Elaborate Design Strategies Toward Novel Microcarriers for Controlled Encapsulation and Release

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Microencapsulation and the controlled release of bioactive agents have long been investigated and exploited to both improve the fundamental understanding of cell functionality and to develop efficient delivery vehicles for drugs, nutrients, and cosmetics. Conventional approaches to the synthesis of particles and capsules for practical applications have achieved only limited control over particle size, shape, functionality, and encapsulation efficiency. To overcome such limitations, a variety of approaches have been developed. Recent advances in microfluidics and other techniques have inspired the design of new microcarriers that efficiently encapsulate bioactive agents to enable the on-demand release or functionalization of encapsulants. Here, the current state of the art in the area of elaborate design strategies for microcarriers that enable efficient encapsulation and controlled release for biological applications is described. This is discussed in three sections, which are categorized by microcarrier type: particle-type carriers, capsule-type carriers, and foldable microcarriers. In each section, new routes to fabricating microcarriers are discussed together with their functionalities; techniques based on droplet microfluidics, lithography, micromolding, and imprinting are covered. In addition, the synthetic routes and the microcarriers are evaluated by comparison with alternative routes. Finally, future perspectives for these new strategies are outlined briefly.

1. Introduction

A variety of bioactive agents, including synthetic drugs, proteins, and cells, have been encapsulated in biocompatible microcarriers in the form of either particles or capsules for a wide range of biological applications, including fundamental studies of biological systems, drug delivery, tissue engineering, and immunosolation of cells. Each application requires specially designed microcarriers to fully achieve its goal. For example, fundamental studies of cell function or the development of artificial cells require biological system that resemble those of live cells;[1] symmetric or asymmetric vesicles provide useful platforms for studying cell membranes and cell functions. The isolation of biological targets provides an understanding of the causes and effects that underlie behavior in complex biological systems. Drug delivery vehicles should satisfy the specific conditions of a target site in the body; the size of a vehicle is critical to the ability to pass through a narrow channel in an organ, and the vehicle shape is important for determining the interactions among vehicles and cells.[2] The targeted delivery and stimulus-triggered release of drugs at a desired rate of release are very important for maximizing the effects of a drug.[3] Certain specific diseases can be efficiently treated using combination therapies that require the sequential release of several distinct drugs, while avoiding cross-contamination.[4] Tissue scaffolds should be highly porous and flexible to accommodate cells and maintain their viability.[5] The scaffolds should also be degradable to facilitate removal after the cells have grown sufficiently. Scaffolds must be designed to assume specific shapes that mimic the structures present within a target organ via assembly. Effective immunosolation requires the selective permeability of nutrients and wastes while protecting the encapsulated cells from the immune system.[6] Conventional encapsulation approaches based on bulk emulsification cannot be used to control the size, shape, and structure of a microcarrier, significantly limiting their applicability. In addition, conventional methods achieve only limited encapsulation efficiency, thereby wasting valuable bioactive agents. To overcome the shortcomings of conventional approaches and achieve advanced functionalities, new technologies have been introduced into elaborate microcarrier designs. For example, microfluidics have enabled the production of monodisperse emulsion drops; single- or multiple-emulsion drops may be prepared with unprecedented control that enables them to serve as templates for microparticles and capsules. Nanoparticles comprising drugs and polymers may be prepared via controlled precipitation in microfluidic devices. Photolithography and micromolding techniques may be modified and exploited toward the design of nonspherical microcarriers of drugs and cells. Moreover, imprinting techniques have been used to prepare relatively small microcarriers for the delivery of drugs into cell interiors.
This paper reviews the state of the art of microcarriers with advanced functionalities produced using new strategies. Such microcarriers can be categorized into three distinct types: particles, capsules, and foldable microcarriers, as summarized schematically in Figure 1. Particles comprise a single body of materials without distinct layers and they enable drugs or cells to be directly embedded in the body, as shown in the leftmost panel of Figure 1. By contrast, capsules are composed of empty core and shell membranes and permit the encapsulation of freely suspended materials in the interior of an enclosed membrane, as shown in the middle panel. Membranes can be tailored to be dense or porous, depending on the target application. Foldable microcarriers can be transformed from planar sheets to enclosed capsules through stimulus-responsive folding, thereby providing for the dynamic encapsulation and release of materials, as shown in the rightmost panel. Each type of microcarrier may be produced via a variety of synthetic routes and used for different application, depending on the shape, structure, material, and function. In the following three sections, we describe the fabrication strategies and functionalities of these three distinct microcarriers of particles, capsules, or foldable carriers, and we evaluate each type of microcarrier in light of alternative approaches. In the last section, we outline the future directions and outlook for these new microcarrier strategies.

2. Particle-Type Carriers

Particles have been used as carriers dispersed in a fluid, which can encapsulate micro- to nanoscopic encapsulants within their matrices. Degradation of the matrix under specific environmental conditions releases the encapsulant to provide on-target delivery. Highly swollen polymeric networks can be employed as compatible nests for living organisms or cells because hydrophilic polymers can mimic both the chemical and physical aspects of a biological tissue. Such networks provide cell-culturing scaffolds for in vivo transplantation and the networks guard against the immune system. Toward accomplishing such functions and applications, a variety of particle types have been investigated as cell and drug carriers. Conventional approaches are limited due to a lack of control over particle size, shape, and structure. In this section, we provide an overview of recent achievements in novel methods for fabricating particle-type carriers, which enable a high degree of control and flexibility relative to conventional approaches. This section is divided into two parts, according to the particle type: microgels for cell encapsulation are discussed first, followed by a review of dense microparticles for drug delivery. In the first part, we review novel cell-laden hydrogel particles of various shapes and structures that provide controlled environments for cell-to-cell interaction studies and artificial extracellular matrices for tissue constructs. Cell-laden microgels have been produced through three distinct methods: microfluidic emulsion-templating (Section 2.1.1), micromolding (Section 2.1.2), or photolithography (Section 2.1.3). In the second part of this first section, we review advanced drug carriers that facilitate the triggered or programmed release of single or multiple encapsulants. The release occurs upon the spontaneous or stimulus-responsive degradation of a particle matrix, wherein the size and shape of the particles, as well as the material itself, determine the rate of degradation. Such particles are prepared via emulsion-templating (Section 2.2.1), imprinting (Section 2.2.2), or lithography techniques (Section 2.2.3). Particles have also been produced via controlled nanoprecipitation in microfluidic devices to provide nanocarriers for cancer chemotherapy (Section 2.2.4).
2.1. Encapsulation of Cells in Microgels

Cell encapsulation and immobilization in hydrogel particles has been investigated for tissue engineering and regenerative medicine.\[^{5}\] Highly swollen microgels have pores as large as a few nanometers, thereby providing selective permeability; the size of a pore is determined by the crosslinking density. Therefore, the microgels enable the penetration of nutrients and oxygen while protecting the cells from harmful factors and the immune systems, thereby supporting long-term survival in the body. Cellular encapsulation in microgels can also facilitate investigations of single cell behaviors or cell-to-cell interactions by isolating cells from biological systems.

2.1.1. Spherical Microgels Templated by Emulsion Drops

One facile approach to encapsulating cells in microgels is by templating the gels using emulsion drops.\[^{7}\] Aqueous drops can be produced in a continuous oil phase and stabilized with the assistance of surfactants or particles, which provide isolated volumes for encapsulating water-soluble material. Water-in-oil (W/O) emulsion drops are spherical in shape, which minimizes the surface energy. A water drop containing a hydrogel precursor can transform into a gel microparticle through polymerization. When the water drops contain cells, the cells can be captured in hydrogel network of resultant microgels. In traditional approaches, bulk emulsification methods, such as mechanical stirring or homogenization, have been used to produce W/O emulsion drops. Although bulk methods permit commercial-scale emulsion production for the delivery of detergents, food additives, cosmetics, and pharmaceutics, the poor control over the size of the drops makes it difficult to use them in advanced applications such as cell encapsulation. In this section, we discuss microfluidic approaches to the controlled production of monodisperse emulsion templates and cell-laden microgels derived from templates. Certain advanced applications of cell-laden microgels that are difficult to achieve using traditional approaches will be discussed.

Two Distinct Types of Microfluidic Drop Makers and Cell Viability in Microgels: Monodisperse W/O emulsion drops can be produced using microfluidic devices as microgel templates. Typically, two distinct drop maker designs have been employed, using either a T-junction or a cross-junction. T-junctions include one main channel that delivers a continuous phase and a second channel for injecting a dispersed phase, as shown in Figure 2a.\[^{8}\] The two channels generally intersect perpendicularly. The dispersed phase is emulsified at the junction by the drag force exerted by the continuous phase during drop formation, thereby producing monodisperse emulsion drops. The sizes of the drops depend predominantly on the flow rate of the continuous phase and the width of the channels, whereas the frequency of drop generation depends on the flow rate of the dispersed phase in a stable mode.

Cross-junction designs use hydrodynamic flow-focusing to create a narrow aperture, as shown in Figure 2b.\[^{9}\] A dispersed phase is injected through a single channel, which is strongly confined by the continuous phase in a narrow jet. The jet can be emulsified into monodisperse drops via its breakup, driven by either the Plateau–Rayleigh instability or the drag force exerted by the continuous phase. The breakup mode under Rayleigh–Plateau instability conditions provides relatively small drops at a high frequency of generation compared to the breakup mode under the drag force in either T-junctions or cross-junctions. In both designs, channel surfaces should have a higher affinity for the continuous phase to avoid wetting problems. For example, hydrophobic channels are appropriate for stably producing W/O emulsion drops.

When W/O emulsion drops contain pre-polymers in combination with cells, the precursors can be crosslinked by ionic gelation or photopolymerization to form cell-laden microgels. For example, Tan et al. used a T-junction device to produce W/O emulsion drops containing an alginate precursor and CaCO\(_3\) nanoparticles.\[^{10}\] The precursor in the drops polymerized by ionic gelation as acetic acid diffused from the continuous phase to avoid wetting problems. For example, hydrophobic channels are appropriate for stably producing W/O emulsion drops.

Figure 1. Three distinct types of microcarrier: particles, capsules, and foldable microcarriers.
continuous oil phase to water. Rapid transfer yielded an encapsulated cell viability of 74%. Although the size of the microgels could be controlled using a T-junction, the minimum microgel size remained comparable to the main channel width.

To make smaller microgels, a hydrodynamic flow-focusing geometry was employed. In this system, a narrow jet produced drops with diameters approximately twice the diameter of the jet at the breakup driven by Plateau–Rayleigh instability. Alternatively, high shear stress in a narrow orifice can produce small drops, even under the breakup driven by drag force. For example, Eun et al. encapsulated *Escherichia coli* (*E. coli*) in agarose microgels using a hydrodynamic flow-focusing geometry. The resultant microgels were smaller than 50 μm.[16] The high uniformity of the cell-laden microgels provided a reproducible microenvironment that facilitated various quantitative studies of cell behavior. These methods required high cell viability during microfluidic fabrication as a prerequisite. Optimal cell viabilities have been achieved using natural polymer gels as an extracellular matrix. Such gels provide cell-compatible conditions in that they are nontoxic, permit the rapid diffusion of nutrients and waste, facilitate strong cellular adhesion to the matrix, and yield a high void fraction. For example, alginate, derived from brown algae, is popular natural gel material for cell encapsulation, as described in Figure 2c. Another natural gel generally used for encapsulation of cells is agarose which is derived from red algae. For example, *E. coli* could be encapsulated in agarose microgels; the cells exhibited migration, growth, and differentiation in the microgels. In addition, these non-toxic microgels can facilitate the separation of mutant cells which are sometimes generated during the differentiation; the mutated *E. coli* can be confirmed by fluorescence labeling, which could be rapidly isolated by injecting the microgel into a flow cytometer of fluorescence-activated cell sorting (FACS). This method reduced the overall required sample amounts as a factor of at least 10 and required time as a factor of at least 8 compared with those required in traditional cell mutant generation methods based on batch processes; reduction in sample amount is attributed to the small volume of microgels and reduction in analysis time to obtain statistics is attributed to the continuous and automatic analysis of FACS technique.

Biocompatible synthetic polymers with acrylate or amine groups have been employed as alternatives to natural polymers. Unlike natural polymers harvested from living organisms, synthetic polymers can be prepared with a high degree of homogeneity and with a consistent quality, which is very important for repetitive cell studies. However, most synthetic polymers require free-radical polymerization and the residual reagents in a polymer gel can reduce cell viability, making such synthetic polymers incompatible with cell-based applications. To avoid these issues, Rossow et al. demonstrated the radical-free gelation of precursors in cell-loaded drops using the thiol-ene click chemistry.[17] hyperbranched poly(ethylene glycol) PEG molecules were crosslinked by a dithiolated PEG unit without an initiator. The yeast cells were encapsulated without significantly reducing the cell viability. This radical-free polymerization achieved a cell viability of 90%, higher than that achieved in the presence of photopolymerization; the mutated *E. coli* can be confirmed by fluorescence labeling, which could be rapidly isolated by injecting the microgel into a flow cytometer of fluorescence-activated cell sorting (FACS). This method reduced the overall required sample amounts as a factor of at least 10 and required time as a factor of at least 8 compared with those required in traditional cell mutant generation methods based on batch processes; reduction in sample amount is attributed to the small volume of microgels and reduction in analysis time to obtain statistics is attributed to the continuous and automatic analysis of FACS technique.

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In an effort to enhance cell adhesion and cell spreading on synthetic hydrogel matrices, Tsuda et al. employed a self-assembling peptide (SAP) functionalized with short biologically active motifs.[18] the SAP is originally developed by Zhang et al.[14] Microgels of the SAP were fabricated by emulsifying an aqueous sucrose solution containing 10% (w/v) endothelial
cells and 0.25% (w/v) SAP in mineral oil containing salt powders using a hydrodynamic flow-focusing device. The SAPs in the W/O emulsion were crosslinked by ionic polymerization upon diffusion of the salts from the continuous phase. The resultant SAP microgels yielded cell viability values as high as 95%, as shown in Figure 2d (living cells are stained green, dead cells are stained red). The cell migration and cell-to-cell interactions were observed after incubating for 3 days, as shown in Figure 2e. These results indicated that the SAP gel networks were cell-compatible due to both strong adhesion of the cells to the SAPs and to the rapid diffusion of oxygen and nutrients through the matrixes.

All emulsion templates for cell encapsulation require an immiscible oil phase that can threaten living cells. The oil phase prevents nutrient supplements from reaching the cells, and the oil molecules themselves diffuse slowly into the water drops and interfere with cell function. The rapid phase transfer of cell-laden microgels from oil to an aqueous medium is very important for achieving a high cell viability. In all W/O emulsion preparations, microgels must be washed and centrifuged prior to transferring to an aqueous phase, leading to a long residence time in the oil. Recently, the on-chip washing and phase transfer of microgels was demonstrated by integrating oil skim and washing channels into a microfluidic device, as shown in Figure 2f; drop makers in Figure 2a,b can be combined with this on-chip washing unit to compose full devices. The continuous oil phase was drained out through the oil skim channel, and the aqueous medium was introduced through the washing channel, creating parallel flows of the washing solution and the residual oil in a single channel. The thin oil flow exposed the microgels to the washing solution, and the hydrophilicity of the microgels favored spontaneous transfer to the water phase, significantly reducing the residence time in oil. This was demonstrated using alginate microgels.19 Two distinct aqueous drops containing sodium alginate and CaCl₂ were prepared separately and then coalesced to polymerize alginate. The microgels in the drops subsequently flowed through the oil skim and the washing channels in the device, thereby transferring the alginate microgels into the washing solution. In such microfluidic device, phase transfer occurred immediately, leading to a high cell viability. On-chip transfer of the alginate microgels containing colon cancer cells, HCT116, achieved a cell viability of 80%, whereas off-chip transfer achieved only a 50% viability.

