gelation of alginate in the fused core. Reprinted with permission from ref. 122 (a) and 123 (b). Copyright Royal Society of Chemistry (a, b).

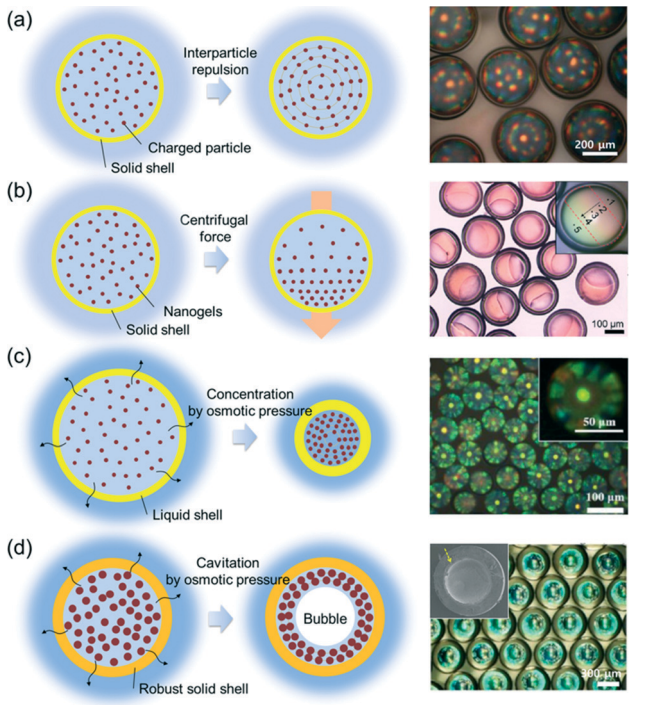

Fig. 12 (a–d) Sets of schematic showing the formation of colloidal photonic structure and optical microscopy image of resulting photonic capsules: (a) crystallization of charged colloids by electrostatic interparticle repulsion, (b) centrifugal force-induced formation of ordered nanogels with a concentration gradient, (c) osmotic-pressure-induced concentration of colloids confined in a spherical liquid shell, and (d) osmotic-pressure-induced cavitation in a microcapsule and consequent concentration of particles along the inner surface of a microcapsule. Reprinted with permission from ref. 87 (a), 125 (b), 127 (c), and 130 (d). Copyright American Chemical Society (a–d).

drops are excellent templates to create stable photonic ink capsules. The inner drop can be used to form and store the colloidal array, and the outer drop can be solidified to form stable membranes to protect the array.

To produce photonic microcapsules containing colloidal arrays, W/O/W double-emulsion drops are prepared to have a core consisting of an aqueous colloidal suspension and a shell consisting of a photocurable resin. If the colloids are highly charged, they spontaneously form CCAs with long-range order within the core even at low concentration to minimize the repulsive energy among the colloids, as shown in Fig. 12a.[87] The colloidal array adopts an onion-like configuration by forming a hexagonal array along the interface of the inner drops, which gives the photonic drops rotation-independent colours. Although such crystals formed by electrostatic repulsion are highly susceptible to ionic impurity and dilution, rigid solid shells with low permeability protect the crystals from the surrounding, thereby assuring the long-term maintenance of the isotropic structural colours from CCAs.

Soft nanogels can be contained in the aqueous core of the solid microcapsules, instead of highly charged colloids. However, the nanogels do not form ordered arrays at low concentration due to insufficient interparticle repulsion. One way to induce order is the concentration of the nanogels using a centrifugal force.[125] As the microcapsules are centrifuged, the nanogels sediment along the direction of the force and the concentration gradually varies within each microcapsule. Therefore, the resulting nanogel array in the microcapsule possesses a gradual increase of structural periodicity from the bottom to the top and therefore exhibits a colour gradient, as shown in Fig. 12b. This anisotropy of the colloidal array gives the photonic capsules rotation-dependent structural colour.

