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Functional Polymeric Microparticles Engineered from Controllable Microfluidic Emulsions

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CONSPECTUS

Functional polymeric microparticles with typical sizes of 1−1000 μm have received considerable attention for many applications. Especially in biomedical fields, polymeric microparticles with advanced functions such as targeted delivery, controlled encapsulation, or "capture and release" show great importance as delivery systems for active molecules and drugs, as imaging agents for analytics and diagnostics, as microreactors for confined bioreactions, and more. Generally, the functions of these microparticles rely on both their structures and the properties of their component materials. Thus, creating unique structures from functional materials provides an important strategy for developing advanced functional polymeric microparticles.

Several methods, such as dispersion polymerization, precipitation polymerization, copolymer self-assembly, and phase-separated polymer precipitation can be used to make functional microparticles, but each has limitations, for example, their limited control over the particle size and structure. Using emulsions as templates, however, allows precise control over the size, shape, composition, and structure of the resulting microparticles by tuning those of the emulsions via specific emulsification techniques. Microfluidic methods offer excellent control of emulsion droplets, thereby providing a powerful platform for continuous, reproducible, scalable production of polymeric microparticles with unprecedented control over their monodispersity, structures, and compositions. This approach provides broad opportunities for producing polymeric microparticles with novel structure−property combinations and elaborately designed functions.

In this Account, we highlight recent efforts in microfluidic fabrication of advanced polymeric microparticles with well-designed functions for potential biomedical applications, and we describe the development of microfluidic techniques for producing monodisperse and versatile emulsion templates. We begin by describing microparticles made from single emulsions and then describe those from complex multiple emulsions, showing how the resulting microparticles combine novel structures and material properties to achieve their advanced functions. Monodisperse emulsions enable production of highly uniform microparticles of desired sizes to achieve programmed release rates and passive targeting for drug delivery and diagnostic imaging. Phase-separated multiple emulsions allow combination of a variety of functional materials to generate compartmental microparticles including hollow, core−shell, multicore−shell, and hole−shell structures for controlled encapsulation and release, selective capture, and confined bioreaction. We envision that the versatility of microfluidics for microparticle synthesis could open new frontiers and provide promising and exciting opportunities for fabricating new functional microparticles with broad implications for myriad fields.

1. Introduction

Polymeric microparticles are particles in size range of 1−1000 μm, usually with solid, porous, or compartmental polymeric interiors. Polymeric microparticles with advanced functions, such as targeted delivery and controlled encapsulation or capture and release, show great importance in numerous applications, including drug delivery, diagnostic imaging, and confined microreaction.

In general, the overall functions of microparticles are largely governed by both their structures and the properties of their component materials. To ensure that each individual microparticle has uniform and controllable performance, uniformity in size and shape is essential, since most of their physical/chemical properties are size- and shape-dependent. For example, uniform sizes and shapes are critical for microparticles to achieve quantitative

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encapsulation and uniform release kinetics for desired delivery.[1] Compared with microparticles with the simplest solid structures, construction of microparticles with porous networks can provide permeable structures with large specific surface area for load and release and as scaffolds for tissue engineering. Alternatively, creation of hollow or liquid cores in microparticles can produce compartmentalized structures with large inner volume that are more useful for active encapsulation and confined microreaction. Further engineering of their internals into multicompartments allows separate encapsulation of multiple incompatible components without cross-contamination for applications such as triggered microreaction and drug co-delivery.[2] Moreover, addition of holes in their shell can facilitate mass transport through the shell for loading and release of substances based on size or functional selectivity of the hole.[3] These diverse architectures can be integrated with myriad functional materials to endow the microparticles with elaborately tailored functions. Thus, combination of unique structures and integrated properties of functional materials creates vast opportunities for developing advanced functional polymeric microparticles.

Polymeric microparticles can be fabricated by various methods. Generally, solid or porous microparticles can be fabricated by a batch process such as dispersion polymerization and precipitation polymerization; however, they usually have polydisperse sizes and limited microsize range.[1] Compartment microparticles can typically be produced by copolymer self-assembly, phase-separated polymer precipitation, and techniques that utilize sacrificed solid-core templates such as layer-by-layer deposition.[4] Usually, these techniques also provide limited control over particle size and are restricted to particular materials. More importantly, controllable generation of microparticles with complex internal components remains difficult to achieve with these techniques.

Utilization of emulsions as templates allows production of versatile microparticles with their size, shape, and structure largely relying on those of the emulsions. Among various emulsification techniques, the microfluidic technique enables controllable generation and manipulation of microsized emulsion drops,[5−7] which provides a powerful platform for continuous, reproducible and scalable production of polymeric microparticles with unprecedented control over their monodispersity, shapes, structures, and compositions. The monodisperse emulsions from microfluidics efficiently guarantee the production of highly uniform spherical or even nonspherical microparticles that are challenging to conventional methods.[8] Moreover, the most highlighted feature is that the microfluidics can generate complex emulsions with precisely controlled internal components for controllable production of monodisperse microparticles with well-tailored inner architectures. These phase-separated emulsions allow flexible integration of various functional materials in the aqueous and organic phases and at the interfaces to produce microparticles with diverse functions.

In this Account, we highlight the development of polymeric microparticles with well-designed functions that benefit from the controllable microfluidic emulsions for potential biomedical applications. This is introduced by starting with the synthesis of uniform microparticles from single emulsions for drug delivery and bioimaging, and then the engineering of hierarchically compartmentalized microparticles with diverse structures from versatile double and triple emulsions for controlled encapsulation/release, selective capture, and confined bioreaction is discussed. While focusing on the microparticle functions, we also describe the microfluidic techniques for controllably generating the emulsion templates.