Cell-Laden Microgels with Multicomponents: Microfluidics have also enabled the independent injection of multiple components into the microgels. Microgels of uniform size and containing multiple components are difficult to achieve using bulk emulsification techniques. In microchannels, the low Reynolds number generates a stable laminar flow in microfluidic devices with negligible mixing; the Reynolds number is a dimensionless number indicating the ratio of inertial forces to viscous forces. This flow facilitates the production of monodisperse microgels with heterogeneous structures composed of multiple components. Anisotropic microgels can be used to achieve novel functionalities while avoiding damage to living organisms. For example, magneto-responsive cell-laden microgels can be produced through the parallel injection of two distinct alginate flows, as illustrated schematically in Figure 3a. Two distinct solutions of alginate precursors were separately injected into a cross-junction to form a pair of emulsion drops in an oil phase, where one solution contains mammalian cells and the other contains superparamagnetic nanoparticles. In situ

Figure 3. Microgels with multiple components. a) Schematic illustration of a microfluidic device used to generate Janus microgels. Controlled coalescence of CaCl₂-dissolved water drops with Janus alginate drops induced rapid gelation with insignificant mixing, resulting in Janus microgels. b) Schematic illustration of fabrication method of multicomponent microgels using centrifugal force; a tapered glass capillary with two separated channels is used to inject distinct alginate solutions and Janus drops are generated upon application of centrifugal force. c) Optical microscope image of Janus alginate microgels encapsulating Jurkat cells and magnetic nanoparticles in their own hemispheres. Panel (c) reproduced with permission.© 2012, Wiley-VCH. d) Schematic illustration of a microfluidic device developed for the independent injection of two different solutions of a gel precursor into single-phase drops; precursor emulsion drops are cooled down as they pass the cooler, leading to polymerization of emulsion drops to microgels.

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The shape, elasticity, and chemical composition of an emulsion drop are important factors for artificial extracellular matrices. Although the chemical properties of the extracellular matrix have been widely studied, the physical aspects, such as the shape and elasticity, are poorly understood due to a lack of control over such properties. The elasticity of a microgel was controlled by Kumachev et al. to prepare a set of agarose microgels with various elasticities via the independent injection of two solutions: a concentrated 3.0 wt% agarose solution and a dilute 0.5 wt% agarose solution. The relative flow rates of these two solutions determined the concentration of agarose in the mixed drops and, therefore, the elastic modulus of the agarose microgel. This combinatorial mixing can be used to generate a library of cellular microenvironments, which affect the fates of cells.

2.1.2. Nonspherical Microgels Produced using a Micromolding Technique

Emulsion drops have provided useful templates for producing spherical microgels. Although the throughput of microfluidic emulsification approaches is not comparable to bulk approaches, approximately 1000 drops can be produced per second, and the production rate may be enhanced by parallelizing the T-junctions or cross-junctions. Nevertheless, drop-based approaches present a few limitations. First, an immiscible oil phase must be used to generate water drops, and this phase can reduce the viability of cells, as discussed above. The microgel particle shape is limited to spherical or another shape with high isotropy.

Micromolding techniques provide an alternative to emulsion templating. These methods use solid molds with a hole pattern that isolates aliquots of an aqueous precursor solution, thereby producing nonspherical microgels directly in water. Micromolding procedures are illustrated schematically in Figure 4a. An aqueous precursor solution is deposited on top of a mold and residual solution is removed, leaving the holes occupied by the solution. As the mold is dipped into an aqueous salt solution, the precursors are crosslinked in the holes through ionic polymerization that produces microgels. The microgels are dispersed in water after being released from the mold. During these steps, the precursor solution should remain in the holes of the micromold to completely isolate the microgels; therefore, the surface properties of the mold are very important. For example, Qiu et al. used a PDMS template as a hydrophobic mold for fabricating alginate microgels. An alginate solution was poured into a PDMS mold, and the residual solution was removed by scraping the PDMS surface with a microscope slide. The whole mold was then immersed in 1.0 M aqueous solution of CaCl$_2$ to permit ionic gelation of the precursor solution. Release of the microgels from the PDMS mold may be performed either by stretching or swelling the PDMS mold. The resultant alginate particles with hexagonal shapes are shown in Figure 4b. E. coli incorporated into these alginate microgels exhibited migration in the microgel, suggesting that this micromolding technique did not involve any cytotoxic steps.

Instead of a PDMS mold, Dang et al. used a commercially available polypropylene mesh as a mold to fabricate alginate microgels and facilitate the release of resultant microgels from the mesh.
In addition, the top surfaces of the microgels could be modified by dipping the mold into an aqueous solution containing poly-L-lysine. Positively charged poly-L-lysine was coated onto the surface of the negatively charged alginate through electrostatic attractions. Such modifications are useful for the attraction-mediated assembly of microgels in 3D tissue constructs. The encapsulation of insulin-secreting cells (INS-1) into the alginate microgels and incubation for 3 days yielded a cell survival rate exceeding 90%, as shown in Figure 4c. The encapsulated INS-1 cells secreted insulin upon stimulation with glucose for more than 10 days.

Microgels composed of multiple components are difficult to produce via micromolding because a mold becomes fully occupied after a single dip into an aqueous solution, and additional components cannot be easily included. To overcome this limitation, size-controllable molds have developed for micromolding applications to broaden the compositional variety of microgels prepared via micromolding. Tekin et al. used a
2.1.3. Lithographically Featured Microgels

Higher degrees of freedom over the shapes of microgels may be obtained using photolithography techniques. In this method, pre-polymers in an aqueous solution are locally polymerized by selective exposure to UV light through a photomask, rather than physically isolating particles in a mold. UV light-induced activation of the photoinitiator produces free radicals that initiate polymerization of the unsaturated groups in the pre-polymers. Pre-polymers with one unsaturated group form saturated linear polymers through a chain reaction, whereas pre-polymers with more than one unsaturated group form a crosslinked network. Combinations of both pre-polymers produce similar networks with a lower crosslinking density. Because crosslinked hydrogel networks maintain their shape, photolithography can produce 2D microgels with shapes identical to the shape of the windows in the photomask, thereby enabling the production of microgels with complex shapes.

**Fabrication of Microgels Using Conventional Photolithography**:

Most photolithography uses highly viscous photoresists spin-coated onto a substrate and organic solvents evaporated by baking prior to UV exposure. The solid content in the final photoresist is extremely high, producing dense particles upon photopolymerization. By contrast, aqueous solutions of pre-polymers have a low polymer content and a low viscosity, making it difficult to coat the solutions onto a substrate. Instead, 2D microchannels with a constant height have been employed to confine aqueous solutions without flow during photopolymerization, as shown in the first schematic diagram of Figure 5a.

The pre-polymers in the aqueous solution are locally polymerized upon UV exposure through the photomask, and the polymerized microgels are released from the microchannels, as shown in the second and third diagrams. Du et al. used this technique to prepare cell-laden microgels with various shapes and subsequently used these microgels as building blocks for the construction of 3D tissue via bottom-up assembly. For example, square-shaped microgels were fabricated as building blocks using PEG-methacrylate (PEGmA) pre-polymers. The microgels were then swollen with a dilute pre-polymer solution. The microgels were dispersed in mineral oil, resulting in formation of free interface between the microgels and the continuous oil. Under continuous agitation, the microgels are coalesced to minimize the interfacial area, leading to adhesion...
of microgels to one another. The addition of more energy through a high agitation rate or a long agitation time induced the microgels to form a linear assembly, regardless of the size or aspect ratio. Finally, the pre-polymers, which had swelled the microgels, could be crosslinked under a second round of UV exposure, leading to one continuous structure of the self-assembled microgels. Interestingly, a pair of microgels whose shape is designed to form a complementary lock-and-key structure (a circular disk and a cross) exhibited directed assembly to form a complex organizational structure, as shown in Figure 5b. This is important for 3D tissue construction.

Microgels with complex shapes can be sequentially assembled to construct vascular-like microchannels. For example, PEG-diacrylate (PEGDA) particles with a donut shape were fabricated in a form of array on substrate by photolithography. The substrate was then immersed in mineral oil and the particles were brought into contact by mechanical swiping with a needle along the surface of the substrate, thereby forming a hollow cylindrical structure. Vascular structures may be mimicked using donut-shaped microgels with two distinct layers: an inner layer containing smooth muscle cells and an outer layer containing endothelial cells, as shown in the inset of Figure 5c; these microgels could be prepared by two sequential photolithography steps. Through the sequential assembly of such core-shell microgels, vascular structures consisting of a hollow core, a middle layer of muscle cells, and an outer layer of endothelial cells, were obtained as shown in Figure 5c.

Continuous Fabrication of Microgels by Flow Lithography: Photolithography has enabled the production of nonspherical microgels, thereby providing building blocks with a variety of shapes for constructing advanced structures; however, the microgel production throughput is too low for the construction of large-scale organs. The limited throughput may be improved by combining photolithography with microfluidic techniques to enable the continuous production of lithographically featured particles, as shown schematically in Figure 5d. This is called flow lithography. Microfluidic channels composed of the elastomer poly(dimethylsiloxane) (PDMS) are oxygen-permeable and can maintain a relatively high concentration of oxygen at the channel wall. As UV-curable pre-polymers, such as PEGDA, flow through PDMS channels, the free radical polymerization reaction is terminated by the oxygen molecules, thereby inhibiting polymerization near the wall. This enables the continuous production of microgels in microfluidic channels while avoiding clogging. The microparticle shapes are identical to the windows of a photomask, as in photolithography, although relatively long exposure times relative to the flow rate can produce microgels with shapes that are elongated along the direction of flow. To prevent this, advanced systems have been developed for “stop-flow lithography”, which temporally stop the flow during UV exposure. Panda et al. used stop-flow lithography to produce cell-laden microgels. Fibroblast cells (NIH-3T3) dispersed in culture media containing a PEGDA pre-polymer in concentrations of 10–40% (w/v) and a photoinitiator in concentrations of 1–5% (w/v) were used as a precursor solution, and uniform cell-laden PEG microgels with square shapes were prepared by stop-flow lithography. By designing additional rail channels, microgels can be guided and assembled into 2D array. As an example, HeLa-laden microgels and HEK293-laden microgels were produced separately in a device and subsequently co-assembled into a 3 × 3 heterogeneous array using railed microfluidics, as shown in Figure 5e. The HeLa and HEK293 are stained to appear green and red, respectively. Co-culturing of the cells in this heterogeneous array of microgels provided useful information for cell screening and study of cell-to-cell interaction.

2.2. Drug-Loaded Particles for Triggered Release

Microgels for cell encapsulation and culture are, ideally, highly swollen hydrogel networks that provide for the rapid diffusion of nutrients and wastes, and a high void fraction that promotes cell migration, growth, and differentiation. By contrast, particles for drug delivery and release purposes are, ideally, dense degradable polymers that encapsulate small drug molecules without a leakage, releasing the drug only in the desired environment to enable efficient delivery and dosing. Hydrogels can be used as drug carriers only for drug molecules that chemically bond to or are strongly adsorbed to the gel matrix. The size and shape of a drug carrier must be carefully tuned to facilitate circulation through narrow channels in blood vessels, as well as to control the interactions between the carriers and host cells. Drug carriers have been produced using techniques similar to those used for cell-laden microgels: microfluidic emulsion-templating, lithography, micromolding, or imprinting. In addition to these techniques, controlled precipitation in microfluidic devices has also been exploited to prepare drug-loaded nanoparticles.

2.2.1. Drug Carriers Templated by Emulsion Drops

Emulsion drops enable the efficient isolation and confinement of materials to provide useful templates for particle production. Drug-loaded dense particles may be prepared from emulsion templates using oil-in-water (O/W) emulsion drops. Drops of a volatile organic solvent containing hydrophobic drugs and biodegradable polymers will evaporate in a continuous water phase and shrink to form a spherical particle. A variety of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA), polyactic acid (PLA), and polycaprolactone (PCL), have been used in such applications. The hydrolysis of ester groups in the backbones of these polymers induces spontaneous degradation in water. After evaporation has reached completion, the remaining polymers form a particle that is densely packed with the drug, achieving a high efficiency of encapsulation. Most drug molecules cannot escape from the matrix immediately after particle formation because the interstices between the physically interpenetrated polymer chains are narrow. Spontaneous degradation of the polymer matrix induces the slow release of the drugs. The degradation rate depends strongly on environmental conditions, such as the pH and temperature. The sizes and shapes of the particles and the type and molecular weight of the polymer also influence the degradation rate. Monodisperse particles with a homogenous morphology are required to attain a reproducible drug release profile. Microfluidics approaches can easily generate monodisperse O/W emulsion drops for this purpose, where the stable production of O/W emulsion drops requires a hydrophilic channel surface. As an example, dichloromethane (DCM) containing PLGA and
released the drugs at a higher level over a longer period of time than the polydisperse particles. The smaller particles released the drugs more rapidly than the larger particles. Fast release from the small particles was attributed to the large surface area-to-volume ratio. The polydisperse particles formed using bulk methods exhibited an initial burst of drug release, releasing most of the drugs within a short timeframe. The monodisperse particles did not display an initial burst release and provide sustained release at a constant dose level, which is highly desirable for a wide range of drug delivery applications.

Drug-loaded particles could be formed into nonspherical shapes by shearing emulsions. Heslinga et al. mechanically an amphiphilic local anesthetic drug, bupivacaine, was emulsified in an aqueous solution of poly(vinyl alcohol) (PVA) using a microfluidic device with a flow-focusing geometry, and DCM was selectively evaporated. During evaporation, the monodisperse drops shrunk and consolidated into drug-loaded PLGA particles, as shown in Figure 6a. The particle size was proportional to the size of the emulsion drops and the cube root of the volume fraction of polymer in the initial drops. The drug release profiles of the monodisperse PLGA particles are shown in Figure 6b. The drug release profile from a polydisperse PLGA particle sample characterized by the same average size, formed by bulk emulsification, is presented for comparison. The monodisperse particles released the drugs at a higher level over a longer period of time than the polydisperse particles. The smaller particles released the drugs more rapidly than the larger particles. Fast release from the small particles was attributed to the large surface area-to-volume ratio. The polydisperse particles formed using bulk methods exhibited an initial burst of drug release, releasing most of the drugs within a short timeframe. The monodisperse particles did not display an initial burst release and provide sustained release at a constant dose level, which is highly desirable for a wide range of drug delivery applications.