An alternative way to concentrate colloids is the selective removal of water from the core of W/O/W double-emulsion drops using osmotic pressure. By exerting higher osmotic pressure in the surrounding than in the core, water in the core can be pumped out through liquid shells to the surrounding water phase. Because of this, the colloids are concentrated in the shrunken, spherical core, which crystallizes the colloids, as shown in Fig. 12c.[64,126] The colloidal crystals are then stabilized by forming solid membranes through the polymerization of resin in the shell drop. The osmotic-pressure-driven concentration maintains a uniform concentration of colloids within the core or a small radial gradient of concentration. Therefore, the resulting photonic microcapsules exhibit rotation-independent structural colours. Colloidal glasses with only short-range order can be formed within the shrunken drops by exerting a high osmotic pressure.[127] Rapid concentration does not allow the rearrangement of colloids and arrests the random array in the core. The use of colloids composed of a rigid polymer core and a soft gel shell enables the formation of colloidal glass even at a low rate of concentration.[128] The sticky gel shells interrupt colloidal rearrangement, promoting the formation of a colloidal glass.

The colloidal glasses render the photonic microcapsules non-iridescent, unlike colloidal crystals; this non-iridescent property is beneficial for display applications among others. If water is further pumped out after the solidification of the shell, the microcapsules are buckled. The colloidal array contained in the resulting nonspherical shell reduces the iridescence in comparison with its spherical counterpart.[129]

The osmotic pressure difference across liquid or flexible solid shells can reduce the volume of the core by pumping out water. By contrast, the pressure difference across the rigid solid shell can produce a gas bubble in the core by draining water out, while maintaining the size and shape of microcapsules.[130] When these aqueous cores contain colloids, the drainage concentrates the colloids near the inner surface of the solid shell. Therefore, a layer of colloidal crystals is formed along the inner surface, and a large void is formed at the centre, as shown in Fig. 12d. The resulting photonic capsules with a void can be designed to have an average density comparable to the water medium in which they are dispersed, which provides a long-term suspension stability of the photonic microcapsules.

Most photonic ink capsules exhibit invariant structural colour as internal photonic structures are stabilized. Microcapsules containing an aqueous suspension of magnetic nanoparticles show structural colour only when they are subjected to the external magnetic field.[131] The nanoparticles form strings along the direction of the external field that have consistent interparticle distance. Therefore, structural colour is developed by 1D photonic structures under the external field. Arrays of the microcapsules can be used as a display panel as they show colour images when the magnetic field is regioselectively applied.

4.4 Capsule-type microsensors

Microcapsules produced from double or multiple emulsions have been further designed to be environment-responsive for new types of microsensors. Such capsule-type microsensors have a great potential in many applications due to their small sizes. Microcapsules are small enough to be injected in the target volume, which can potentially report conditions of the local microenvironment. Moreover, a microcapsule array dispersed in the volume can report on the spatial distribution of conditions. Capsule-type sensors are currently limited to measuring osmotic condition or temperature at this early stage of development.

The osmotic conditions of aqueous solutions have usually been measured by using colligative properties such as the depression of the freezing point or elevation of the boiling point. However, such conventional methods require at least a millilitre of the sample and are not available for measuring the osmotic pressure *in situ* or *in vivo*. Microcapsules composed of solid semipermeable membranes have recently been designed to directly measure the osmotic pressure with a small volume of the sample. For example, microcapsules with an ultra-thin solid membrane can be used because they are buckled in surroundings with slightly higher osmotic pressure than the core.[50] Such sensitive membranes are produced to have a thickness of approximately one hundred nanometres by evaporation-induced solidification of polymers from double-emulsion drops with a thin shell.[132] When a set of optically-labelled microcapsules containing aqueous cores of different osmolarities is dispersed in the sample, some of them are buckled as shown in Fig. 13a, from which osmotic pressure of the surrounding can be estimated within a certain range. Because the buckling of these microcapsules can be simply confirmed by optical microscopy, no delicate equipment is required for the measurement.

Photonic microcapsules can be designed to have a colloidal crystal-laden core and a semipermeable, elastic shell by the osmotic pressure-medicated concentration of colloids and subsequent polymerization of the resin.[64] The volume of the photonic core is determined by the osmotic pressure of