2. Functional Microparticles from Controllable Single Emulsions

Single emulsions are mixed systems consisting of two immiscible liquids, with drops of one liquid dispersed in the continuous phase of another one. They are widely used for template synthesis of polymeric microparticles. Typically, bulk emulsification can produce single emulsions but with polydisperse sizes. More uniform drops can be produced by membrane emulsification, but they are still quasi-monodisperse due to the quasi-monodisperse size distribution of membrane pores.[8] Recent advances in microfluidics enable generation of monodisperse emulsion drops for producing microparticles with even greater control over their size and monodispersity.[9] Moreover, the produced water and oil drops can dissolve a variety of water-soluble and oil-soluble materials for synthesizing diverse microparticles. This section mainly focuses on the controllable monodisperse microparticles developed from microfluidic single emulsions for drug delivery and bioimaging, which usually require well-controlled particle size and size distribution to achieve predictable and improved performances.

2.1. Microfluidic Generation of Controllable Single Emulsions. To form controllable monodisperse emulsions, microfluidic capillary devices[9] and polydimethylsiloxane (PDMS) devices[10] are usually used. Capillary devices are

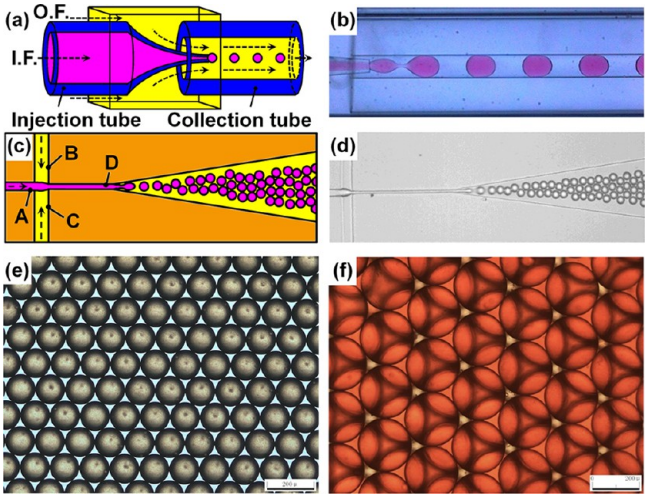

FIGURE 1. Microfluidic generation of monodisperse single emulsions. (a–d) 3D capillary device with two cylinder capillaries aligned inside a square capillary (a) and 2D PDMS device with cross-junction microchannel (c) for generating single emulsions (b, d). (e, f) Monodisperse O/W (e) and W/O (f) emulsions from microfluidics.[3,37] Scale bars are 200 μm.

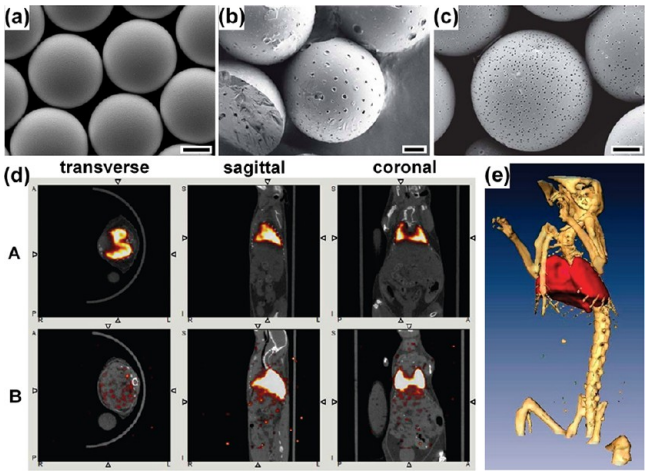

FIGURE 2. Functional microparticles for drug delivery and bioimaging. (a–c) SEM images of PLGA microparticles (a)[14] and PLA microparticles with size-tunable pores (b, c).[15] (d) Lung perfusion SPECT scan using ^{99m}Tc-microparticles (A), and the same data but shown in the transverse direction through liver with a much more sensitive radioactivity threshold to depict thyroid and liver activity (B).[16] (e) Surface-rendered microSPECT/CT image (with radioactivity shown in red) of rat taken 5 min after tail vein injection of ^{99m}Tc-microspheres.[16] Scale bars are 10 μm.

3D geometries with coaxially assembled capillaries on glass slides. Typically, two cylinder capillaries, used as the injection and collection tubes, are aligned inside a square capillary to construct a coflow geometry (Figure 1a). To form single emulsions, the inner fluid (I.F.) flowing through the injection tube is broken into drops at the tapered orifice by the outer fluid (O.F.) coflowing through the square tube (Figure 1b).

PDMS devices are 2D geometries with microchannels created by soft lithography.[11] They offer more flexible microchannels than capillary devices for drop generation and manipulation but usually require modification of the microchannel wettability. In a typical PDMS device with flow-focusing cross-junction (Figure 1c,d), liquid that flows in channel A is broken into drops at the orifice D by two flows of a second liquid from two outside channels (B and C).

Both devices produce highly monodisperse drops (Figure 1e,f) with their coefficient of variation (CV) less than 5%. The drop size can be well-controlled by adjusting flow rates or device dimensions. Moreover, enhanced-throughput production of the emulsions can be achieved by parallelizing emulsification geometries.[12] These features allow mass production of microparticles with well-defined size and size distribution from microfluidic emulsions.

2.2. Microparticles for Drug Delivery and Bioimaging. Microparticles have an enormous impact on medical technology such as drug delivery and bioimaging, since they enable flexible delivery of existing drugs with improved performance.[13] Typically, microparticle size strongly affects their drug release profile, biodistribution, and administration route.[1] From microfluidic drops, uniform microparticles with controlled sizes can be generated to tailor these size-dependent features for desired release.