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Figure 6. Drug carriers templated by emulsion drops. a) Scanning electron microscopy (SEM) image of monodisperse poly(lactic-co-glycolic acid) (PLGA) particles. b) The cumulative release of drug from the PLGA particles. Particles with two different average sizes were prepared by either microfluidic or bulk emulsification techniques. Panels (a,b) reproduced with permission. Copyright 2009, Wiley-VCH. c) Janus microgels composed of BODIPY-coupled pectin hemispheres and fluoresceinamine-coupled alginate hemispheres. Alginate hemispheres were selectively degraded by alginate lyase in the continuous phase, leading to the selective release of fluoresceinamine. In the same fashion, pectin hemispheres could be degraded by polygalacturonase. Panel (c) reproduced with permission. Copyright 2012, American Chemical Society. d) Confocal microscopy images of nanoeulsion-embedded pNIPAAm microgels containing a hydrophilic dye, DAPI, in the gel matrix and a hydrophobic dye, Nile red, in the emulsion. Emission from the hydrophobic dye is shown in the leftmost image, and emission from the hydrophilic dye is shown in the middle images. The merged images showing emission from both dyes is shown in the rightmost image. e) Temperature dependence of the volume of an emulsion-embedded microgel (empty circle), and the fluorescence intensities of DAPI (filled circle) and Nile red (filled square) in the surrounding fluids, released from the microgel. Heating resulted in the collapse of the microgels, leading to the release of both dyes due to physical dissociation of DAPI from the gel matrix and expulsion of the Nile red-dissolved emulsion from the microgel. Panels (d,e) reproduced with permission. Copyright 2011, Wiley-VCH.
stirred emulsion drops during evaporation of organic solvent, leading to deformation of the drops. Therefore, elongated spheroïd particles were generated when the evaporation was completed. The shape of particles influences degradation rate and interaction of the particles with cells.

Although microgels cannot encapsulate small drug molecules due to their large mesh size, drugs may be chemically bonded to the gel matrix to enable encapsulation. Drugs can then be released upon degradation of the matrix or dissociation of the bonds. For example, pectin or alginate can be modified with drugs or a dye and used as a carrier precursor. The drugs or dyes can be selectively released upon degradation of the pectin or alginate by the enzymes polygalacturonase (PG II) or alginate lyase (AL), respectively. To demonstrate this, Janus microgels consisting of a BODIPY-coupled pectin hemisphere and a fluoresceinamine-coupled alginate hemisphere were prepared using a hydrodynamic flow-focusing device. Acetic acid dissolved in the continuous phase diffused into the drops, polymerizing both BODIPY TR cadaverine-conjugated pectin and the fluoresceinamine-conjugated alginate through ionic gelation. Addition of AL to the continuous water phase selectively degraded the alginate hemisphere to release fluorescein into the continuous phase while retaining BODIPY in the pectin hemisphere, as shown in Figure 6c.

Microgels can deliver hydrophilic drugs encapsulated via chemical or physical adsorption to the microgel matrix. Hydrophobic drugs are difficult to encapsulate in hydrophilic hydrogels. To deliver both hydrophilic and hydrophobic drugs using a single microgel carrier, Jagadeesan et al. incorporated a hexadecane nanoemulsion into a pNIPAAm microgel network. The hydrophobic dye, Nile red, was used as a model hydrophobic drug and was dissolved in hexadecane. The hexadecane was then emulsified in an aqueous solution containing the n-isopropylacrylamide monomers by bulk homogenization, leading to the formation of an O/W nanoemulsion. The nanoemulsion was re-emulsified in a continuous oil phase using a microfluidic device, thereby preparing W/O emulsion drops. The monomers in the drops were polymerized by UV exposure. During this step, the nanoemulsion became trapped in the gel network because the emulsion drop size was larger than the network pore size. The hydrophilic dye, DAPI, was used as a model hydrophilic drug and was physically adsorbed onto the pNIPAAm network in the continuous phase water–dimethyl sulfoxide (DMSO) mixture. Both hydrophobic and hydrophilic dyes were distributed in the resulting pNIPAAm microgels, as shown in Figure 6d. The release of both dyes from the microgels was triggered by increasing the temperature above the LCST of pNIPAAm, thereby collapsing the network. Network shrinkage expelled the nanoemulsion by squeezing, and the physically adsorbed hydrophilic dye dissociated to release both dyes into the continuous phase. The temperature dependence of the microgel volume and the fluorescence intensities of both dyes in the surrounding fluid were measured, as shown in Figure 6c.

2.2.2. Imprinting Techniques for Preparing Nonspherical Drug Carriers

The sizes and shapes of drug-loaded particles are important factors for determining the interactions between particles and cells or the flow of particles through narrow channels in the body. Although emulsion-templating can provide a wide range of particle sizes, from a few micrometers to hundreds of micrometers, the fabrication of small particles requires delicate control over the flow rate, and the resultant particles are limited to spherical shapes. In an effort to prepare small particles with nonspherical shapes, imprinting techniques were used for the production of drug delivery vehicles. As with the micromolding technique, the imprinting technique used a micromold to isolate a solution containing precursors or polymers. Unlike micromolding, isolation was achieved by squeezing the solutions on a substrate using the mold. The solution in the mold is subsequently solidified via photopolymerization or drying, and the particles were released from the substrate by dissolving a sacrificial layer. These procedures are presented schematically in Figure 7a.

Chemically and Biologically Triggered Release of Drugs: Innovative approaches to trigger the release of encapsulants have been developed by the Desimone group, who employed non-wetting micromolds prepared from the fluorocarbon elastomer, perfluoropolyether (PFPE). The molds had a low surface energy and induced complete solution dewetting outside of the holes, yielding particles with a well-defined shape. Imprinting enabled the production of monodisperse particles using a variety of materials. Natural materials, such as proteins, hormones, antibodies, or enzymes, as well as synthetic polymers may be used in this technique. For example, albumin particles with 5, 2, or 0.2 μm in size were fabricated by lyophilizing an aqueous solution in a mold and harvesting the particles in a chloroform and induced complete solution dewetting outside of the holes, yielding particles with a well-defined shape. Imprinting enabled the production of monodisperse particles using a variety of materials. Natural materials, such as proteins, hormones, antibodies, or enzymes, as well as synthetic polymers may be used in this technique. For example, albumin particles with 5, 2, or 0.2 μm in size were fabricated by lyophilizing an aqueous solution in a mold and harvesting the particles in a chloroform suspension. The particles were alternatively transferred onto poly(cyano acrylate) or poly(vinyl pyrrolidinone) films to form two-dimensional arrays. The resultant albumin particles were readily dissolved in an aqueous solution, as shown in Figure 7b to enable the rapid release of the encapsulants into water.

Biodegradable PLGA particles containing a hypertension drug, felodipine, were prepared by Acharya et al. using gelatin molds replicated from silicon microstructures. A PLGA solution containing the drug was imprinted and dried at room temperature. The felodipine-loaded PLGA particles were then released in water upon dissolution of the gelatin mold. The resultant PLGA particles showed a high drug loading efficiency of 80% and a low fraction of initial release, approximately 10%; many drug carriers show an uncontrolled initial release by burst. In a similar way, the cancer drug, docetaxel, was loaded with a variety of concentrations into PLGA particles. The resultant particles were evaluated for their drug delivery efficacy in SKOV3 cell (ovarian carcinoma) viability assay. The results were compared with the efficacy of a commercial formulation of docetaxel, Taxotere. The PLGA particles exhibited a longer anti-cancer effect at docetaxel loading levels of 10 or 20%, whereas they exhibited a higher anticancer effect at docetaxel loading levels of 30 and 40%.

Environment-specific drug release was accomplished using drug-loaded degradable particles fabricated from polymers with cleavable crosslinkers. As an example, the disulfide crosslinker N,N'-cystaminebisacrylamide was added to a solution containing the monomer trimethylolpropane ethoxylate triacrylate (TMTPA) and doxorubicin. The monomer solution was imprinted using a PFPE mold (Fluorocur) and subsequently photopolymerized. The resultant particles were released from the mold, as
The silyl ether crosslinker remained stable in the presence of enzymes and degraded only under acidic conditions. The particles with disulfide bonds, which were designed to control over the degradation rate and avoided the formation of disulfide bonds but no doxorubicin, as shown in Figure 7 d. Viability of HeLa cells that had internalized particles containing disulfide bond-containing particles was supported by the high composition B) remained high. The non-cytotoxicity of the drug release profile from particles without disulfide bonds. The viability of the HeLa cells treated with the particles was comparable to that obtained using free doxorubicin. The half-life of the same particle containing diethyl silyl ether was 0.091 days, whereas the particles with di-tert-butylsilyl groups selectively degraded without cytotoxicity, whereas the particles with di-tert-butylsilyl groups remained undegraded, as shown in Figure 7e (the particles crosslinked with dimethyl silyl ether or di-tert-butylsilyl and internalized into HeLa cells. The particles with the dimesyl silyl ether groups selectively degraded without cytotoxicity, leading to the release of anti-mouse IgG. Panel (f) reproduced with permission. Copyright 2010, American Chemical Society. f) Cumulative release of the anti-mouse IgG from nanoparticles composed of poly(ethylene glycol) diacrylate (PEGDA) and peptide. The enzyme, cathepsin B, was added over 25 h to trigger degradation of the peptide, leading to the release of anti-mouse IgG. Panel (f) reproduced with permission. Copyright 2008, Elsevier.

shown in Figure 7c, and treated with UltraAvidin for efficient cell internalization. The disulfide crosslinker could be cleaved in a reducing environment, for example, in the presence of the reductant dithiothreitol (DTT). DTT had a negligible effect on the drug release profile from particles without disulfide bonds. The particles with disulfide bonds, which were designed to be internalized into HeLa cells in vitro, killed the HeLa cells as they released doxorubicin. The viability of the HeLa cells treated with the particles was comparable to that obtained using free doxorubicin, as shown in Figure 7d (denoted composition A); this proved the triggered release of doxorubicin from particles inside HeLa cells. By contrast, particles without disulfide bonds did not afford the effective release of doxorubicin, and the viability of the HeLa cells treated with these particles (denoted composition B) remained high. The non-cytotoxicity of the disulfide bond-containing particles was supported by the high viability of HeLa cells that had internalized particles containing disulfide bonds but no doxorubicin, as shown in Figure 7d (denoted composition C). Parrott et al. achieved a high level of control over the degradation rate and avoided the formation of toxic byproducts by crosslinking hexanuf-shaped particles using a silyl ether crosslinker that degraded under acidic conditions. The silyl ether crosslinker remained stable in the presence of enzymes and degraded only under acidic conditions. The particles containing the dimethyl silyl ether linkage exhibited a pH-dependent rate of degradation. The half-life values were found to be 2.19 h at pH 5.0, in the lysosome, 1.08 days at pH 6.0, in the endosome, and 2.94 days at pH 7.4, under physiological conditions. To demonstrate the selective degradation, a set of particles was crosslinked with either dimethyl silyl ether or di-tert-butylsilyl and internalized into HeLa cells. The particles with the dimethyl silyl ether groups selectively degraded without cytotoxicity, whereas the particles with di-tert-butylsilyl groups remained undegraded, as shown in Figure 7e (the particles crosslinked with dimethyl silyl ether or di-tert-butylsilyl groups encapsulated green or red dyes, respectively, to facilitate imaging). Modification of the silyl ether permitted control over the degradation rate. For example, the half-life of particles with 5 μm in diameter containing dimethyl silyl ether at pH 5 was 0.091 days, whereas the halflife of the same particle containing diethyl silyl ether was 1.24 days. Further increases in the half-life were achieved using diisopropyl silyl, which extended the half-life to 30.7 days. Although the pH-triggered release of a drug can be widely used for the in vivo delivery of drugs, release under more specific conditions is sometimes desired. For example, enzymatically triggered degradation can be used to achieve site-specific targeting. As an example, Glangchai et al. produced PEGDA nanoparticles containing the enzymatically degradable peptide...
Gly-Phe-Leu-Gly-Lys-diacrylate (GFLGK-DA) using an imprinting technique. Degradation of the resultant sub-micrometer particles was triggered by Cathepsin B, which is potentially useful for specific environment- or disease-triggered release of the drugs. As a demonstration, plasmid DNA and antibodies were loaded into nanoparticles prepared using PEGDA-GFLGK-DA. The particles, which retained the encapsulants without degradation, exhibited degradation and release of the encapsulants immediately after Cathepsin B was added to the PBS solution, as shown in Figure 7f, where Cathepsin B was added at 25 h; Cathepsin B is usually overexpressed in lung, ovarian and colorectal tumor cells, potentially providing targeted release of the encapsulated materials.

**Effects of Drug Carrier Particle Shape and Elasticity:** Drug release and particle internalization into cells can be further improved by designing the particle shape. Drugs are generally released from the surfaces of a drug carrier; therefore, the shape of a carrier can influence the release profile of a drug. In general, particles with a high aspect ratio reminiscent of bacteriophages or sperm cells are easily internalized into cells. Drug carrier shapes may be carefully selected to achieve a controlled drug release profile and a high efficiency of particle internalization into cells. The effect of particle shape on the drug release profile was investigated using cylindrical PLGA particles with different aspect ratios, prepared by the imprinting technique. An organic solvent was dried in a gelatin mold to consolidate the drug and PLGA into particles, which were then released upon dissolution of the mold in water at 40 °C, as shown in Figure 8a. The release profiles of the encapsulated felodipine from the particles with different sizes and aspect ratios are shown in Figure 8b, where the diameters and heights of the particles are denoted in the plot in units of μm. The results were consistent with the expectation that a larger surface area-to-volume ratio induced the more rapid release of a drug. The release profile of the disk-type particle fluctuated less than the release profile of the short cylinder-type particles.

The shape of a drug carrier can influence the rate of carrier internalization into cells. This was investigated by Gratton et al., who prepared a variety of TMPTA particles with different sizes, shapes, and aspect ratios and observed their internalization into HeLa cells. For example, three distinct particles...
of cubic, circular disk, and rod shapes are shown in Figure 8c. During a 4 h observation period, eight distinct particles exhibited remarkably different efficiencies of cellular internalization, as shown in Figure 8d. Cylindrical particles 150 nm in diameter and 450 nm in height (black squares) or 200 nm in both diameter and height (green triangles) provided a particularly interesting comparison. Particles with an aspect ratio of 3 were most rapidly internalized with a rate 4 times the internalization rate of particles with an aspect ratio of 1 and a similar volume. Moreover, it is noteworthy that cylindrical particles 150 nm in diameter and with an aspect ratio of 3 (black squares) exhibited a higher rate of internalization than particles 100 nm in diameter and with the same aspect ratio (red circles). These results demonstrated that the size of a drug carrier is important for determining the internalization rate. The size effects were also observed among the cubic particles with two different sizes: 2 μm (pink triangles, rotated 90° counterclockwise) or 3 μm (violet triangles, rotated 90° clockwise). Optimization of both the size and shape is required for the efficient internalization of particles into cells.