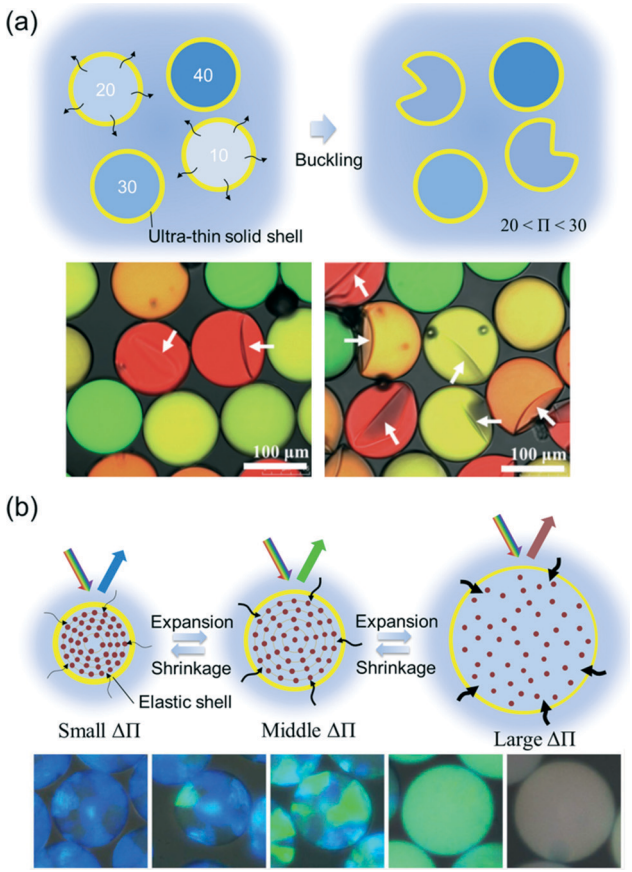

Fig. 13 (a) Estimation of osmotic pressure from selective buckling of microcapsules containing aqueous cores with different osmotic pressures: a schematic illustration (top) and confocal microscopy images showing selective buckling of red capsules (bottom left) and all capsules except green (bottom right). (b) Measurement of osmotic pressure using osmochromatic microcapsules composed of a colloidal crystal-laden core and an elastic shell: a schematic illustration (top) and a series of optical microscopy images showing the osmotic-pressure-dependent change in volume and colour (bottom). Reprinted with permission from ref. 132 (a) and 64 (b). Copyright Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (a) and Nature Publishing Group (b).

the surroundings as the osmotic pressure difference across the shell is balanced by the membrane elasticity at equilibrium. Therefore, the concentration of colloids in the photonic core is also varied with the osmotic pressure, resulting in osmotic-pressure-dependent structural colours, as shown in Fig. 13b; this property is called osmochromaticity. The osmotic pressure of the surroundings can be estimated from the structural colour of a single osmochromatic capsule, thereby providing a facile way to measure osmotic pressure.

Many types of thermocouples have been developed. However, most of them are not suitable for measuring the temperature of microenvironments. Microcapsules containing temperature-sensitive photonic structures can report the temperature at which they are dispersed through their colours. Various colorimetric sensors based on photonic structures have been developed in a film format.[133] To make the thermochromic photonic structure in a capsule format, double-emulsion drops have been used as templates. For example, highly concentrated nanogels made of p(NIPAm-*co*-AAc) are encapsulated in microcapsules templated by W/O/W double-emulsion drops. The negative charge of the nanogels leads to the formation of CCAs through electrostatic repulsion at low temperature, thereby developing structural colour. However, when the temperature increases, the nanogels shrink, which reduces the volume fraction of particles in the microcapsules. Therefore, the crystals melt as the temperature increases, by which structural colour is lessened, as shown in Fig. 14a.[134] This temperature-dependent change is fully reversible, enabling the rough estimation of temperature from the saturation of the structural colour.

To maintain structural regularity and pronounced structural colour across an entire range of temperature sensing, nonclose-packed colloidal crystals, or CCAs, have been immobilized in thermoresponsive gels; such structures are referred to as polymerized crystalline colloidal arrays (PCCAs). In PCCAs, the interparticle distance is reversibly changed as the thermoresponsive matrix experiences a volume change, while maintaining ordered structures of CCAs. To make PCCAs in a granule format, O/W/O double-emulsion drops are prepared using a capillary microfluidic device. The aqueous shell is composed of highly charged PS particles dispersed in an aqueous solution of PNIPAm precursors. Upon the formation of these double-emulsion drops, the PS particles form CCAs in the liquid shell, and are then captured in a PNIPAm shell by photopolymerizing the precursors. Finally, the resulting microcapsules are transferred into a water phase to have a water core and gel shell in a continuous water medium. The microcapsules exhibit reversible colour and spectrum shifts responsible for temperature change, as shown in Fig. 14b;[65] these PCCA shells have faster volume change dynamics and therefore a shorter response time than PCCA particles templated from W/O single-emulsion drops.