Generally, microparticle size strongly affects their drug release rate. With controllable monodisperse size, microparticles would allow more controllable and uniform release kinetics. A typical example is the monodisperse biodegradable microparticles fabricated by solvent removal of microfluidic drops containing poly(lactic-*co*-glycolic acid) (PLGA) and drugs (Figure 2a).[14] The smaller PLGA microparticles (11 μm) release drugs faster *in vitro* than the larger ones (41 μm), due to their larger surface-area-to-volume ratio. Moreover, these microparticles exhibit significantly reduced initial burst release and slower sustained release compared with microparticles with similar average sizes but broader size distributions prepared from conventional emulsification methods. Such size-dependent release kinetics may allow combined release patterns by mixing monodisperse microparticles with different size combinations. Further adjustment of the release behavior can be achieved by creating microparticles with size-tunable pores via encapsulation of pore-forming agents in the emulsion templates (Figure 2b,c).[15]

The size and uniformity of microparticles also greatly influence their biodistribution *in vivo*. For example, upon intravenous injection, the particle size determines which

organ or tissue traps the microparticles while their uniformity determines the uptake efficiency. Thus, microparticles with well-defined size would allow targeted intravascular delivery to different organs or tissues by clogging small blood vessels.[16] This size-dependent passive-targeting concept is typically demonstrated by using monodisperse $[^{99m}Tc(CO)_3]^+$-radiolabeled poly(L-lactide) (PLA) microparticles (^{99m}Tc-microparticles) from microfluidics for lung perfusion imaging of rats.[16] Upon intravenous tail vein injection, ^{99m}Tc-microparticles with size (~9 μm) larger than that of the typical rat lung capillary (~5.7 μm) can be retained by these capillaries (the first capillaries they encounter) for lung targeting. The biodistribution results (Figure 2d,e) show their homogeneous and efficient retention (99.4%) in the lung region, indicating excellent performance. In this case, particle aggregation is minimized by providing a hydrophilic particle surface via PEGylation, which can also increase the blood circulation time of particles.[17] With such passive-targeting, targeted drug delivery can be realized by further encapsulating the microparticles with drugs.

3. Functional Microparticles from Controllable Double Emulsions

Multiple emulsions are complex nested systems, with liquid drops of decreasing sizes placed one inside another. As the simplest multiple emulsions, double emulsions are usually with dispersed drops containing smaller drops inside. They are widely used as templates for creating compartmental microparticles; where their outer and inner drop, respectively, becomes the particle shell and core. Both bulk mixing and membrane emulsification produce double emulsions with poorly controlled internal compositions.[8] More precisely and flexibly controlled double emulsions can be generated from microfluidics for fabricating highly controllable microparticles.[9] The microfluidic double emulsions allow elaborate incorporation of various materials, including water-soluble and oil-soluble organic materials and inorganic materials, into particle shell and core to achieve desired functions. This section describes some novel concepts and strategies for developing functional microparticles for controlled encapsulation or capture and release and confined bioreaction.

3.1. Microfluidic Generation of Controllable Double Emulsions. One of the significant advantages for microfluidic techniques over other emulsification techniques is their excellent extendability by combining drop-making geometries for controllably producing double emulsions or even higher-order multiple emulsions.[9] As typically illustrated in Figure 3a, by use of an extended capillary device that combines two coflow geometries, a two-step emulsification process (Figure 3b,c) can be employed for generating monodisperse double emulsions (Figure 3d,e). The separated emulsifications allow accurate and independent control over the number and size of inner drops by adjusting flow rates and device dimensions (Figure 3f).[18]

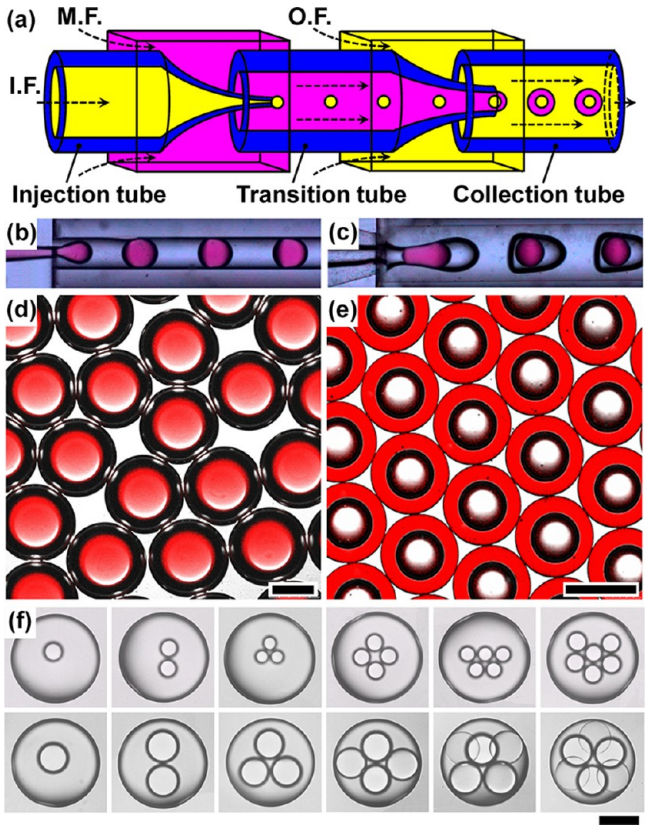

FIGURE 3. Microfluidic generation of controllable double emulsions. (a–c) Capillary device with two coflow geometries (a), and the two-step emulsification process for producing double emulsions (b,c).[38] (d,e) CLSM images of monodisperse O/W/O (d) and W/O/W (e) emulsions with oil phase containing fluorescent dye LR300.[37,38] (f) Optical micrographs of double emulsions containing inner drops with controlled number and size.[18] Scale bars are 200 μm.

Double emulsions with more diverse structures can be developed by encapsulation of inner drops containing different contents. This can be achieved by using more flexible devices constructed from three basic building blocks (Figure 4a).[19] Different combinations of the building blocks enable a versatile route to create branched devices for producing multicomponent multiple emulsions with highly controlled yet exceptionally diverse structures. Typically, as illustrated in Figure 4b, different drops formed in two drop makers can be merged by the connectors and further encapsulated in the drop maker downstream to produce