Drug-loaded particles should pass through blood vessels during the in vivo delivery of a drug. The sizes of particles should be carefully selected to avoid vessel clogging or removal by filtering. In addition to size, the elasticity of a particle is important for circulation. To investigate this effect, Merkel et al. fabricated red blood cell-shaped hydrogel particles using an imprinting technique, as shown in Figure 8e. The elasticity of the 2-hydroxyethyl acrylate particles was modulated by changing the concentration of the crosslinker, PEGDA. The elasticity or deformability of the particles strongly affected the circulation of the particles. Narrow microfluidic channels were employed to evaluate the deformability of the particles, as shown in Figure 8f. The results showed that 1% crosslinked particles with 6 μm in diameter passed through 3 μm × 3.5 μm channels by elongating up to 239 ± 26%, and the particles readily recovered their original shapes in the absence of confinement. Additional in vivo experiments showed that the elasticity of the particles dramatically affected their circulation time and biodistribution. An 8-fold decrease in the elasticity increased the half-life of elimination by a factor of 30.

2.2.3. Lithographically Featured Drug Carriers Using Microfluidics

Imprinting techniques have enabled the production of non-spherical drug carriers; however, the technique is limited in its ability to incorporate multiple components and achieve microscopic morphologies. As discussed above, lithography techniques have been used to fabricate nonspherical particles as an alternative to micromolding or imprinting. Flow lithography has enabled the continuous production of lithographically featured particles. Moreover, flow lithography can produce heterogeneous particles by controlling the UV exposure dose or including parallel laminar flows in a single channel toward providing advanced functionalities in drug carriers. Therefore, lithography is a promising approach to preparing drug carriers. Hwang et al. used flow lithography to prepare 2D triangular microparticles with controlled degradability. To this end, diacylate PLA-b-PEG-b-PLA was dissolved in a mixture of water and ethanol at 10, 20, and 30 wt%, along with a photoinitiator and N-vinylpyrrolidone. The solution was introduced into microfluidic channels for flow lithography to prepare the microparticles shown in Figure 9a. The resultant microparticles exhibited notable differences in their degradation rates, depending on the PLA-b-PEG-b-PLA content. Particles with a lower PLA-b-PEG-b-PLA content showed more rapid degradation and, therefore, the faster release of encapsulants, where hydrolysis of the ester group in PLA induced degradation. Heterogeneous microparticles that are sequentially and selectively degraded were produced either by applying a grayscale photomask to a homogeneous diacrylate PLA-b-PEG-b-PLA flow or by forming multiple parallel flows with different diacrylate PLA-b-PEG-b-PLA contents. A grayscale photomask creates a spatial distribution of UV intensity that induces a different degree of crosslinking for the short-term exposure time. A single particle can therefore have both a highly crosslinked region and a poorly crosslinked region to independently adjust the rates of degradation in different regions of the microparticle. This method is applicable to the stepwise release of single drugs.

By contrast, parallelized multiple flows may be used to prepare heterogeneous microparticles, thereby enabling the encapsulating of multiple drugs in separate regions of the microparticle. For example, flow lithography can be applied to a channel with three parallel flows comprising 30 wt% PEGDA, 20 wt% diacylate PLA-b-PEG-b-PLA, or 30 wt% PEGDA. The resultant particles will include three distinct regions, wherein the central region is degradable and the other two regions are not degradable. A single microparticle will then break up into two microparticles upon degradation of the middle region, as shown in the top images of Figure 9b. Similarly, three parallel flows were constructed, composed of diacylate PLA-b-PEG-b-PLA in three different concentrations of 30, 20, and 10 wt%. The resultant heterogeneous particles included three degradable regions that each degraded at a different rate, as shown in the bottom images of Figure 9b. The region composed of 10% diacrylate PLA-b-PEG-b-PLA degraded first, and the middle region composed of 20% diacrylate PLA-b-PEG-b-PLA degraded second, enabling the sequential release of multiple encapsulants.

The sequential release of multiple drugs is very important for effective treatments of disease or wounds. To provide additional flexibility with regards to the encapsulation and release of multiple distinct drugs, An et al. prepared composite microgels containing a nanoemulsion using flow lithography. The composition was prepared by pre-emulsifying silicone oil in an aqueous solution containing 33% (v/v) PEGDA and 100 mM SDS under constant agitation, followed by high-pressure homogenization to create a nanoemulsion. A lipophilic dye and a photoinitiator were added to the nanoemulsion prior to performing flow lithography. The photopolymerized composite microgels showed microscopic heterogeneity due to the presence of the nanoemulsion in the hydrogel matrix. Multiple drugs could then be encapsulated in each of three distinct regions: hydrophobic drugs in the nanoemulsion, amphiphilic drugs at the emulsion interface, or hydrophilic drugs in the hydrogel matrix. The release rates of the drugs depended strongly on the release mechanism. For example, an oil-soluble dye, PKH26, was loaded into the nanoemulsion and displayed a slow release profile due to its limited solubility in aqueous media, as shown in Figure 9c. Release occurred over several days. By contrast,
the temperature-dependent adsorption and desorption of active ingredients at the O/W interface can facilitate the triggered release of materials. For example, an acrylate-functionalized biotinylated nucleotide (Ac-BT) adsorbed at the O/W interface at 45°C and desorbed at 15°C. Ac-BT or molecules attached to Ac-BT could then be encapsulated at 45°C and released at 15°C. This result was confirmed by conjugating Ac-BT to streptavidin functionalized with a cyanine dye (SA-Cy3), as shown in Figure 9d. The release of the Cy3 complex occurred over several hours. Although the nanoemulsion was trapped in the PEGDA matrix, it could be released upon degradation of PEGDA, which is accelerated by high pH conditions, as shown in Figure 9e. The release of the nanoemulsion proceeded over 2 h in a 0.1 M NaOH solution.

2.2.4. Controlled Precipitation of Drug-Loaded Nanoparticles

Imprinting and lithography techniques can significantly reduce the sizes of particles to the sub-micrometer scale, which is otherwise very difficult to achieve using emulsion-templating methods; however, drug delivery to a final destination frequently demands that the size of a carrier particle be smaller than this in many cases. In general, small particles have an enhanced ability to reach their target. For example, cancer drugs should be delivered to tumors that have leaky blood vessels with an inner diameter of 200 nm or less. Therefore, controlled production of nanoparticle carriers is very important for cancer therapy.

Nanoprecipitation provides a practical strategy for producing drug-loaded nanoparticles. Amphiphilic polymers dissolved in a good organic solvent precipitate upon mixing with a poor solvent, i.e., water, because the polymers are not soluble in the mixture, as illustrated in Figure 10a. During precipitation, amphiphilic polymers drive the formation of micelles through their alignment. Hydrophobic regions of the polymers aggregate to form a core, whereas the hydrophilic regions stretch to form a corona. Nanoprecipitation can be used to produce drug carriers with a fairly uniform size. The average nanoparticle size can be controlled via several factors, such as the solvent/water ratio, the type of organic solvent, or the polymer concentration during precipitation. For example, Farokhzad et al. used PLGA-b-PEG amphiphilic polymers to encapsulate docetaxel for chemotherapy. Encapsulation was achieved by adding an acetonitrile solution containing carboxylated
nanoparticles (Dtxl-NP-Apt) exhibited a high level of cytotoxicity toward the targeted LNCaP prostate epithelial cells in vitro, and effective tumor regression was observed. The delivery and release of docetaxel were confirmed by comparing these results to docetaxel-loaded nanoparticles that were not conjugated to Apt (Dtxl-NP), bare nanoparticles without docetaxel (NP), free docetaxel (Dtxl), or saline. The toxicity properties of these control samples were tested in BLAB/c nude mice, as shown in Figure 10c. Dtxl-NP exhibited a slight reduction in the tumor size, whereas the other three control samples did not affect the growth of the tumors.

Tumor-targeting in vivo requires “stealth”, particularly for immune evasion or cell-specific targeting. Conveying both stealth properties and cell-specific targeting to a nanoparticle is challenging. To achieve both, nanoparticles were prepared by co-precipitation of Apt-copolymerized PLGA-b-PEG and normal PLGA-b-PEG. The ratio between the two block-copolymers was adjusted to vary the density of Apt at the particle surface, as illustrated schematically in Figure 10d. Because Apt enhanced cell-targeting properties, whereas the PEG corona improved the anti-biofouling properties, the Apt density on the nanoparticle surfaces should be optimized. As the Apt surface density increased, the NPs accumulated in the liver due to a poor anti-biofouling properties, as shown in Figure 10e. By contrast, a decrease in the Apt surface density reduced NP loading in the tumors, as shown in Figure 10f. Optimal conditions were achieved at a 5 wt% NP-Apt, which provided the highest NP loading in the tumor while maintaining acceptably low levels of NP accumulation in the liver. These results suggest that a balance between tumor-targeting ligands and the anti-biofouling surface is important.

Nanoprecipitation in Microfluidic Devices:

Microfluidic devices provide precise control over mixing in a microchannel and are useful platforms for producing nanoparticles through precipitation. Because diffusional mixing is dominant in low-Reynolds-number flows, stable and controlled mixing in confined microfluidic channels can reduce the size distribution of nanoparticles relative to the distributions achieved through bulk precipitation. For example, Karnik et al. injected an acetonitrile solution containing PLGA-b-PEG polymer and water into a microfluidic device with a hydrodynamic flow-focusing geometry, as illustrated in Figure 11a. The acetonitrile solution stream was focused and confined by the water streams. As the two streams mixed across their interface by diffusion downstream, PLGA-b-PEG nanoparticles
an organic solution is added drop-by-drop to water, which provides sufficient time for the amphiphiles to assemble into large aggregates due to the slow solvent exchange. By contrast, the flow-focusing geometry enabled the continuous mixing of organic solutions with water to generate many nucleates over a wide interface. Rapid solvent exchange prevented the growth of particles due to a lack of supplementary amphiphiles. Ali et al. replaced the flow-focusing geometry with a Y-shaped channel to form parallel flows and prepare monodisperse hydrocortisone particles. The nanoparticles could be varied between 20 and 35 nm by adjusting the acetonitrile-to-water flow ratios and the polymer concentration, as shown in Figure 11b. The size distribution narrowed relative to the distribution obtained by bulk mixing, as shown in Figure 11c. The efficiency of drug encapsulation was slightly enhanced relative to the efficiency achieved from the sample prepared by bulk mixing. The formation of smaller nanoparticles in a microfluidics device was attributed to continuous mixing. Under bulk mixing conditions, an organic solution is added drop-by-drop to water, which provides sufficient time for the amphiphiles to assemble into large aggregates due to the slow solvent exchange. By contrast, the flow-focusing geometry enabled the continuous mixing of organic solutions with water to generate many nucleates over a wide interface. Rapid solvent exchange prevented the growth of particles due to a lack of supplementary amphiphiles. Ali et al. replaced the flow-focusing geometry with a Y-shaped channel to form parallel flows and prepare monodisperse hydrocortisone nanoparticles.

Figure 11. Controlled precipitation in a microfluidic device. a) Schematic illustration of a microfluidic device with a hydrodynamic flow-focusing geometry for nanoprecipitation. b) Size dependence of the nanoparticles on the flow rate ratio between an organic solution and water. The concentrations of the polymers in the organic solution are reported. c) Size distributions of the nanoparticles prepared either by microfluidic mixing with two different flow ratios or by bulk mixing. Panels (a–c) reproduced with permission. Copyright 2008, American Chemical Society. d) Fluorescence microscopy images of an LNCaP cell with internalized nanoparticles containing poly(lactic acid) (PLA)-treated Pt(IV)-monosuccinate. Cisplatin was released upon degradation of PLA after reducing Pt(IV)-monosuccinate, which formed a complex with nuclear DNA (Pt-GG). This was confirmed by antibody-derived green fluorescence, and the nuclei were stained with blue dye. Panel (d) reproduced with permission. Copyright 2010, National Academy of Sciences, USA. e) Schematic illustration of a microfluidic device with a 3D hydrodynamic focusing geometry for nanoprecipitation. f) Transmission electron microscopy (TEM) images of nanoparticles composed of PLGA-b-PEG with different polymer chain lengths. The polymers with high molecular weights could be used in the 3D focusing device to prepare nanoparticles without clogging. Panels (e,f) reproduced with permission. Copyright 2011, Wiley-VCH. g) Optical microscopy images of a multi-inlet vortex mixer for the fabrication of β-carotene-loaded nanoparticles composed of poly(ethylene oxide) (PEO)-b-polystyrene (PS). h) Size dependence of the nanoparticles on the Reynolds number (Re) at four different mixing ratios of water and the organic solution. Panels (g,h) reproduced with permission. Copyright 2011, Springer.
An aqueous flow and an ethanolic solution of hydrocortisone were used to form laminar streams in a microfluidic channel that yielded precipitation. The nanoparticles were transferred to the water using a stabilizer to avoid additional aggregation.

Multiple distinct drugs with different hydrophobicities could be loaded into the nanoparticles through precipitation, thereby enabling the delivery of multiple chemotherapeutic drugs with different solubilities. The hydrophilic Pt(IV)-monosuccinate was conjugated to PLA, which was encapsulated by the PLGA-PEG nanoparticles, together with the hydrophobic drug, docetaxel, by the nanoprecipitation method. The encapsulation efficiency of the PLA-conjugated Pt(IV) was much higher than that of the hydrophobic Pt produgs. The particles released hydrophilic cisplatin through reduction of the Pt(IV)-monosuccinate upon degradation of PLA in LNCaP cells, as shown in Figure 11d. The released cisplatin forms Pt-d(GpG) complex with the nuclear DNA in the nuclei of LNCaP cells, as confirmed by overlaying the antibody (R-C18)-derived green fluorescence from the Pt-d(GpG) with the nuclei stained with blue dye.

In 2D microfluidic devices, hydrophobic polymers frequently adhere to hydrophobic surfaces and clog the channels. This is a serious problem, particularly for polymers with a high molecular weight. Because polymers with a higher molecular weight are useful for increasing drug loading in particles, this adhesion should be prevented in the design of better drug carriers. Such adhesion may be avoided by using a 3D hydrodynamic flow-focusing geometry for the stable long-term production of nanoparticles in a microfluidic device. The devices composed of a wide channel with three sequential inlets and a cross-junction are shown schematically in Figure 11e. A horizontal laminar flow consisting of three distinct layers formed in the wide channel: a top sheath layer, a polymeric solution layer, and a bottom sheath layer, all of which were laterally confined by the continuous water phase in the flow-focusing geometry. This configuration permitted the 3D flow-focusing of a polymeric solution. The polymeric solution flowed through the center of the channel without contacting the wall, thereby preventing adhesion. The 3D flow focusing was used to continuously produce uniform nanoparticles over a long period of time, as shown in Figure 11f. PLGA with a range of molecular weights was used to evaluate the device. Controlled precipitation was achieved using a vortex mixer with multiple inlets, as shown in Figure 11g. Organic and aqueous solutions were mixed using a whirlpool. The nanoparticle size and drug encapsulation efficiency were controlled by adjusting the Reynolds number. Using this design, Shen et al. produced β-carotene-loaded nanoparticles using either poly(ethylene oxide)-b-polystyrene or PEG-b-pol(ε-caprolactone). Under Reynolds number conditions below 2000, the particle size decreased as the Reynolds number increased. Under Reynolds number conditions above 2000, a vortex formed in the channel and turbulent flow induced homogeneous mixing. Therefore, the nanoparticle size was independent of the flow rate in this range, as shown in Figure 11h. Rapid mixing by vortex enabled the high loading of drugs into the nanoparticles via sudden supersaturation of the drugs and polymers in the mixture. More than 85% of the β-carotene was loaded into the nanoparticles at Reynolds numbers exceeding 2000. By contrast, at Reynolds numbers smaller than 2000, more than 70% of β-carotene precipitated and formed large crystals in the suspension, thereby wasting the drug.