Cholesteric liquid crystals (CLCs) can also be employed as a core material for capsule-type temperature sensors, instead of colloidal structure, due to their thermochromic property. CLCs are nematic liquid crystals whose director is rotated

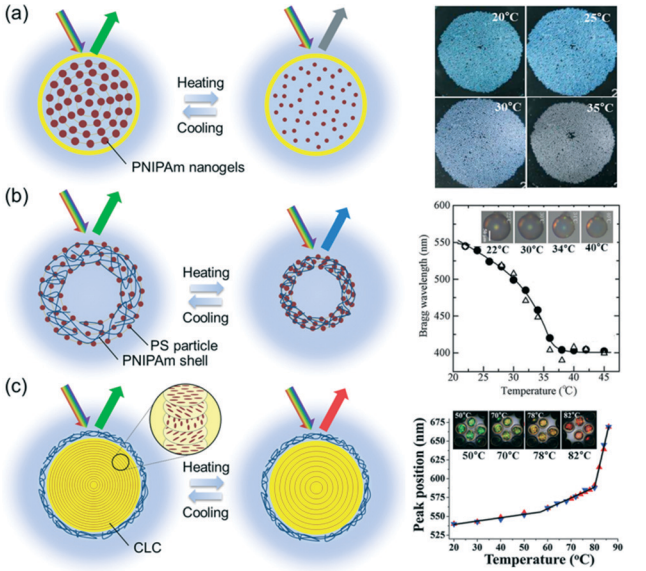

Fig. 14 (a) Microcapsule containing charged nanogels made of PNIPAm in its core which shows a temperature-dependent change of colloidal structure and colour saturation; as the temperature increases, the colloidal crystals melt and the structural colour becomes timid. (b) Microcapsule whose shell is composed of a nonclose-packed colloidal array captured in a PNIPAm matrix which shows a temperature-dependent shift of structural colour; as temperature increases, colloidal lattice shrinks and the structural colour blue shifts. (c) Microcapsules containing cholesteric liquid crystals (CLCs) in its core which shows a temperature-dependent shift of structural colour; as the temperature increases, helical structures are relaxed and structural colour red shifts. Reprinted with permission from ref. 134 (a), 65 (b) and 66 (c). Copyright American Chemical Society (a) and Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (b, c).

along a perpendicular axis in the presence of chiral dopant, forming a helical structure. When the helical pitch is comparable with the wavelength of visible light, CLCs exhibit structural colours. In general, the helical pitch increases with the temperature because the thermal energy relaxes the helix; if the dopant solubility in the host LC increases with the temperature, the helical pitch can decrease as the temperature increases. To make thermochromic microcapsules using CLCs, O/W/O double-emulsion drops are used, where the oil core consists of a CLC and the water shell is an aqueous solution of polymer precursor. As the precursor is photo-polymerized, the CLC is enclosed by the solid shell. The CLC-laden microcapsules are transferred into the water. The microcapsules exhibit a reversible shift in structural colour and spectrum as the temperature is varied, as shown in as shown in Fig. 14c.[66] The thermochromic property of CLC microcapsules can be maintained even in a dried state because of the low volatility of CLCs, which is difficult to achieve with thermochromic microcapsules based on colloidal photonic structures.

4.5 Other applications

4.5.1 Encoded microparticles. A library of microparticles with identification tags has been used in suspension arrays

for biological screening and multiplex immunoassays, as an alternative to conventional 2D arrays. The suspension arrays show fast binding kinetics, a large surface area and high statistical power. As one of the approaches to writing a code in microparticles, multiple-emulsion drops have been used. For example, core–shell microparticles composed of a quantum dot (QD)-loaded core and a hydrogel shell are prepared from O/W/O double-emulsion drops whose inner phase is a photocurable suspension of QDs and outer phase is an aqueous solution of hydrogel precursors.[135] The microparticles exhibit photoluminescent spectra from QDs, which are dictated by the type and the relative amount of QDs contained in the core. Therefore, various optical codes can be written in the core of microparticles. The hydrogel shell prevents direct contact between the QDs and biomolecules in the surrounding solution, which provides high biocompatibility. In addition, the microparticles can be further rendered to be magneto-responsive by employing dual-cored double-emulsion drops whose other core is a photocurable suspension of magnetic nanoparticles.