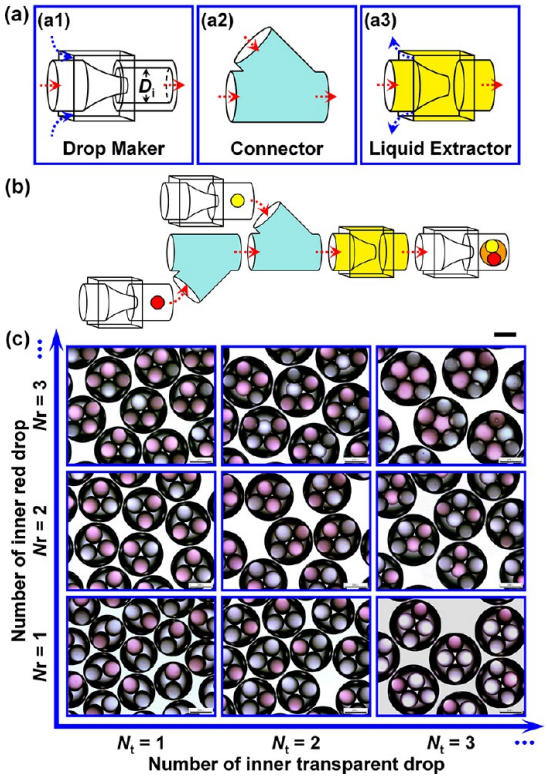

FIGURE 4. Capillary device for controlled production of quadruple-component double emulsions.[19] (a) Functional building blocks: (a1) drop maker, (a2) connector, and (a3) liquid extractor. (b) Branched device constructed from the building blocks for generating quadruple-component double emulsions. (c) Optical micrographs of quadruple-component double emulsions exhibiting precise control over the number and ratio of inner red and transparent drops. Scale bar is 200 μm.

quadruple-component double emulsions. Accurate control over the number, ratio, and size of different inner drops can also be achieved (Figure 4c). Moreover, inner drops of other contents can also be introduced by adding more branch channels. These highly controlled double emulsions provide excellent platforms for fabricating compartmental microparticles with versatile structures.

3.2. Microparticles for Controlled Release. Microencapsulation and controlled release are of considerable interest for drug delivery. One of the major challenges in this field requires the delivery systems to encapsulate drugs with desired dose and release them at a desired site or moment with controllable rate to achieve more efficient delivery and fewer side effects. Compartmental microparticles containing interior with large capacity and shell with controlled permeability or decomposition behavior provide unique advantage for such smart drug delivery. Compared with other techniques, microfluidic double emulsions provide tremendous advantages for developing compartmental microparticles with advanced functions. For example, integration of inner and outer drops with different functional components allows separate design of the shell and core for flexible controlled release. Control of the size, number, and content of inner drops allows precise manipulation of the encapsulation characteristics for diverse or even incompatible components. Such excellent controllability also provides nearly 100% encapsulation efficiency for drugs. Moreover, the O/W/O and W/O/W emulsions can offer a favorable particle core with increased solubility for oil- and water-soluble drugs to increase their loads. Stimulated by these advantages, functional microparticles with high potential for controlled sustained release, burst release, and synergetic release have been developed.

3.2.1. Microparticles for Stimuli-Responsive Sustained Release. Self-regulated release of actives such as hormones is important for biological function regulation of humans. For drug delivery systems, similar functions are required to release drug in a controlled manner. For example, a delivery system that enables glucose-responsive insulin release can achieve more efficient drug delivery for diabetes therapy and relieve the pain of patients from insulin injections. Usually, such smart delivery can be realized by hollow microparticles containing aqueous interior and controllably permeable shell.

Stimuli-responsive polymeric materials are widely utilized as particle shell for controlled release.[20] Typically, monomers for synthesizing stimuli-responsive hydrogels can be dissolved in the middle aqueous layer of O/W/O emulsions for constructing a particle shell with tunable permeability for drug diffusion. For example, hollow microparticles with poly(*N*-isopropylacrylamide-*co*-3-acrylamidophenylboronic acid-*co*-acrylic acid) hydrogel shell that are stable in water have been fabricated from microfluidics for glucose-responsive drug release. They can release insulin much faster when glucose concentration changes from 0.4 to 3.0 g/L at physiological temperature *in vitro*.[21] Moreover, their reversible glucose-responsive volume transitions enable constant monitoring of glucose levels for self-regulated release and reusable drug loading and release.[22]

3.2.2. Microparticles for Stimuli-Responsive Burst Release. Burst release allows one-shot triggered release of contents with a strong propagating boost for fast and widespread delivery. This release behavior provides a Trojan-horse-like strategy to protect the contents before triggering to reduce side effects and then rapidly release them at a desired site upon triggering to achieve high local concentration.

Usually, burst release from microparticles requires breaking the particle shell. One of them involves partial shell