3. Capsule-Type Carriers

The flexibility and functionality of particle-type carriers may be extended through the use of microcapsules consisting of mostly liquid cores and solid shell membrane. The inner compartment of a capsule can be used to encapsulate large amounts of bioactive agents that are then released upon the breakup of the shell membrane. Membrane engineering through optimization of the composition and structure can be used to design impermeable shell membranes, or selectively permeable shell membranes that permit the passage of molecules smaller than a critical size. Techniques for fabricating such microcapsules have been intensively investigated and developed for use as microcarriers. The core–shell geometry of a capsule complicates the fabrication of such structures relative to the fabrication of particle-type carriers. A variety of approaches have been used for preparing particle-type carriers, such as photolithography, micromolding, or imprinting, none of which are applicable to the preparation of capsules without implementing significant modifications to the methods. One approach to producing enclosed membranes is through templating with emulsion drops. Both single- and double-emulsion drops were used to produce microcapsules with solid membranes or vesicles with molecular membranes.

In this section, we first review the formation of microcapsules with solid membranes via interfacial adsorption using single-emulsion drops and via bulk solidification using facile double-emulsion drops. Additionally, we review the production of vesicles with bilayer membranes composed of amphiphilic lipids or block-copolymers using the same single- and double-emulsion templates and pulsed-jetting techniques.

3.1. Microcapsules with Solid Membranes

Microcapsules with solid membrane were prepared using templates of single- or double-emulsion drops. Because single-emulsion drops do not have a core–shell geometry, special physical or chemical treatments are required to prepare membranes at the surfaces of the drops. By contrast, double-emulsion drops intrinsically have a core–shell geometry that enables the direct formation of compartments for separating the core and continuous phases. Certain type of microcapsules with a gel core and a gel shell cannot be templated using double-emulsion drops. The stepwise formation of first a core and then a shell, using a re-injection procedure, has enabled the production of hydrogel capsules with tunable permeability properties.

3.1.1. Capsules Templated by Single-Emulsion Drops

As discussed in the second section, single-emulsion drops are useful templates for producing spherical microgels or particles through polymerization of aqueous precursors or the evaporation of organic solutions from a drop, respectively. In addition to particles, single-emulsion drops can provide templates for
producing core–shell structures through the formation of a membrane at an interface. Solid membranes can be produced in three distinct ways: 1) physical adsorption of materials at an interface, 2) the chemical reaction of two different reactants present in the drops or the continuous phase, or 3) evaporation-induced concentration of a material from beneath an interface. Once a membrane is integrated and locked, the core–shell structures can be transferred into a single phase. W/O emulsion drops are predominantly used for biological applications, and the resultant capsules may be transferred into an aqueous medium. These processes are shown schematically in Figure 12a.

Physical Adsorption of Materials at Interfaces: The free interface between two immiscible fluids can be stabilized by the adsorption of colloids. Colloid anchoring at the interface reduces the area of the high-energy free interface. Therefore, colloids that are adsorbed at the interface between a drop and a continuous phase are strongly trapped and undergo only 2D migration along the spherical interface of the drop. Such 2D motion is limited because colloids tend to be densely packed at an interface. A large number of adsorbed colloids will reduce the total interfacial energy. Therefore, emulsion drops may be armored by a colloidal shell, which provides useful templates for porous membranes. The interlocking of colloids at an interface produces a mechanically stable membrane and preserves the structure, even after phase transfer. Microcapsules with an interlocked colloidal array membrane are called colloidosomes. The colloidal structures may be stabilized via two distinct methods: thermal fusion of the adsorbed particles or adsorption of polymers onto the colloid surfaces. Thermal annealing of polymeric particles arranged in a spherical shell, at a temperature slightly above the glass transition temperature, can induce interfusion of the particles to create a single continuous membrane with a regular array of pores. The pore size can be controlled according to the annealing temperature and annealing time. Higher temperatures and longer annealing times produce smaller pores. For example, colloidosomes annealed for different times of 5 min, 20 min, and 2 h at 105 °C exhibit different pore sizes, as shown in Figures 12b–d. However, thermal annealing can damage encapsulants. A safer method for interlocking colloids involves the adsorption of oppositely charged polyelectrolytes to the surface of the colloid. For example, colloids adsorbed at an interface can present negative charges on the aqueous side, and these colloids may be bridged by the adsorption of positively charged poly-L-lysine. Polymer-assisted stabilization of a membrane enhances the elastic properties of the membrane to create a flexible and deformable membrane, unlike those prepared by thermal annealing.

Figure 12. Capsules templated by single-emulsion drops. a) Schematic illustration of membrane formation at the interface in single-emulsion drops via physical adsorption or chemical reactions. b–d) SEM Images of a colloidosome templated using W/O emulsion drops, which are annealed at 105 °C for b) 5 min, c) 20 min, and d) 2 h. Panels (b–d) reproduced with permission. Copyright 2005, American Chemical Society. e,f) Optical microscopy images of microcapsules with composite film membranes of polymers and colloids. Panels (e,f) reproduced with permission. Copyright 2004, American Chemical Society. g) SEM Images of hollow metal–organic framework capsules prepared using an interfacial reaction between metal ions in water drops and organic ligands in a continuous phase. The inset shows the cross-section of a membrane. h) Release of ethylene glycol from the capsules. Experimental data agreed well with the Fickian diffusion model (dotted line), yielding a diffusivity of 5 × 10⁻¹¹ cm² s⁻¹ in the membrane. Panels (g,h) reproduced with permission. Copyright 2011, Nature Publishing Group.
The resultant colloidosomes show selective permeability according to the size of the material. Materials smaller than the pore size can diffuse through the membrane, whereas materials larger than the pores cannot. For example, colloidosomes composed of polystyrene particles with 1.3 μm in diameter did not permit the diffusion of particles that were 1 μm in diameter, whereas they did allow the diffusion of particles with 100 nm in diameter. This selective permeability is very useful for achieving the immunosolubilization of cells: the porous membrane protects the cells from the immune system, such as from antibodies or white blood cells, while permitting the diffusion of cell-function stimuli, nutrients, and wastes through the membrane, thereby supporting cell viability and functionality. Excess amounts of polyelectrolytes in a water drop can close macropores on a membrane and produce a high osmotic pressure in the core, leading to capsule inflation. Capsule membranes are highly flexible and tough, as shown in Figure 12e, where the membrane is a composite film composed of colloids and polymer, as shown in Figure 12f. The composite membrane was slightly permeable to salt ions and counterions through its very small pores. Therefore, although low salt concentrations in the continuous phase were insufficient to disintegrate the membrane, a high salt concentration above 1 M deflated the capsules and ruptured the membrane, thereby releasing the encapsulants. In addition to the osmotic pressure, the temperature, pH, or solvent may potentially provide useful stimuli for triggering the rupture of the composite membranes, providing the stimulus-triggered release of encapsulants.

Interfacial Reaction-Induced Formation of Membranes: Physical adsorption is driven by interfacial energy reduction and electrostatic attraction, and components of the membrane are stabilized by weak non-covalent bonding. By contrast, reagents that are stored separately in a drop and in the surrounding fluid can react at an interface to form a relatively strong solid membrane with components that are covalently or non-covalently bonded. Therefore, interfacial reactions on a drop can produce core-shell structures without interlocking steps. A variety of chemical reactions have been employed to prepare capsules from monodisperse single-emulsion drops. For example, the membrane of a metal–organic framework (MOF) was generated along the surface of a W/O emulsion drop via coordination bonds between metal ions in the water drop and organic ligands in the continuous oil phase. Specifically, an aqueous solution of copper acetate was emulsified in a 1-octanol solution of 1,3,5-benzenetricarbosylic acid (H₃BTC) in a microfluidic device. Emulsification brought two precursors into contact at the interface, which nucleated and grew a crystalline MOF composed of [Cu₃(BTC)₂] via a ligand exchange mechanism. This reaction led to the formation of a rigid membrane, as shown in Figure 12g. Although the membrane was composed of polyhedron subunits of a crystal structure, the interstices were completely sealed by small crystallites without macropores. Because the [Cu₃(BTC)₂] crystal lattices include microchannels, small molecules could diffuse through the membrane, whereas large molecules could not, thereby providing selective permeability. For example, ethylene glycol (MW 62.07 g/mol) was released from the core of the capsules, as shown in Figure 12h, whereas a large dye molecule, Rose Bengal (MW 973.67 g/mol), remained encapsulated without release. The diffusivity of ethylene glycol through the membrane was estimated to be 5 × 10⁻¹¹ cm²s⁻¹ based on a Fickian diffusion model.

Similarly, inorganic microcapsules may be produced via a sol-gel reaction at the interface in O/W emulsion drops. Although most sol-gel reaction precursors (metal alkoxides) are highly reactive with water, thereby complicating the use of O/W drops for membrane formation in microfluidics platforms, chemical modifications of the precursors with a long acid chain can reduce the rate of hydrolysis to enable the usage of water as a continuous phase. For example, titanium n-butoxides (TBT) in octanoic acid can be used to prepare monodisperse oil drops in a surfactant-laden water phase in a microfluidic device, while avoiding clogging. The carboxylates in the octanoic acid reduce the reactivity of TBT by chelating to regions of the coordination sites. Emulsification brings the TBT into contact with the continuous water phase to form TiO₂-Ti linkages at the interface and, therefore, a TiO₂ membrane. The inner oil phase can be removed by washing with ethanol or calcining at high temperatures. Quantum dots or iron oxide nanoparticles may be embedded in the TiO₂ membrane by dispersion in the oil phase prior to emulsification. The incorporation of nanoparticles provides optical or magnetic functionalities to the biocompatible inorganic capsules for imaging and targeting in biological applications while avoiding direct contact between the toxic nanoparticles and the immediate cellular environment.

Evaporation-Induced Formation of Membranes Near the Interface in a Single Emulsion: Previous approaches to the production of core–shell structures from single-emulsion drops have used interfacial adsorption or reactions to prepare a shell at the interface. The capsule size is then nearly identical to the size of the drops. By contrast, evaporation of a drop can induce the accumulation of materials below the interface of the drops, thereby forming a membrane. For example, Zhang et al. reported the production of monodisperse microcapsules using supramolecular host–guest chemistry by evaporating W/O drops. Host molecules, Cucurbit[8]uril (CB[8]), form dynamic complex with two guest molecules—electron-deficient methyl viologen (MV²⁺) and electron-rich naphthol (Np) derivatives. The host and guest molecules were separately injected into a T-junction and emulsified into water drops in fluorinated oil. As the drops passed through a tortuous channel, the components mixed to form a 1:1:1 ternary complex of the host and two guests in the water drops through non-covalent interactions. The water drops subsequently evaporated and shrank to form microcapsules consisting of a water core and shell made of an accumulated ternary complex. After complete evaporation of both the oil and the water, the capsules were dispersed in water. The resultant membranes were porous and provided selective permeability. The capsules retained large molecules, such as fluorescein isothiocyanate (FITC)-dextran, with a molecular weight of 500 000, whereas FITC-dextran with a molecular weight of 10 000 freely diffused through the membrane. The membrane complexes could be disintegrated by the one-electron reduction of electron-deficient guest molecules to enable the on-demand release of encapsulants. Such release occurred upon dispersal in an aqueous solution containing sodium dithionite (Na₂S₂O₄) under a nitrogen atmosphere.
3.1.2. Capsules Templated using Double-Emulsion Drops

Single-emulsion drops provide templates for producing core–shell structures; however, these emulsions are limited in their applicability to certain sets of materials and involve complex production steps due to the need for phase transfer. Such transfers are not desirable in most biological applications. To overcome these shortcomings, double-emulsion drops, drops-in-drops, have been prepared and employed to fabricate microcapsules in a more controlled and versatile fashion. The intrinsic core–shell geometry enables the direct production of capsules through solidification of a middle phase, as shown schematically in Figure 13a. Solidification can be achieved in one of three distinct ways: 1) polymerization, 2) freezing, and 3) evaporation-induced consolidation. Unfortunately, double-emulsion drops are more difficult to produce than single-emulsion drops. Double-emulsion drops are unstable with respect to the coalescence of the inner drops with the continuous phase, and the emulsions frequently experience structural rupture prior to completing the solidification steps. Therefore, careful investigations of the production and stabilization of double-emulsion drops are needed before the technique may be more widely adopted.

Microfluidic Approaches to the Production of Double Emulsions: Double-emulsion drops can be prepared via two distinct methods in PDMS or capillary microfluidic devices: one-step emulsification or sequential emulsification. These approaches are complementary. A series of two single drop makers enables the production of double-emulsion drops via two sequential emulsifications. Monodisperse single drops are generated in a first drop maker, and these drops are injected into a second level of single drops at a second drop maker. Water-in-oil-in-water (W/O/W) double-emulsion drops may be prepared using this sequential emulsification by modifying the microfluidic device channel surfaces to be hydrophilic in the first junction and hydrophobic in the second junction, to avoid wetting problems. The opposite modification is required for preparing inverse O/W/O double-emulsion drops. Sequential emulsification enables independent control over the frequencies of drop generation in the first and second junctions, thereby providing for the precise manipulation of a number of core drops in each shell drop, although delicate control over the flow rates is usually required. Multiple-emulsification drops can be generated by increasing the number of drop makers in the same fashion. For example, quintuple-emulsion drops may be prepared by five sequential drop makers. The device fabrication entails complex procedures due to the need for surface patterning, and full control over the relative diameters of the core and shell are difficult to achieve because the dispersed phases are used as the continuous carrier fluid of the inner drops prior to the sequential emulsification.

Figure 13. Capsules templated by double-emulsion drops. a) Schematic illustration of capsule formation via solidification of the middle layer of a double-emulsion drop. b) Capillary microfluidic device for the generation of water-in-oil-in-water (W/O/W) double-emulsion drops through sequential emulsification and UV illumination for in situ photopolymerization of a middle layer. c) Optical microscopy image of monodisperse microcapsules containing crystalline colloidal arrays in aqueous cores. The inset shows the broken membranes of capsules. Panels (b,c) reproduced with permission. Copyright 2008, American Chemical Society. d) The release behavior of encapsulants from microcapsules with membranes prepared by freezing wax oil from W/O/W double-emulsion drops. The membrane thawed upon heating, leading to coalescence among the inner water drops with the continuous phase. The encapsulants in the core were thereby released. Panel (d) reproduced with permission. Copyright 2010, American Chemical Society. e) Capillary microfluidic device for the generation of W/O/W double-emulsion drops with an ultrathin middle layer. f,g) Confocal microscopy images of microcapsules with ultra-thin and biodegradable membranes. The inset of (g) shows a cross-section of the membrane. h) Fraction of unreleased capsules. The capsules released encapsulants as the membrane was degraded by hydrolysis. The insets show confocal microscopy images of capsules collected 39, 55, or 71 days after capsule production. Panels (e–h) reproduced with permission. Copyright 2011, Royal Society of Chemistry.
single channel, and the stream is simultaneously broken into double-emulsion drops. This approach obviates the need for sequential emulsification and provides a high degree of control and stability in the generation of double- or multiple-emulsion drops using an even simpler device design. Robust device operation is easy to achieve, and very thin shells can be produced. One drawback is that this method does not yet permit control over the number of innermost drops, unlike sequential emulsification approaches. Therefore, one-step and sequential emulsifications are complementary to each other, and the method is selected according to the ultimate purpose.