The number of distinct optical codes that are achievable from a single core with photoluminescence is limited. To increase the number, W/O/W double-emulsion drops are produced with controllable numbers of distinct water cores. With a capillary microfluidic device with multiple channels for inner phases, distinct aqueous solutions of chemical dyes are independently emulsified in a photocurable resin, which is then further emulsified into a continuous water phase through sequential emulsification. The number of inner drops of each colour is set by the relative frequency of generation of the inner drop to the outer drop. The independent control over the numbers of distinct water cores in the outer drop enables the formation of codes through the combination of the numbers and colours of cores in the resulting microparticles.[136] The number of possible codes is 816 for three different colours and number control up to 15. The number is increased to 53 130 for five different colours and number control up to 20. The surface of the microparticles can be decorated with a dense array of silica particles, which allows the simple chemical treatment for biological assays.

Optical codes from photoluminescence or absorption spectra fade due to bleaching and can overlap with the fluorescence spectra of biomolecules for assays, which possibly reduces the precision of the analysis. The limitations can be partially solved by using codes from reflectance spectra of photonic nanostructures. Microparticles composed of multicores of photonic crystals and a hydrogel shell are prepared from O/W/O double-emulsion drops.[137] The inner phase is a photocurable suspension of silica particles which spontaneously form a crystal lattice due to interparticle repulsion. The peak position of the reflectance spectrum is determined by the size of silica particles in the suspension. Therefore, multiple peaks can be introduced in the spectra by incorporating multiple distinct cores. In addition, cylindrical double-emulsion drops containing the serial arrangement of cores are prepared by confining them in a narrow channel, which yields rod-like microparticles through *in situ* polymerization. The rod-like microparticles provide a larger number of codes than spherical microparticles because the arrangements of distinct cores also serve as identification tags. The rod-like microparticles containing four cores are shown in Fig. 15a.

4.5.2 Particle-type adsorbents. Microparticles templated from double-emulsion drops have been used as adsorbents for oil spilled in water. For the adsorption, the microparticles are designed to have hydrophobic porous structures. At the same time, a hydrophilic surface is desired for high dispersion stability in water. To create such amphiphilic microparticles with internal pores, O/O/W double-emulsion drops are employed.[138] The inner oil phase is a homogeneous mixture of a photocurable, hydrophobic resin and inert oil, and the middle oil phase is a less hydrophobic resin containing silica particles. As the double-emulsion drops are formed, the silica particles in the outer drop migrate to the interface between