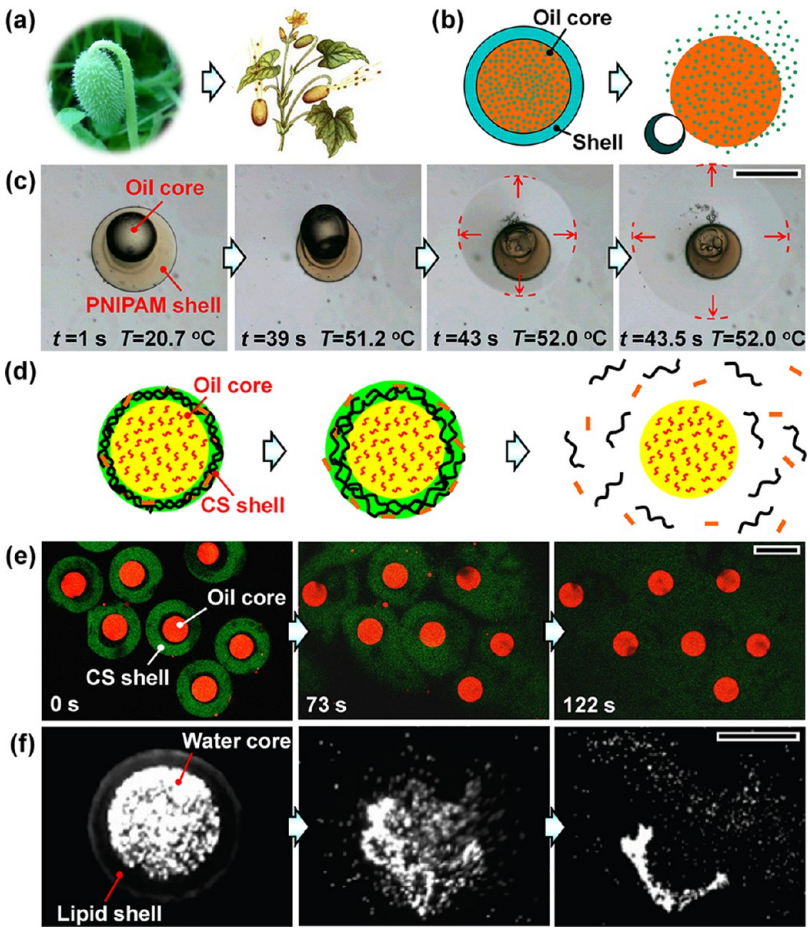

FIGURE 5. Functional microparticles for stimuli-triggered burst release. (a) Squirting cucumber that can eject seeds with a liquid jet.[36] (b) Bursting microparticles that enable burst release of lipophilic drugs loaded in the oil core by stimuli-induced shrinking of the hydrogel shell. (c) Optical micrographs showing the thermo-triggered burst release of core–shell PNIPAM microparticle.[23] (d,e) Schematic illustration (d) and CLSM images (e) showing the acid-triggered burst release (pH = 3.1) of core–shell chitosan (CS) microparticles by shell decomposion.[25] (f) CLSM images showing the thermo-triggered burst release of fluorescent beads from core–shell microparticles of fatty acid glycerides.[26] Scale bars are 250 μm in panels c and e and 50 μm in panel f.

rupture by internal stress for drug release. In nature, plants, such as squirting cucumber, can squirt their seeds under disturbance, together with a liquid jet to achieve widespread dispersion (Figure 5a). Inspired by this interesting phenomenon, oil-core/hydrogel-shell bursting microparticles with similar release behavior have been developed from O/W/O emulsions (Figure 5b).[23] The oil core benefits the encapsulation of poorly water-soluble drugs, such as anticancer drug paditaxel, and the hydrogel shell enables stimuli-induced shrinking behaviors for triggered burst release. Their typical release behavior is demonstrated by bursting microparticles with thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM) shell as an example (Figure 5c).[23] Upon heating above the volume phase transition temperature of the PNIPAM shell, the shell dramatically shrinks and extrudes the oil core, resulting in final shell rupture for fast release of the oil core. Moreover, simple change of the shell materials enables bursting microparticles to be responsive to other stimuli for applications in different situations. For example, incorporation in the shell of gold[22] or magnetic[23] nanoparticles allows volume shrinking induced by near-infrared light or an oscillating magnetic field.[22,24] The embedded nanoparticles allow local heating of only the microparticles for release and reduce the burning of nearby tissues. Furthermore, targeted delivery of the magnetic-nanoparticle-embedded microparticles can also be realized under guidance of a magnetic field that focuses on the lesion site.[24]

Another style for burst release involves complete shell breakage by a chemical or physical approach. For example, microparticles with a shell consisting of chitosan cross-linked by terephthalaldehyde via forming Schiff base can be chemically decomposed in acidic media for drug release, due to the unstable Schiff base at low pH (Figure 5d,e).[25]

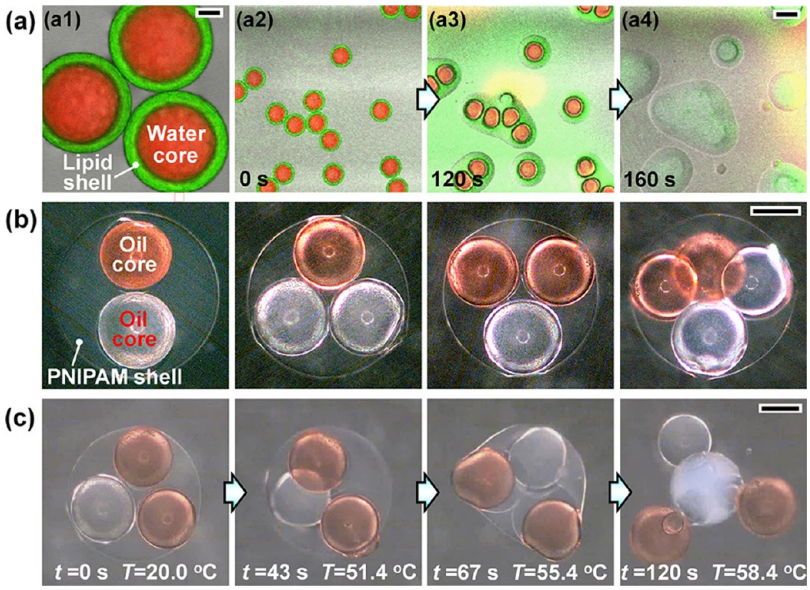

FIGURE 6. Functional microparticles for stimuli-responsive synergetic release. (a) CLSM images of core–shell microparticles with lipid shell containing paclitaxel (green) and water core containing doxorubicin (red) (a1) for synergetic release of both drugs upon shell melting at 37 °C (a2–a4).[27] (b, c) Dark-field optical micrographs of multicore–shell PNIPAM microparticles controllably coencapsulated with different oil cores (b) for thermo-induced synergetic release (c).[28] Scale bars are 20 μm in a1 and 100 μm in the rest.

This acid-triggered release could be used for drug delivery in the stomach. Alternatively, physically induced burst release is illustrated by microparticles made from microfluidic melt emulsification.[26] From W/O/W emulsions with melted fatty acid glycerides (melting temperature $T_m = 33–35$ °C) as the oil shell, water-core-containing microparticles can be fabricated by cooling the oil shell into a solid one. The solid lipid shell can be melted into liquid again at 37 °C, which then leads to shell breakage and burst drug release (Figure 5f). These bursting microparticles hold great potential as drug delivery systems for controlled release.