Membrane Formation through Polymerization of a Middle Layer in Double Emulsion: Polymerization provides a simple way to solidify the middle phases of double-emulsion drops. In a middle phase consisting of monomers or pre-polymers and initiators, the precursors undergo polymerization upon activation of the initiator to prepare a crosslinked polymeric network in the middle phase. Depending on the type, we can use photo- or thermoinitiators to induce photopolymerization through UV illumination or thermal polymerization through temperature increases. In many cases, photopolymerization is employed to induce rapid and facile solidification without destabilizing the double-emulsion drops. In situ polymerization can be achieved by illuminating the drops flowing through the channel with UV, which reduces the interval between drop formation and polymerization and prevents rupture of the double-emulsion drop prior to solidification. As an example, Kim et al. reported the encapsulation of a colloidal suspension in a polymeric membrane using the in situ photopolymerization of monomers in the middle phase of double-emulsion drops, as shown in Figure 13b. W/O/W double-emulsion drops were produced via sequential emulsification in a capillary microfluidic device, the middle phase of which included ethoxylated trimethylolpropane triacrylate (ETPTA) that was rapidly polymerized upon UV exposure in the downstream region of the device. The resultant membrane was composed of crosslinked dense polymers that enhanced the physical rigidity and chemical resistance relative to most membranes prepared using single-emulsion drop templates. Importantly, the membrane was impermeable to small molecules or ions, although water molecules could pass through it. The low degree of membrane porosity was difficult to achieve using single-emulsion drop templates. Therefore, this approach provided a high encapsulation efficiency and long-term storage without loss of materials. For example, the colloidal suspension encapsulated in the capsules showed a diffraction pattern due to the regular arrangement of colloids induced by the repulsive interparticle potential, as shown in Figure 13c. This pattern was preserved even after the capsules were dispersed in an aqueous NaCl solution because the membrane did not permit diffusion of the sodium ions into the interior of the capsules. In these rigid capsules, the encapsulants could be released by mechanical rupture of the membrane. A broken capsule is shown in the inset of Figure 13c. Photopolymerization of a gel precursor in the middle phases of O/W/O double-emulsion drops, that is, microcapsules consisting of an oil or water core and a hydrogel shell, could be prepared through an additional phase transfer step into water. For example, acrylamide or n-isopropylacrylamide was used as the monomer in an aqueous middle phase. The monomers were polymerized under UV illumination to prepare the hydrogel shell. Subsequently, both the inner and the continuous oil phases (or only the continuous oil phase) were replaced with water. Although photopolymerization of the monomers can prepare a hydrogel network, rapid free-radical polymerization frequently leads to a heterogeneous morphology. To overcome this problem, photocrosslinkable polymer precursors were synthesized and used as a gel phase in place of the monomers. For example, pNIPAAm functionalized with reactive side groups, dimethylmaleimide, could be homogenously gelled by UV illumination for any reaction rate, thereby enabling the production of gel capsules with a high reproducibility.

Thermal polymerization may also be used to solidify the middle phase; however, high temperatures are not favorable in emulsion systems due to the decreased stability of the emulsion. Double-emulsion drops are more unstable than single-emulsion drops, and coalescence among the inner drops into coacervate phase is favored when the middle phase is slightly elevated temperatures. Instead of a temperature increase, reaction accelerators can be used to induce the relatively fast polymerization of monomers at room temperature. For example, n-isopropylacrylamide in aqueous drops containing a thermo-initiator, such as ammonium persulfate (APS) or potassium persulfate (KPS), can be polymerized by the slow diffusion of a reaction accelerator, such as N,N,N′,N′-tetramethylethylenediamine (TEMED), from a continuous phase to prepare pH-sensitive drug carriers.

In a similar fashion, a variety of gel precursors, such as alginate or chitosan, can be employed in the aqueous middle phase of O/W/O double-emulsion drops to prepare capsules. For alginate capsules, O/W/O double-emulsion drops are prepared and injected into a single channel, together with an aqueous solution of CaCl2. As soon as the double-emulsion drops contact the aqueous solution, calcium ions diffuse into the middle phase and rapidly polymerize the sodium-alginate precursors to form calcium-alginate without rupturing the core–shell structure. The alginate capsules are then dispersed in a water phase in the form of an oil core and a hydrogel shell, which is potentially useful for delivering lipophilic drugs. In chitosan capsules, an oil phase containing terephthalaldehyde may be used to form the innermost drops of O/W/O double-emulsion drops, and a water phase containing chitosan may be used to form shell drops. As terephthalaldehyde diffuses from the innermost drops into the shells, the chitosan molecules in water crosslink in the neutral medium to form a membrane at the inner O/W interface. This crosslinking is a reversible process. Although the chitosan membrane is stable under neutral conditions, it decomposes under acidic conditions, thereby releasing the encapsulating materials. The biocompatible chitosan capsules are potentially useful as pH-sensitive drug carriers.

Selective Freezing of the Middle Phase: Without chemical reactions or gelation, the middle phase of a double-emulsion drop can be selectively solidified by freezing if the middle phase has a higher freezing point than the innermost and continuous phase. For example, waxes, such as paraffin, melt above approximately 45 °C. W/O/W double-emulsion drops can be prepared in a microfluidic device at temperatures higher than the melting point. The middle molten wax phase may be selectively frozen by cooling the double-emulsion drops down
to room temperature, thereby forming a liquid core and a stable solid membrane. The resultant capsules retained their encapsulants without loss at room temperature for a few months. The encapsulants could be released upon the temperature-triggered bursting of the membrane. Heating the capsules above the wax melting point liquidized the solid membrane, leading to movement of the inner water drop within the outer wax drop. As the inner interface met the outer interface, the inner drop coalesced with the continuous phase to release the encapsulants. This process of temperature-induced release is shown in Figure 13d. Although this approach provided a simple tool for encapsulating and triggering release, several limitations are apparent. Operating microfluidic devices at high temperatures requires special equipment, and controlling the flow of highly viscous molten wax is difficult. Also, the materials used in the middle phase are limited to waxes or thermo-sensitive gels.

Evaporation-Induced Consolidation: A variety of microcapsules can be produced by the evaporation-induced consolidation of a middle phase in W/O/W double-emulsion drops. A volatile oil phase containing polymers or colloids preferentially evaporates, concentrating and finally consolidating the materials. The core diameter of a capsule is maintained, while the shell becomes thinner than the initial middle layer of the double-emulsion drops due to shrinkage during evaporation. Evaporation-based solidification approaches are applicable to any type of polymer or colloid as long as dispersion in organic solvents is possible. By contrast, polymerization or freezing-based approaches are limited to certain membrane materials. For example, ethyl acetate containing ethyl cellulose as the middle phase of W/O/W double-emulsion drops can be used to prepare microcapsules consisting of a large water core and an ethyl cellulose membrane through evaporation of ethyl acetate. Some drawbacks of this method include the low stability of double-emulsion drops due to coalescence of inner drops into a continuous phase. Coalescence frequently leads to rupture prior to the completion of evaporation, which tends to require longer times than other approaches.

The stabilities of double-emulsion drops can be enhanced by reducing the thickness of the middle layer until reaching the lubrication regime. Thin shells strongly confine the inner drops, dramatically reduce the migration velocity, and, therefore, reduce the probability of coalescence. In addition, if the middle layer is very thin, complete evaporation requires a shorter time, thereby improving the attractiveness of this template for capsule production through evaporation-based solidification. Double-emulsion drops with thin shells were produced by Kim et al. by emulsifying a biphasic flow in a capillary microfluidic device, as shown schematically in Figure 13e. When two immiscible fluids flow through a single capillary channel, the fluid with a lower affinity to the wall flows through the center of the channel without contacting the wall, whereas the other fluid flows along the wall. Core–sheath biphasic flows form either a jet or a train of drops, depending on the diameter of the channel, the flow rates, the viscosities, and the interfacial tension. Both jets and drops may be emulsified into a third fluid at the tip of a single capillary channel, leading to the formation of double-emulsion drops consisting of a large core and an ultra-thin shell. A typical shell thickness is a few hundred nanometers. Using double-emulsion drops as templates, microcapsules with ultra-thin membranes could be produced by the rapid evaporation of the middle phase without rupturing the core–shell structure. For example, a toluene solution containing biodegradable polymers, such as PLA or PLGA, could be used as the oil phase. Evaporation of the toluene will then decrease the shell thickness to form a polymeric membrane with sub-100 nm thickness. All materials dissolved in the inner drops were encapsulated by the ultrathin membrane without loss. Small green dye molecules, 8-hydroxy-1,3,6-pyrenetrisulfonic acid, trisodium salt (MW 524.4 g/mol) were encapsulated, as shown in Figure 13f,g, where the PLA membrane was doped with the hydrophobic red dye, Nile red. The membrane thickness was characterized by SEM to be 80 nm, as shown in the inset of Figure 13g. As the membrane degraded via hydrolysis of the ester groups in the PLA chains, the capsules released the encapsulants, as shown in Figure 13h. The release occurred over a period of 70 days at room temperature. The release rate could be further controlled by the thickness of the membrane, the molecular weight, and the type of polymer. Long-term release is highly desirable in a wide range of delivery applications because it obviates the need for repeated injections of a drug. Although PLA is not sensitive to external stimuli, such as the temperature or pH, microcapsules prepared with ultrathin PLA membranes are highly responsive to osmotic pressure differences across the membrane. Because dense PLA membranes are semipermeable, osmotic pressure differences due to ion or polymer concentration differences between the aqueous core or surrounding fluid induce water flux through the membrane. Under conditions in which the osmolarity of the surrounding fluid is higher than that of the core, capsules will deflate under the outward flow of water. Large pressure differences can then break up the membrane at a highly folded region, thereby releasing the encapsulants. By contrast, a high osmolarity in the core induces the inward flux of water, which inflates the capsules. A fully inflated PLA capsule will release its encapsulants due to the formation of a nanopore upon lateral extension of the membrane. Therefore, microcapsules with ultrathin membranes can encapsulate any water-soluble material without loss under negligible osmotic pressure differential conditions, and the release of the encapsulants can be triggered by applying a positive or negative osmotic pressure.

Porous Membranes: Dense membranes can encapsulate small molecules without leaking and release them as the membranes are ruptured or degraded. By contrast, membranes with regular pores can encapsulate molecules larger than their pores while permitting the diffusion of molecules smaller than their pores across the membrane. The permeability of small molecules depends on the relative sizes of the molecules and the pores, the structure or connectivity of the pores, and the thickness of the membrane. These factors control the rate at which small molecules diffuse across the membrane. Selective permeability is potentially useful for the immunoisolation of cells, and the sustained release of encapsulants is very important in drug delivery systems. Regular pores on a membrane may be formed by preparing colloidsomes via the adsorption and fixation of colloids at the interfaces in single-emulsion drops. However, spherical crystal colloidal monolayers can have defects or vacancies that can form an aberrant pore that is larger than the other regular
pores, thereby increasing the critical size for selective permeability. In addition, the fabrication of regular pores involves complex and delicate procedures, such as mechanical stabilization and phase transfer, which frequently damage the particle structures. To address the issues associated with single-emulsion approaches to forming regular pore structures for semi-permeable membranes, colloidosomes with membranes that are composed of silica nanoparticle multilayers were prepared using a double-emulsion approach.\cite{72} The volatile organic middle phase of the W/O/W double-emulsion drops contains silica nanoparticles, and the particles stabilize both the inner and outer interfaces by adsorption. Evaporation of the organic solvent results in the packing and consolidation of the nanoparticles; after complete evaporation, the interstices of the assembled nanoparticles fill with water to create interconnected pores between the interior and exterior of the capsules, as shown schematically in Figure 14a. The pore size was determined to be approximately 5% of the particle size for randomly closed-packed spheres; therefore, structural defects do not significantly affect the permeability of the membrane or the critical size for selective permeability. The selective permeability of the membrane was confirmed using two fluorescence probes: calcein (MW 622.55 g/mol) and FITC-dextran (MW 2,000,000 g/mol). Calcein dissolved in the continuous phase freely diffused through the membrane, whereas FITC-dextran remained in the continuous phase, as shown in Figure 14b,c. The surface morphology of the capsule membrane is shown in the inset of Figure 14b.

Figure 14. Three distinct approaches to producing microcapsules with selective permeability using double-emulsion drops. a) Formation of a colloidosome via the assembly of nanoparticles in the middle phase of W/O/W double-emulsion drops upon evaporation. b,c) Confocal microscopy images of colloidosomes dispersed in aqueous solutions of b) a low molecular weight fluorescence probe, calcein, or c) a high molecular weight probe, fluorescein isothiocyanate (FITC)-dextran (MW = 2,000,000 g/mol), respectively. The small probes freely diffused through the interstices between nanoparticles in the membrane, whereas the large probes remained in the continuous phase. The inset of (b) shows the surface morphology of the membrane. Panels (b,c) reproduced with permission.\cite{72} Copyright 2008, Wiley-VCH. d) The formation of a porous membrane templated by W/(W/O)/W double-emulsion drops, in which small water drops in the middle oil phase produced pores on the membrane during evaporation of the organic solvent. e,f) Fluorescence microscopy images of e) W/(W/O)/W double-emulsion drops and f) the corresponding capsule containing an aqueous solution of FITC–dextran (MW 20,000 g/mol) in the core after evaporation. The dextran molecules diffused through the pores on the membrane. The images were collected at the indicated times after drop generation. g,h) Fluorescence microscopy images of g) a W/O/W double-emulsion drop and h) the corresponding capsule containing an aqueous solution of FITC–dextran (MW 20,000 g/mol) in the core. Panels (e–h) reproduced with permission.\cite{72} Copyright 2009, Wiley-VCH. i) Formation of microcapsules with perforated membranes templated using nonspherical double-emulsion drops with multiple inner drops. Removal of the colloids produced regular pores on the membrane which interconnect the inner drops with the continuous phase. j) Optical microscopy image of microcapsules with 8 compartments in a snub disphenoid configuration. k) SEM image showing a cross-sectional image of the perforated membrane. Panels (j,k) reproduced with permission.\cite{74} Copyright 2011, Wiley-VCH.
Although evaporation-induced emulsion assembly in the middle phase of the double-emulsion provided a facile approach to preparing porous membranes without additional steps for pore generation, the resultant structures were mechanically fragile due to the discontinuous structure of the colloidal assemblies. The stability of the capsules was improved by preparing a continuous membrane perforated by pore-templating materials. For example, Choi et al. used a volatile middle oil phase containing polymers as a membrane material and small water drops as the pore templates. The small water drops were prepared by homogenization prior to generating the W/O/W double-emulsion drops in the capillary microfluidic device.