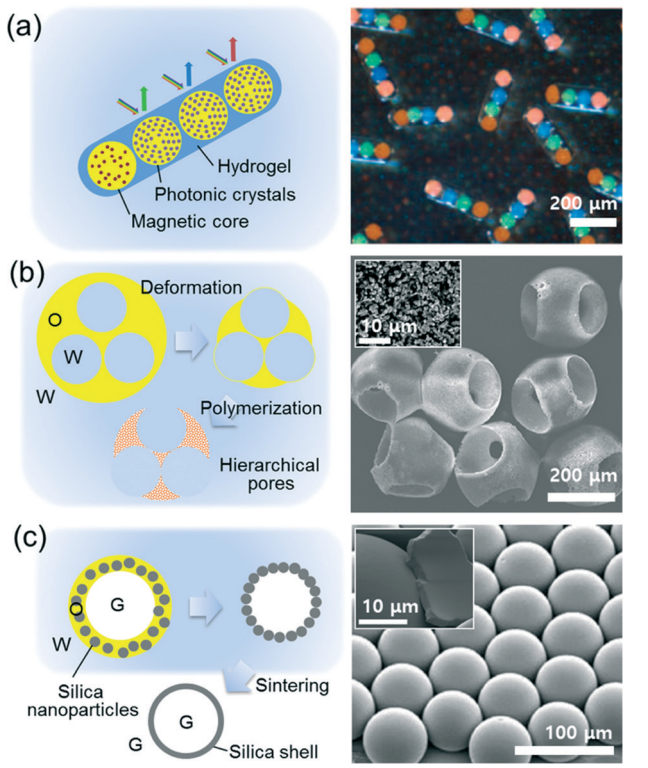

Fig. 15 (a) Rod-like microparticles with an identification tag and magnetic function. The tag is composed of serial cores with structural colours. (b) Microparticles with hierarchical pores for adsorption of spilled oil in water. The microparticles are templated by W/O/W double-emulsion drops whose oil shell is photocurable monomers partially miscible with water. Inset in the right panel shows pores with submicron scale. (c) Bubble capsules prepared by G/O/W drops. The membrane of silica nanoparticles is first prepared by the consolidation of middle phase, which is then sintered to form a continuous shell. Inset in the right panel shows smooth membrane without porosity. Reprinted with permission from ref. 137 (a), 140 (b) and 73 (c). Copyright Nature Publishing Group (a) and American Chemical Society (b, c).

the drop and surrounding water to minimize interfacial energy. Upon UV irradiation, the homogeneous core becomes heterogeneous due to phase separation of the polymerized resin and the inert oil, thereby leaving behind hydrophobic, porous internal structures after washing off the oil. The silica particles are trapped at the surface during the polymerization, which renders the microparticles hydrophilic. The amphiphilic microparticles are dispersed in water without forming aggregates. When the surrounding water contains oil drops, the microparticles are partially exposed to the oil, and adsorb and store the oil in the internal pores.

Amphiphilic microparticles with hydrophobic pores can be prepared from W/O/W double-emulsion drops containing closely-packed multi-cores. The inner phase is an aqueous suspension of graphene oxide and the middle phase is a photocurable suspension of silica particles. In the same manner to the previous example, silica particles are spontaneously anchored at the interface, which renders the resulting microparticles hydrophilic after photopolymerization of the suspension. The hydrophilic graphene oxide in the cores is reduced by hydrothermal treatment, which yields hydrophobic spongy graphene. Thin parts of the membrane are fragile, which are torn apart to expose the spongy graphene to the surroundings. Therefore, the amphiphilic microparticles can adsorb and store oils in its cores, while maintaining a high dispersion stability in water. The microparticles can be further rendered to be magneto-responsive by dispersing magnetic nanoparticles in the middle phase, which helps recover the microparticles after use.[139]

Highly porous microparticles can also be prepared from W/O/W double-emulsion drops using a photocurable resin that is partially miscible with water. As the double-emulsion drops are formed, water diffuses into the oil shell and the resin molecules diffuse to the water. This mass transfer leads to the partial extrusion of the water core into the surrounding medium, while maintaining a thin membrane. In addition, upon subsequent polymerization, the water that is diffused in the oil drop forms fine drops through phase separation, thereby producing small pores in polymerized shell. When multiple inner core drops are densely packed in the outer drop, the resulting microparticles have macropores from the core drops and mesopores from the phase separation, as shown in Fig. 15b. Such a hierarchical porous structure is beneficial for rapid mass transfer and adsorption, which is used for oil removal and protein adsorption.[140]

Microparticles have also been designed to adsorb specific ions from water. For this, molecular receptors should be introduced in microparticles, instead of forming hydrophobic pores. For example, benzo-18-crown-6-acrylamide that is copolymerized in PNIPAm gel can selectively capture lead ions from water.[141] To facilitate mass transfer and convective mixing, the gels are tailored to microgels by using an O/W/O double-emulsion template. The inner phase is a volatile organic suspension of magnetic particles and the middle phase is an aqueous solution of the gel precursors. The organic solvent is first vaporized from the core and gels are then formed by photopolymerization. The resulting microgels have an aggregate of magnetic nanoparticles that is trapped in the microgels. Therefore, the microgels can be used for adsorption of lead ions, which are then recovered by a magnetic field. Moreover, the thermoresponsive property of the PNIPAm gel enables the release of lead ions at high temperature.

4.5.3 Bubble capsules for acoustic contrast agents. Microbubbles have been used as acoustic contrast agents. Microfluidics has provided monodisperse bubbles, which potentially show uniform resonance.[142] However, microbubbles are unstable against coalescence among themselves and dissolution in the medium. To enhance the stability, bubbles have been encapsulated by solid shells through microfluidic techniques. For example, G/O/W whose oil shell is an organic suspension of silica nanoparticles is used to form a solid membrane of nanoparticle aggregates through evaporation of the organic solvent.[73] The membrane stability can be further enhanced by sintering the nanoparticles at high temperature. The sintered silica forms a continuous shell without porosity as shown in Fig. 15c, which completely isolates the gas core from surrounding, thereby providing high stability of bubble; these bubbles can also be used as fillers for lightweight materials. As an alternative, G/W/O is used as a template, of which the water phase contains graphene oxide and surfactants for stabilization of the G/W interface. As the water evaporates, the graphene oxide forms a solid shell through consolidation, thereby isolating gas core from the surrounding oil.[143]