3.2.3. Microparticles for Stimuli-Responsive Synergetic Release. Recently, co-delivery of different components such as anticancer drugs and DNA or antigen and adjuvant to achieve a combined curing efficacy has attracted increasing interest for disease treatment. To ensure optimized efficacy, precise encapsulation of each component with desired dosage is critical. Moreover, when co-delivery of incompatible actives or reactants is desired, isolated encapsulation before release becomes essential. Microparticles with separate compartments have unique advantages for isolated coencapsulation and on-demand synergetic release. The major challenge for fabricating such microparticles is the creation of separate compartments into a single particle, especially with precise control over the multicompartment structures for optimizing the encapsulation of each component.

Compared with techniques that employ stepwise encapsulation of one particle in another for fabricating multicompartment microparticles, phase-separated double emulsions offer more advanced platforms for one-step fabrication of controllable microparticles with separate compartments. From microfluidic W/O/W emulsions, core–shell microparticles with hydrophilic doxorubicin hydrochloride contained in the aqueous core and hydrophobic paclitaxel incorporated in the solidified lipid shell are developed for co-delivery (Figure 6a).[27] Burst release and sustained release of both drugs can be achieved at 37 °C by using a lipid shell with different T_m. The effectiveness of the co-delivered drugs is investigated by *in vitro* cell viability studies, in which viability rates of 0% are observed for both immortal human cervix cancer cells and mouse hybridoma cells after 20 h.

Alternatively, multicomponent double emulsions provide different inner drops as efficient templates for co-delivering drugs with same hydrophilicity or hydrophobicity. For example, PNIPAM bursting microparticles with controllable multiple compartments (Figure 6b) can be developed from multicomponent O/W/O emulsions for synergetic release of hydrophobic drugs (Figure 6c).[28] With microfluidics, the concentration of each drug in these carriers can be optimized by tuning the size of shell and core[27] or the number of inner different cores.[28] All this research highlights the power of microfluidic double emulsions for designing multicompartment microparticles for coencapsulation and synergetic controlled release.

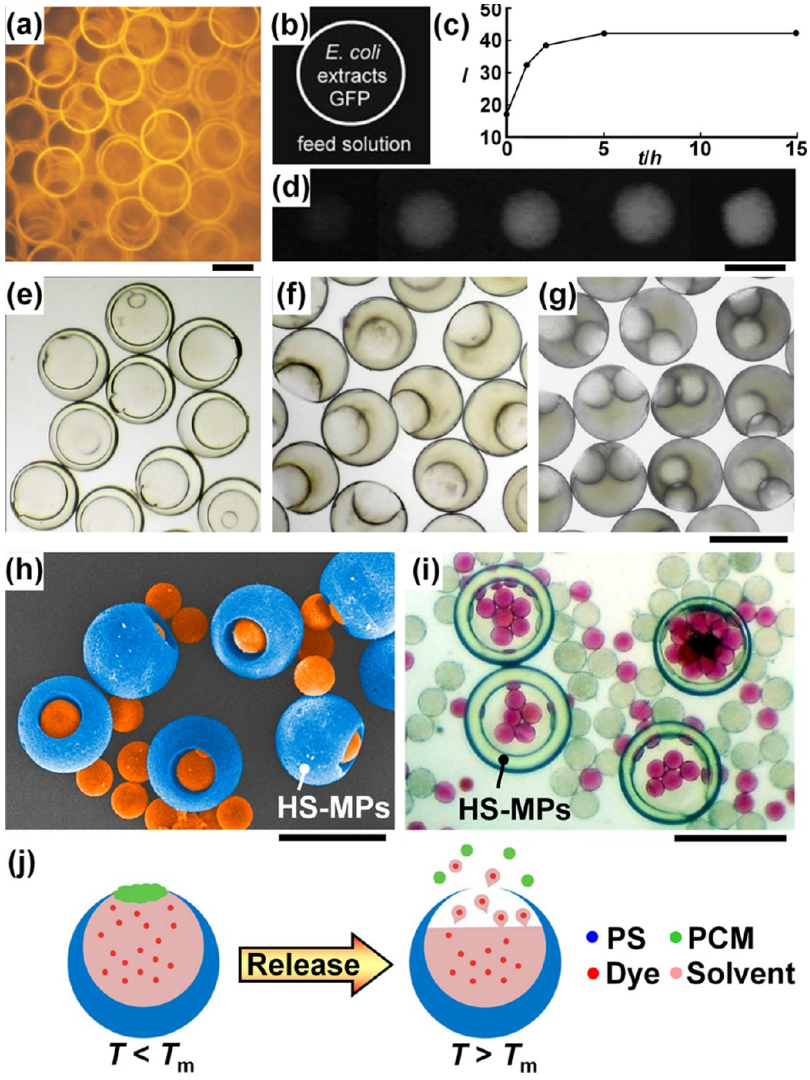

FIGURE 7. Functional microparticles for confined bioreaction and controlled capture and release. (a) Fluorescent image showing liposomes of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine labeled with Fluorescent DiI-C_{18}. (b–d) Schematic illustration (b), fluorescent intensity (*I*) changes (c), and sequential fluorescent images (d) for the GFP expression process in liposomes of diphytanoylphosphatidylcholine encapsulating *E. coli* extracts for GFP expression.[31] (e–g) Optical images of hole–shell microparticles (HS-MPs) with controllable structures.[3] (h) SEM image showing bowl-shaped hole–shell microparticles (blue) capture microspheres (orange) based on "lock–key" size-match.[3] (i) Optical micrograph showing that fishbowl-shaped hole–shell microparticles selectively capture smaller microspheres (red) from larger microspheres (gray) for size-classification.[3] (j) Schematic illustration of hole–shell microparticles with a thermoresponsive gate for controlled release.[32] Scale bars are 50 μm in panel a, 20 μm in panel d and 200 μm in panels e–i.