As the volatile solvent evaporated, the polymers and the small water drops were consolidated together to form a polymeric membrane that embedded the small water drops, as shown schematically in Figure 14d. The water drops formed interconnected channels that linked the interior and the exterior of the resultant capsules, yielding permeable membranes. The resultant capsule, which originally encapsulated FITC-dextran (MW 20,000 g/mol) in the water core, released the encapsulants after the organic solvent had fully evaporated, as shown in Figure 14e,f. By contrast, the capsule prepared from W/O/W double-emulsion drops without small water drops in the middle phase, retained the FITC-dextran, even after the organic solvent evaporated, as shown in Figure 14g,h. This proved that the small water drops templated the interconnected pores.

The water drops can be replaced with colloids to template the pore generation if the colloidal size exceeds the thickness of the membrane and the colloids are exposed to both the interior and the exterior of the capsule. Colloids can produce monodisperse regular pores, which is difficult to achieve using small water drops. To this end, Kim et al. used W/O/W double-emulsion drops with multiple inner drops covered by a small volume of a middle phase as the capsule templates, as shown in Figure 14i.

The inner drops were densely packed due to the capillary forces exerted by the middle phase of the nonspherical envelope, which drained the middle phase from the overhanging surface of the inner drops. The middle phase, therefore, formed a very thin film. For example, the microcapsules templated by double-emulsion drops with 8 innermost drops are shown in Figure 14j. The 8 drops were densely packed into a smub disphenoid configuration. A photo-curable suspension containing 900 nm hydrophobic silica particles was used as the middle phase. The particles became trapped in the thin film, exposing one part of the particles to the inner drops and the other part to the continuous phase. The resulting thin film was therefore perforated by the selective removal of silica particles, as shown schematically in Figure 14i. The pore size was determined by the templating particle size and film thickness. A cross-sectional SEM image of the perforated membrane is shown in Figure 14k, demonstrating that the hydrophobic particles were positioned at the center of the film.

Another possible strategy for producing a perforated membrane involves microphase separation of the block-copolymers in the capsule membranes. Microphase separation can induce a variety of regular structures and microdomains, depending on the block ratio. In particular, perforated lamellae, gyroid, and double diamond structures are promising for the generation of regular and complex channels for material transport due to the connectivity of one or both blocks. In such structures, selective degradation or etching of one block will produce an interconnected complex of pores on the microcapsules. To the best of our knowledge, this technique for generating pores has only been reported in the context of films.

3.1.3. Capsules Prepared by Microfluidic Re-Injection

Double-emulsion drops have provided useful templates for producing a variety of microcapsules due to their core–shell geometry. The preparation of double-emulsion drops requires that the inner and middle phases are immiscible, thereby forming a clear interface. Double-emulsion drops are not always useful templates, especially for certain types of capsules. For example, microcapsules composed of a non-thermo-responsive hydrogel core and a thermo-responsive hydrogel shell could not be produced using double-emulsion drops; rather, they were prepared through the stepwise formation of a core and a shell. In this work, poly(acrylamide) (pAAm) gel particles are prepared using W/O emulsion drops to form a non-responsive core of the capsules using conventional methods discussed in the second section. The gel particles were then transferred to water and re-injected into the second level of O/W emulsion drops in a microfluidic device, as shown schematically in Figure 15a, where the water drops contained crosslinkable pNIPAAm. Although the core gel particles were highly swollen and porous, the pNIPAAm could not diffuse into the interior of the gel particles due to the large molecular weight. Therefore, pNIPAAm formed a distinct shell layer encapsulating the pAAm core upon UV illumination to produce microcapsules consisting of a non-thermo-responsive hydrogel core and a thermo-responsive hydrogel shell. The microcapsule displayed shrinkage properties that differed from those of conventional microcapsules consisting of a water core and a thermo-responsive hydrogel shell. The conventional pNIPAAm shells collapsed into the smaller shells as the temperature was increased above the LCST, thereby reducing the diameters of both the core and the shell. By contrast, the shell thickness of the new capsule decreased while the diameter of the core was retained. This capsule provided a larger volume for material encapsulation. Because the permeability of the pNIPAAm shell could be dynamically tuned according to the temperature, the capsule encapsulated and released water-soluble materials in a reversible fashion. The pNIPAAm shells swelled and were porous at temperatures below the LCST, whereas the shell became deswollen and formed a dense state at temperatures higher than the LCST. For example, rhodamine isothiocyanate (RITC)-dextran (MW 10,000 g/mol) could diffuse through the swollen pNIPAAm shell into the pAAm core at room temperature. A subsequent increase in the temperature collapsed the shell to reduce the impermeability for dextran. The capsules retained dextran as long as the temperature was maintained. Release of the encapsulants was triggered by reducing the temperature below the LCST. As shown in Figure 15b, the capsules were maintained above the LCST for the first 10 s, then they were cooled below the LCST. The time-dependence of the fluorescence intensity was measured in the core and surrounding fluid, as shown in Figure 15c. The fluorescence intensity in the core decreased continuously due to the release of the encapsulants. Simultaneously, the fluorescence intensity...
in the surrounding fluid increased immediately after the rapid release, then decreased due to diffusion and dilution by the large volume of the continuous phase.

3.2. Vesicles

Vesicles are microcompartments enclosed by bilayers of amphiphilic molecules, such as lipids or block-copolymers. Vesicles composed of lipids are called liposomes, and vesicles composed of block-copolymers are called polymersomes. Lipid bilayers have a typical thickness of a few nanometers, whereas block-copolymer bilayers have a typical thickness in the range of a few nanometers to tens of nanometers, depending on the molecular weight of the block-copolymer. Polymersome capsules are relatively durable compared to liposomes. The large molecular weights of synthetic amphiphiles in polymersomes result in a low permeability, a low lateral mobility, and a high stability; however, both liposomes and polymersomes form very thin layers compared with the solid capsules discussed above. Vesicles are highly fragile and very sensitive to osmotic pressure. Nevertheless, vesicles are important materials for a wide range of applications, from fundamental studies of cell function to drug delivery vehicles, and they have several advantages over solid microcapsules. First, vesicle membranes are identical or similar to biological cell membrane and provide useful platforms for studying cell functions. Subunits of the membranes, such as lipids or block-copolymers, are biocompatible and yield the in vivo delivery of bioactive agents without a leakage. Moreover, functionalization of the subunits enables the targeted delivery or triggered release of encapsulants. Conventional approaches to preparing vesicular structures, such as electroformation or bulk hydration of dried amphiphiles, has achieved only limited control over their size and structure. Materials dissolved in a continuous phase are randomly encapsulated during vesicle formation in conventional methods, leading to a low efficiency of encapsulation. Recent advances in microfluidics have enabled the production of vesicles with more control over the vesicle size, structure, and encapsulation efficiency. In this part, we review and discuss three distinct microfluidic approaches for the controlled production of vesicles: 1) single-emulsion templating, 2) double-emulsion templating, and 3) microfluidic jetting.

3.2.1. Vesicles Templated using Single-Emulsion Drops

Vesicles are composed of a bilayer of amphiphiles. The hydrophilic regions of the amphiphiles are exposed to the inner and outer aqueous environments, whereas the hydrophobic regions are hidden inside the bilayer to minimize exposure to the surrounding water. W/O single-emulsion drops cannot directly produce vesicles because they hold only a monolayer
formation and second centrifugation steps to independently control the formation of inner and outer monolayers in the bilayer, resulting in an asymmetric bilayer membrane. The asymmetric properties were confirmed by fluorescence quenching of the fluorophores bound to either the inner leaflet or the outer leaflet. An energy-transfer quenching agent in the continuous phase only reduced the fluorophore fluorescence on the leaflet of amphiphiles at the interface. An additional monolayer on the surface of a single-emulsion drops may be formed by passing the drops through an O/W interface saturated with a monolayer of the amphiphiles, as shown schematically in Figure 16a. As the amphiphile-stabilized W/O drops are forced to pass through the O/W interface under a centrifugal force, the interface deforms into a spherical shape by wrapping the drops with an additional monolayer. This process is governed by the balance between the gravitational forces on the drops and the capillary forces exerted by the deformed interface. Finally, the drops may be transferred to the water phase, with a bilayer membrane formed on their surfaces. The same method was used to prepare an asymmetric bilayer membrane by applying two different amphiphiles to the W/O emulsion drops and the O/W interface, respectively. The asymmetric vesicles resembled cell membranes and were more stable than symmetric vesicles. The role of an asymmetric cell membrane remains an open question; therefore, the controlled synthesis of asymmetric membranes will provide key insights into cell function.

Monodisperse vesicles have been prepared using the single-emulsion approach in microfluidic devices. Briefly, monodisperse W/O emulsion drops were generated in a microfluidic device, and were subsequently centrifuged to force passage through an O/W interface. In the second process, careful control over the centrifugal force was important for preparing vesicles with sizes identical to the sizes of the templating drops. A low centrifugal force formed drops that were concentrated at the top of the interface without a bilayer because the force was insufficient to deform the interface. By contrast, a very high centrifugal force broke the drops into smaller drops on the top of the interface and broadened the size distribution, producing a smaller average particle size. The size distribution of the emulsion drops was characterized by an average size of 11 μm, and the vesicles prepared at different centrifugal forces (RCFs) are shown in Figure 16b. An RCF of 1.5 kG produced vesicles with a size distribution identical to that of the emulsion, whereas higher RCFs produced vesicles that were smaller and more heterogeneous in size. At lower RCFs, the small drops could not pass through the interface due to an insufficient force, resulting in the selective formation of large vesicles. The templating drops and the vesicles obtained at the optimum RCF are shown in Figure 16c,d, respectively. Hu et al. used a similar microfluidic approach to prepare asymmetric vesicles. Two different lipids were used in the first drop formation and second centrifugation steps to independently control the formation of inner and outer monolayers in the bilayer, resulting in an asymmetric bilayer membrane. The asymmetric properties were confirmed by fluorescence quenching of the fluorophores bound to either the inner leaflet or the outer leaflet. An energy-transfer quenching agent in the continuous phase only reduced the fluorophore fluorescence on the leaflet.
the outer leaflet. Vesicles with biotinylated lipids on the outer leaflet were fixed to the avidin-coated substrate due to biotin–avidin binding, whereas the vesicles with biotinylated lipids on the inner leaflet were not fixed. These results demonstrated the successful formation of an asymmetric bilayer using a single-emulsion approach. Although this approach enabled the formation of an asymmetric bilayer, two separate steps: microfluidic emulsification and centrifugation in a test tube, complicated the production of vesicles and increased the sensitivity of the process. Matosevic et al. integrated the two separate steps into a single microfluidic device, as shown in Figure 16e. The device included three parts: 1) a cross-junction, 2) a Y-shaped channel, and 3) a triangular post and oil skim channel. In this device, monodisperse W/O emulsion drops were produced in the cross-junction and subsequently injected through one branch of the Y channel. A water phase was flowed through the other branch of the Y channel to form parallel flows of the emulsion and the water in a single channel. As the emulsion drops arrived at the triangular post, they were forced to migrate toward the water phase due to the channel geometry, thereby creating monodisperse vesicles through phase transfer. The vesicles were produced in the device without the need for an additional centrifugation step. This integrated device produced vesicles with sizes nearly identical to the emulsion drop size and provided an encapsulation efficiency as high as 80%, as shown in Figure 16f.

Monodisperse vesicles may also be obtained using single-emulsion drops without centrifugation steps. For example, monodisperse W/O emulsion drops were prepared in a continuous oil phase of oleic acid containing phospholipids using a microfluidic device, and these emulsion drops were then injected into a mixture of water and ethanol. Because oleic acid rapidly dissolved in ethanol, the excess oil was easily removed. Lipids from the oleic acid migrated onto the surfaces of the water drops to form a bilayer surface membrane. As in the bulk hydration method, monodisperse vesicles could be obtained using frozen water drops. Monodisperse W/O emulsion drops were selectively frozen at −4 °C to −7 °C. An oil phase containing amphiphiles was dried to form a film of randomly stacked lipids that embedded the frozen water drops. Hydration of the lipid film induced the frozen water drops to melt and form vesicles with an uncontrolled number of stacked bilayers.

3.2.2. Vesicles Tempted by Double-Emulsion Drops

Single-emulsion drops have one spherical interface and, therefore, an additional interface is required to form a bilayer. By contrast, double-emulsion drops have two concentric interfaces that permit the direct formation of a bilayer. Unilamellar membranes may be formed if the two interfaces are occupied by amphiphiles and overlap without additional lipids or impurities between the interfaces. Evaporation of an organic solvent in middle phase containing an excess of lipids will result in the formation of multiple lipid bilayers with poor controllability. Overlap of the inner and outer leaflets is accomplished through dewetting of the middle phase on the innermost drop surface. When the middle oil phase consists of a mixture of highly volatile good solvents for amphiphiles and a less volatile poor solvent, double-emulsion drops exhibit dewetting of the middle phase containing the amphiphiles during evaporation. Initially, the inner and outer interfaces are fully occupied by amphiphiles prior to evaporation, thereby maintaining adouble-emulsion structure. As the good solvent preferentially evaporates, the poor solvent concentration increases. The poor solvent is expelled from the dense array of amphiphiles at the inner and outer interfaces, resulting in overlap between the two leaflets in the bilayer to produce an acorn shape. The dewetted drops form bulbs on one side of the inner drop surface that detach from the drops to form unilamellar vesicles. The detached oil drops separate automatically from the vesicles due to density differences. Sometimes, the bulbs remain on the surfaces and form small patches on the vesicle surfaces, depending on the type of poor solvent employed. The process of vesicle formation is shown in Figure 17a. Dewetting in W/O/W double-emulsion drops is illustrated in a series of optical microscopy images in Figure 17b, which were collected at intervals of 1 μm, where a mixture of chloroform and toluene containing biocompatible and biodegradable diblock-copolymers PEG-b-PLA is used as the middle phase. This double-emulsion approach provided highly uniform vesicles, as shown in Figure 17c. Any water-soluble material could be encapsulated in the vesicles with a nearly 100% encapsulation efficiency. A confocal microscopy image of the PEG-b-PLA polymersomes containing a red dye is shown in Figure 17d. The same approach was used to prepare liposomes by replacing the block-copolymers with lipids in the middle phase. The double-emulsion approach is more practical and useful for preparing vesicles by comparison with a single-emulsion approach. As a practical application of vesicles produced using double emulsion, Martino et al. expressed a membrane-related bacterial protein, MreB, by encapsulating the biological machinery for protein expression into a polymersome. Microfluidic production of polymersomes provided a 100% encapsulation efficiency of the protein expression machinery. The high stability of the polymersome membrane enabled the robust encapsulation and incubation of encapsulants during protein expression. The resultant proteins could be released under a negative osmotic pressure that inflated the polymersomes and resulted in a hole in the membrane; the negative osmotic pressure is generated by dispersing the polymersomes in distilled water, which induces higher osmotic pressure in the interior of the polymersomes.

Double-emulsion drops with multiple inner drops transform into polymersomes with multiple compartments through dewetting of the middle phase from the surfaces of the inner drops. For example, double-emulsion drops with two inner drops can transform into polymersomes with a dumbbell shape, as shown in Figure 17e–g. Two innermost drops were independently injected during the formation of double-emulsion drops to encapsulate two distinct materials in their cores. As the number of innermost drops exceeded two, a variety of polymersome configurations were observed, in which the number of cores was determined by the number of inner drops. For example, three cores could be connected in a linear or triangular configuration. Such variations result from strong adhesive forces between two cores with a common single bilayer membrane. Once two cores are connected during dewetting, the connection is fixed, preventing further arrangements. Polymersomes with...
multiple compartments can encapsulate several distinct ingredients while avoiding cross-contamination. Such polymersomes can release encapsulants simultaneously when subjected to an external stimulus that ruptures the membrane.