Gas can be injected after the formation of microcapsules. For example, microcapsules composed of a water core and a solid shell maintain their size and shape during the evaporation of all of the water if the shell is sufficiently rigid to support the pressure reduction in the core. Gas fills the cores during the evaporation and the spherical microcapsules retain the gas when they are dispersed in water. These gas-filled microcapsules show high stability even at high hydrostatic pressure and good echogenic property.[144] In a similar manner, microcapsules can be filled with gas through cavitation by exerting high osmotic pressure with the surrounding water.[145] Gas cores can be generated through chemical reactions by separately storing two reagents in two distinct cores of W/O/W double-emulsion drops.[146] Upon the coalescence of two cores, the reaction produces gas in the fused core. For example, hydrogen peroxide and catalase can be used to produce oxygen gas.

4.5.4 Microcapsules for self-healing. Microcapsules can be used as self-healing materials if they are embedded in the polymer matrix. For self-healing based on the epoxy–amine system, molecules with reactive amines are encapsulated in polymer microcapsules using W/O/W double-emulsion templates,[147] whereas epoxy monomers are encapsulated by a bulk urea-formaldehyde encapsulation protocol.[148] Two different microcapsules are embedded in a polymerized epoxy resin, which release their encapsulants when the resin is cracked. The epoxy polymerizes with the amine curing agent

to form an epoxy polymer which fills the cracks, thereby healing the mechanical failure.

5. Conclusions

Since Nisisako's and Weitz's groups reported their pioneering works on the microfluidic production of double-emulsion drops in 2004 and 2005,[23,45] there has been enormous progress in microfluidics and the material processing technology for producing microcapsules; these two papers have been cited more than 1500 times. In the first half of the past decade, new device designs and materials for multiple-emulsion production have mainly been studied. In the second half, new functions and applications of microcapsules have been suggested. Because of this trend, an interdisciplinary study among fluid mechanics, material science, chemical engineering, biology, medicine and others is getting more important in the field.

Intensive study and development have matured the microfluidic technology for the generation of multiple-emulsion drops. However, we are still in the infancy stage for the functionalization of microcapsules and their use. Although we discuss many functions and applications of microcapsules above, they are only the tip of the iceberg and still primitive. The high flexibility of microfluidic technologies will provide unlimited opportunities for a wide range of conventional and unprecedented applications of microcapsules. For example, the microcapsule sensors can be further designed to have a membrane that actively regulates the material transport and a core that emits NIR signals in response to specific biomolecules. Such a microcapsule could be implanted in patients through injection to monitor the *in vivo* environment without the need for surgery. Furthermore, artificial cells that behave in the same manner to natural cells in many aspects could be implemented. Artificial cells would produce and secrete beneficial chemicals or proteins in the manner by which they are programmed. The only way to design and produce such elaborate microcompartments is to use microfluidics and its high controllability over size, shape, and composition of multiple emulsions and high reproducibility.

Nevertheless, there are many obstacles and challenges. One of the most critical is a low throughput of microfluidics by drop-by-drop production. Although the throughput can be enhanced by a factor of 10 or more by parallelizing drop makers,[149,150] it is still insufficient to satisfy the requirement for many applications. Furthermore, parallelization of multiple-emulsion makers is elusive as complex surface treatment and 3D geometry of channels are prerequisite. Only few works have been reported on this;[43,47,151,152] parallelization of 20 double-emulsion drop makers is demonstrated by designing coaxial annular world-to-chip interfaces.[152] As an alternative route to increase throughput, drop splitting is suggested.[153] Large multiple-emulsion drops can be divided into two identical pieces while maintaining the drop order using Y-shaped channel. Multiple splitting steps in a single device composed of a series of Y-shaped branches produce small multiple-emulsion drops with an enhanced throughput with a factor of 2^N, where N is the number of sequential splitting. However, the splitting method requires high stability of the multiple-emulsion drops, which limits the available set of materials. Therefore, new microfluidic approach should be further studied to achieve high throughput.

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