3.3. Microparticles for Confined Bioreaction. The liposome is a self-closed structure composed of a phospholipid bilayer membrane with inner aqueous compartment, and liposomes are extensively used as carriers for delivering pharmaceuticals.[29] Liposomes can be typically formed by techniques that involve the self-assembly of phospholipids in an aqueous environment under shear or electric field. However, with limited control over the self-assembly behavior, these techniques usually lead to polydisperse liposomes and low encapsulation efficiency. More controllable self-assembly behaviors can be performed with microfluidic W/O/W emulsions, in which the amphiphilic phospholipids dissolved in the middle oil phase can assemble at the oil/water interfaces to produce monodisperse liposomes by solvent extraction (Figure 7a).[30] These uniform liposomes enable more controllable performance with high encapsulation efficiency for drug delivery. Besides, with biomembrane-like structures, the liposomes can also serve as artificial cells to perform confined bioreactions. For example, extracts from *Escherichia coli*, including a plasmid containing the green fluorescent protein (GFP) gene and nutrients for protein synthesis, can be encapsulated in liposomes via

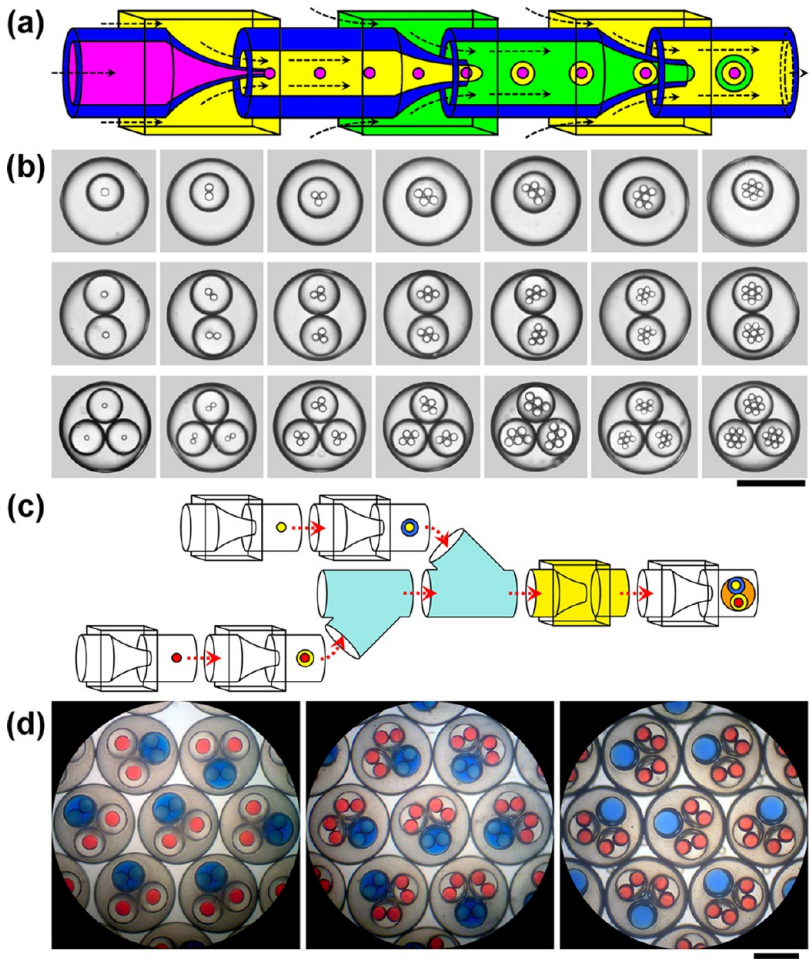

FIGURE 8. Microfluidic generation of controllable triple emulsions. (a) Extended capillary device for generating triple emulsions. (b) Optical micrographs of triple emulsions containing inner and middle drops with controlled number.[18] (c) Branched device extended for generating sextuple-component triple emulsions.[19] (d) Optical micrographs of monodisperse sextuple-component triple emulsions controllably coencapsulating different inner and middle drops.[19] Scale bars are 400 μm.

microfluidics to perform cell-free *in vitro* synthesis of proteins.[30,31] As shown in Figure 7b–d, continuous GFP expression for more than 5 h is observed.[31] The liposomes hold great promise as cell-like bioreactors for synthesizing biomolecules.

3.4. Microparticles for Controlled Capture/Release. Microparticles with hydrogel or porous shell offer excellent performance for encapsulation and controlled release. However, transport of molecules through the cross-linked polymeric networks or the tiny pores of shell is usually a slow diffusion process, which leaves the transport of larger microsized objects such as particles and cells even more difficult. Creation of a well-defined microsized hole in the microparticle shell can provide hole–shell structures with more versatility by facilitating mass transport through the shell.

Such hole–shell particles can be typically prepared by freeze-drying solvent-swollen polymeric particles.[32] With microfluidics, the phase separation behavior of double emulsions can be precisely manipulated to produce hole–shell microparticles with more flexible structures. Typical examples are the versatile hole–shell microparticles fabricated from controllably evolved W/O/W emulsions.[3] Mixed oil solution containing photocurable ethoxylated trimethylolpropane triacrylate monomer and polyglycerol polyricinoleate surfactant with poor solubility in the monomer is used as the oil phase of W/O/W emulsions. The poor solubility of monomer for the surfactant reduces the solvent quality and leads to adhesion of the inner drop with outer phase. This produces controllably evolved W/O/W emulsions from core–shell to different acorn-shaped configurations, which can be precisely manipulated by varying the monomer fraction. Combined with the flow-rate strategy for structure adjustment, such emulsions allow controllable production of versatile hole–shell microparticles

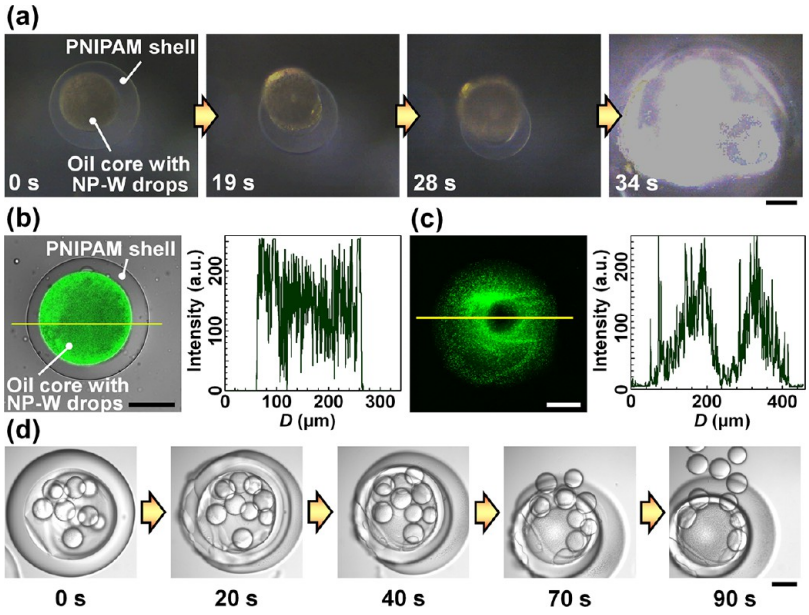

FIGURE 9. Functional microparticles with water drops in the oil core for burst delivery of nanoparticles and hydrophilic substances. (a) Dark-field optical micrographs showing the thermo-triggered squirting behavior of PNIPAM microparticle with oil core containing nanoparticle-loaded water (NP-W) drops by heating from 20 to 50 °C.[36] (b,c) CLSM images and their fluorescence intensity profiles showing the distributions of fluorescently labeled nanoparticles before (b) and after (c) burst release from the PNIPAM microparticle.[36] (d) Optical micrographs showing the thermo-triggered squirting of water drops with controlled number from the PNIPAM microparticle by heating from 25 to 50 °C.[18] Scale bars are 100 μm.

(Figure 7e–g). These microparticles enable capture of living cells, size-match capture of microspheres (Figure 7h), and selective capture of smaller microspheres from larger ones for size classification (Figure 7i).[3] Further corking the hole of such hole–shell structures with phase-change materials allows encapsulation of drugs for thermo-triggered release (Figure 7j).[32] The hole–shell microparticles create new opportunities as microcontainers for controlled capture and release.

4. Functional Microparticles from Controllable Triple Emulsions

Triple emulsions are multiple emulsions with drops containing smaller double emulsion drops. With very good scalability, a microfluidic device also allows controllable production of triple or even higher-order multiple emulsions.[9] The multilayered triple emulsions allow fabrication of more hierarchical microparticles that other techniques usually cannot achieve for advanced functions. To illustrate this advantage, more hierarchical bursting microparticles for delivery of nanoparticles and hydrophilic drugs are introduced as typical examples.

4.1. Microfluidic Generation of Controllable Triple Emulsions. The controllable generation of triple emulsions is typically illustrated by examples involving capillary devices. By addition of a third coflow geometry to the device in Figure 3a, the extended device (Figure 8a) allows further encapsulation of double emulsions to produce triple emulsions.[18] Again, both the size and number of inner and middle drops can be individually and precisely controlled (Figure 8b).

More flexible scale-up strategy based on combinations of the building blocks allows controllable generation of multicomponent multiple emulsions with much more complex structures.[19] For example, by adding another drop maker to each branch channel of the device in Figure 4b, more complex sextuple-component triple emulsions containing two different kinds of double emulsion drops are formed (Figure 8c,d). Even with such complex structures, precise control over their inner structures is still achievable. All these results highlight the remarkable controllability and scalability of microfluidic techniques for producing higher-order multiple emulsions with specified complex structures. These emulsions create exciting opportunities for fabricating hierarchically compartmental microparticles with novel architectures and functions.

4.2. Microparticles for Burst Release of Nanoparticles. Nowadays particles with nanosizes have attracted considerable interest for drug delivery. In some cases, encapsulation of nanoparticles within microparticles can provide additional functions for drug delivery: The nanoparticle-in-microparticle system can provide combined styles for

drug release such as sequential release and reduce unwanted initial burst release.[33] They can also avoid direct contact of nanoparticles with physiological fluids to reduce degradation/aggregation and macrophage clearance.[34,35] With such systems, the encapsulated nanoparticles enable deep lung delivery against exhalation[34] and improved stability in gastrointestinal tract for gene therapy.[35]

Microfluidic triple emulsions facilitate the development of nanoparticle-in-microparticle systems. For example, W/O/W/O emulsions allow production of PNIPAM bursting microparticles with oil core containing water drops for encapsulating nanoparticles that prefer aqueous environment. A simple method employs homogenizer-produced W/O emulsions instead of the oil core of O/W/O emulsions to produce W/O/W/O emulsions for fabricating such bursting microparticles.[36] The nanoparticles in the innermost water drops, shielded by both the oil core and thermoresponsive shell, can be squirted out via thermo-triggering, as typically demonstrated in Figure 9a–c.

Finer control over the internal structures of such microparticles can be achieved by using microfluidic geometry instead of homogenizer to prepare the innermost drops. This produces microparticles containing monodisperse innermost water drops with controlled size and number, which allows drug encapsulation with controlled dosages for burst release (Figure 9d).[18] These microparticles also allow separate delivery of hydrophilic and hydrophobic species in the innermost water drops and the oil core, respectively. More versatile co-delivery could be achieved with multicomponent multiple emulsions as templates.

5. Conclusions and Perspectives

This Account summarizes recent progress on development of functional polymeric microparticles with advanced functions from controllable microfluidic emulsions for potential biomedical applications. These microparticles are fabricated by taking advantage of the controllably kaleidoscopic structures and independently tunable compositions of microfluidic emulsions. This template-based synthesis strategy has attracted a great deal of interest because it allows combination of the shape of emulsion templates and the property of selected functional materials for creating microparticles with elaborately tailored functions for versatile applications. Future efforts should focus on creation of emulsions with novel structures as new templates, deep exploration of the interfacial science in multiphase emulsions for configuration control, and deep understanding of the structure–function relationships for microparticle design. Moreover, since the minimum size of microparticles from microfluidic multiple emulsions is usually tens of micrometers, further minimizing the emulsion size with advanced techniques is required to make the microparticles more efficient for *in vivo* applications. Meanwhile, microfluidic devices that are more robust and scalable for industrial-scale production of microparticles need to be further explored. Such research would benefit the production of brand-new functional microparticles from emulsions for promising new applications.

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FOOTNOTES

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