The sequential release of multiple distinct ingredients can be achieved by designing polymersomes with hierarchical structures, such as polymersomes-in-polymersomes or double polymersomes. Double polymersomes release the first encapsulants from the outer polymersomes and the second encapsulants from the inner polymersomes in the presence of an external stimulus. Similarly, triple polymersomes can sequentially release three distinct encapsulants from the outermost, middle, and innermost polymersomes. Double or triple polymersomes are prepared by controlling the re-injection of polymersomes. The innermost polymersomes must be prepared with a uniform size using a double-emulsion template, and these polymersomes

Figure 17. Vesicles templated by double-emulsion drops. a) Schematic illustration of dewetting of the middle phase from the surface of the innermost drop, and the subsequent separation of the middle phase, leaving unilamellar vesicles. b) A series of optical microscopy images showing the dewetting process, where images were taken at intervals of 1.82 s. Panel (b) reproduced with permission. Copyright 2008, American Chemical Society. c,d) Optical and confocal microscopy images of monodisperse vesicles. e) Schematic illustration of dumbbell-shaped vesicles with two distinct compartments. f,g) Confocal microscopy image of the dumbbell-shaped polymersomes containing two distinct materials in separate lumens. Panels (e–g) reproduced with permission. Copyright 2011, Wiley-VCH.
are, in turn, encapsulated into the innermost water drops of a second level of double-emulsion drops. As the middle phase dewets from the surfaces of the inner drops, the outer polymersomes are prepared, resulting in polymersomes-in-polymersomes. The number of polymersomes encapsulated in an innermost drop can be controlled (one or more) during the generation of double-emulsion drops. If the size of the inner polymersomes is comparable to or larger than the diameter of an oil jet in a capillary microfluidic device, the injected polymersomes preferentially trigger breakup of the jet to form single polymersomes-in-inner drops, as shown in Figure 18a. This process yields double polymersomes containing a single inner polymerosome, as shown in Figure 18b, where the inner and outer polymersomes encapsulate red and green dyes, respectively. By contrast, if the size of the inner polymersomes is smaller than the diameter of an oil jet, several inner drops can be encapsulated into the inner water drops of a double-emulsion drop to form double polymersomes containing several inner polymersomes. The number of inner polymersomes cannot be precisely controlled, but the average number can be controlled according to the volume fraction of the inner polymersomes in the aqueous phase and the average size of the double-emulsion drops. By injecting a mixture of inner polymersomes with different encapsulants, multiple distinct ingredients can be separately encapsulated and released. For example, double polymersomes containing several inner polymersomes, each containing one of three different dyes, are shown in Figure 18c. In this case, the outer polymersomes preferentially release their encapsulants, then the inner polymersomes simultaneously release their own encapsulants when subjected to an external stimulus.

Although hierarchical structures enable the sequential release of encapsulants, control over bilayer stability can provide additional flexibility to the programmed release from the capsules.\(^{[92]}\) For example, the time interval between release from the outer and inner polymersomes can be controlled. The stability of a bilayer membrane can be tuned by incorporating hydrophobic homopolymers into the hydrophobic region of the bilayer. For example, PLA homopolymers may be inserted into a PEG-b-PLA diblock-copolymer bilayer by dissolving the PLA in an amphiphile-laden middle phase. The homopolymer-loaded bilayer membranes exhibit enhanced stability and membrane integrity. Because each membrane in a multiple polymersome can be independently controlled, incorporation of homopolymers into a selected membrane is possible. For example, a triple polymersome may be formed by re-injecting a double polymersome into a third level of a double-emulsion drop. All three membranes could be normal bilayers, or the middle and outer membranes could be tuned to selectively incorporate homopolymers, as shown in Figure 18d. Upon addition to a mixture of water and ethanol, the two triple polymersomes exhibited different release properties. A triple polymersome consisting of normal membranes displayed sequential membrane rupture of first the outer bilayer, then the middle bilayer, and finally the inner bilayer, as shown in Figure 18e. By contrast, a triple polymersome consisting of a normal inner bilayer and reinforced middle and outer bilayers exhibited inner bilayer rupture first, followed by rupture of the outer and middle bilayers, as shown in Figure 18f. A combination of hierarchical structures and control over the bilayer stability could provide a tunable mechanism for the delivery of multiple components through a time-dependent programmed release profile.

3.2.3. Microfluidic Pulsed-Jetting Techniques for Vesicle Production

As an alternative to drop templates, monodisperse vesicles may be prepared via pulsed jet-induced deformation of a bilayer...
capillary force exerted by the deformed oil layer, thereby creating a long neck and inducing breakup into vesicles containing very small volumes of the oil phase, as shown in Figure 19b. After neck breakup, the deformed oil layer recovers its original planar shape by retraction, and the deficiency of amphiphiles at the interface is restored from the relatively thick oil film. Residual oil remains in the bilayers of the separated vesicles. This oil diffuses slowly into the continuous phase to form vesicles with a negligible oil content after a sufficiently long incubation time. The pulsed jets approach can be used to continuously produce vesicles, leading to formation of monodisperse vesicles as shown in Figures 19c. The maximum achievable frequency of vesicle formation is 200 Hz using inkjet pulses. The continuous production may be maintained until the planar bilayer is consumed. The size of the resultant vesicles depends on the pulse volume and the velocity. A low pulse volume and a high velocity can reduce the diameters of the vesicles. Normal operating conditions using a piezo-actuated syringe yield vesicles with a diameter about 5–6 times the size of the nozzle. The diameter can be reduced to 1–2 times the size of the nozzle under extreme conditions; however, smaller nozzles suffer from a larger pressure drop, which limits the minimum size of vesicles to approximately 100 μm. Pulses applied using an inkjet yield a minimum vesicle size of 10 μm in the case that a high-viscosity stationary liquid is employed opposite the inkjet. The aqueous core of the resultant vesicles predominantly contains the aqueous solution injected from the nozzle, and approximately 25% of the volume is from the stationary membrane, as shown schematically in Figure 19a. As an aqueous solution is injected in pulses through a tapered capillary, the high force of the pulsed jet induces local deformation and breakup of the planar bilayer to form a vesicle. The stable and continuous formation of vesicles was accomplished using this approach by first preparing a stable planar bilayer. As with the double-emulsion approach, a planar bilayer may be prepared by overlaying two W/O interfaces. An oil phase containing amphiphiles may be cast to form a freestanding film sandwiched by two aqueous phases. The film will then have two amphiphile-stabilized interfaces. Such a film may be prepared using a hydrophobic double-well structure composed of two circular cells connected by a small waist. An oil solution containing the amphiphiles is introduced into the waist, and two aqueous solutions are then added to each circular cell. The hydrophobic nature of the wall induces spreading of the oil solution over the wall, thereby forming a thin film at the waist and separating the two aqueous phases. The two interfaces of oil film are stabilized through adsorption of the amphiphiles from the oil. Pulses of an aqueous solution are then pumped using a piezo-actuated syringe or inkjet through a nozzle into a stationary fluid. The inertial force of the stream then deforms the planar oil layer, draining the oil out and overlaying two interfaces at which the jet arrives. Although the oil film becomes very thin during deformation, it maintains continuity without rupturing because both interfaces are stabilized by amphiphiles and the oil film displays strong lubrication resistance. The protruding deformation grows once the inertial force is sufficient to overcome the capillary force exerted by the deformed oil layer, thereby creating a long neck and inducing breakup into vesicles containing very small volumes of the oil phase, as shown in Figure 19b. After neck breakup, the deformed oil layer recovers its original planar shape by retraction, and the deficiency of amphiphiles at the interface is restored from the relatively thick oil film. 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The diameter can be reduced to 1–2 times the size of the nozzle under extreme conditions; however, smaller nozzles suffer from a larger pressure drop, which limits the minimum size of vesicles to approximately 100 μm. Pulses applied using an inkjet yield a minimum vesicle size of 10 μm in the case that a high-viscosity stationary liquid is employed opposite the inkjet. The aqueous core of the resultant vesicles predominantly contains the aqueous solution injected from the nozzle, and approximately 25% of the volume is from the stationary membrane.
fluid. The two fluids are rapidly mixed by vortex, as shown in Figure 19b. This method provides for the in situ injection and mixing of two distinct components into the cores of vesicles. Such vesicles are useful for biological or chemical reactions in cell-like environments. For example, Stachowiak et al. used this technique to polymerize the cytoskeletal protein actin and form a densely entangled cytoskeletal network in the vesicle lumen. Purified monomeric actin was injected from the nozzle, and the actin polymerization buffer was the stationary liquid on each side. Vesicle formation induced mixing between the two solutions, thereby initiating polymerization in the lumen while avoiding clogging of the nozzle.

The resultant vesicles were unilamellar and, therefore, the membrane could be perforated by a membrane protein, such as α-hemolysin. Although vesicles are impermeable to small molecules, anchoring of an α-hemolysin protein at the membrane yields nanopores that permit the diffusion of small molecules through the membrane. For example, vesicles dispersed in an aqueous solution containing FITC and α-hemolysin displayed FITC fluorescence in the vesicle lumen upon diffusion of FITC through the pores of the α-hemolysin anchored at the membrane, as shown in Figure 19d. Asymmetric vesicles can be prepared using a modified jetting technique. A planar bilayer in which each leaflet featured a different composition was prepared by placing a thin acrylic solid film in the waist of a double-wall structure to form a divider. The addition of oil and water to the wells facilitated wetting of the oil to the hydrophobic wall of the acrylic divider or wells. Unlike the original method, the interfaces did not initially include lipids. Instead, liposomes, prepared using bulk methods, were injected into the stationary fluids in two of the wells to form a lipid monolayer at the interface via fusion. In this way, two leaflets with different lipid compositions could be prepared by applying two different liposomes. After removing the divider, two parallel interfaces with a different monolayer formed in the waist. As in the original technique, a pulsed jet was applied to the asymmetric bilayer to form protrusions that broke away into vesicles.

The parallelized production of cell-sized vesicles using bilayer deformation techniques was implemented using an array of microfluidic T-junctions with a single main channel and an array of small chambers on the wall of main channel. The small chambers were connected to the relatively large chambers through narrow channels overlaying an aluminum pattern that generated heat under IR laser illumination. Through the main channel, water, lipid-laden oil, and water were sequentially injected without bubbles between the phases. The three fluids were injected into the array of small chambers to create an oil layer sandwiched by two water phases. The aluminum pattern was heated in the large chambers, thereby expanding air bubbles in the chambers, which subsequently forced the fluid to flow out through the smaller chambers. As a result, the oil layer was deformed to produce liposomes similar to those prepared by drop formation in T-junctions. The vesicle size depended on the flow rate of the continuous phase in the main channel and the width of the small chamber. This approach produced monodisperse vesicles with 10 μm in diameter with a high encapsulation efficiency. The vesicle production frequency increased with the number of T-junctions.

4. Foldable Microcarriers

The previous sections reviewed two distinct types of microcarrier: particle-type and capsule-type, for encapsulation and the controlled release of cells or drugs. Advanced functionalities may be introduced into the microcarriers using various strategies to control the shape, structure, and composition. Despite tremendous progress in the fabrication of particle and capsule microcarriers, the triggered encapsulation and release of active ingredients remains a challenge. New types of microcarriers can enable 3D encapsulation of cells or drugs by stimulus-triggered microstructure folding. Microstructures that are composed of multilayers with different volume phase transition characteristics experience a tensile stress between the layers that induces microstructure folding. Tailored microstructures can be completely enclosed via controlled folding to create an isolated space. The structures unfolded upon removal of the stresses to recover the original shape. Foldable microstructures have several advantages over particle and capsule microcarriers: i) the encapsulation step is separated from the fabrication step to avoid exposing the encapsulants to toxic chemical precursors, ii) folding and unfolding can be reversibly controlled by external stimuli, enabling the triggered capture and release of materials in the continuous phase, and iii) release occurs upon carrier unfolding without degradation or rupture to permit the repeated use of a carrier. In this section, we discuss two distinct types of foldable microcarrier. In Section 4.1, we review hinge-assisted folding of 2D microstructures into 3D polyhedron microcarriers, and in Section 4.2, we review bilayer-assisted folding of hydrogel structures into enclosed capsules.

4.1. Hinge-Assisted Folding

Microcarriers based on hinge-assisted folding are composed of hinges and patches. Because folding occurs only at the hinges, patches form hollow polyhedra in a manner analogous to origami folding, as shown schematically in Figure 20a. The type of polyhedron is determined by the initial layout. For example, six square patches produce a cube and twelve regular pentagon patches produce a dodecahedron. Multistep lithography is required for the preparation of polyhedron-shaped microcarriers. A photosresist is first coated onto a substrate with a sacrificial layer, and photolithography is used to form a negative image pattern. A metal film is deposited on the substrate by electrodeposition of an electrolyte solution containing metal ions to form patches. After removing the first photosresist, a second photosresist coating is applied and patterned by photolithography to form a negative image that completes the hinges. Tin/lead solder is subsequently electrodeposited, and particles are released from the substrate upon removal of the residual photosresist and sacrificial layers. Folding at the solder hinge is driven by capillary forces upon heating at 100 °C for 3 min. The high surface tension of the liquid solder enforces folding of the patches to minimize the surface area. The folded structures are fixed by freezing the liquid solder during cooling. Leong et al. used this procedure to fabricate cubic microcarriers of which one patch has a square-shaped opening. The microcarriers provided temperature-triggered folding and the directional
A chrome/copper/polymer trilayer permitted the actuation temperature to be reduced to 40 °C. This carrier was used to demonstrate the in situ capture of glass beads in an aqueous solution, as shown in Figure 20b; captured bead cannot escape from the microcarrier due to its larger size than the openings on the patches. Moreover, mild actuation conditions enabled the delicate biological materials to be encapsulated into the carrier. For example, L929 fibroblast cells and *Triops* embryo was successfully encapsulated without damage.  

Although previous studies provided temperature-triggered folding, controlled unfolding (encapsulant release) is more difficult to achieve. To trigger both unfolding and folding, Randhawa et al. used the sequential dissolution of hinges prepared from a chrome/copper metal bilayer and a polymer layer. Folding was triggered by the dissolution of the polymer in an aqueous acetic acid solution, whereas unfolding was triggered by the dissolution of release of chemicals through an opening on one patch. In addition, the metal patches were magnetoresponsive, enabling remote control over the carrier migration; however, solder requires high temperatures to melt, which provides an obstacle for the in situ encapsulation of temperature-sensitive materials. To reduce the actuation temperature and prepare all-polymer carriers, a patch was made using an SU-8 photoresist, and the hinge solder was replaced by PCL. The low melting point of PCL, 60 °C, reduced the actuation temperature to 58 °C. The polymeric materials were more biocompatible and optically transparent. Further reductions in the actuation temperature could be achieved using a thin metal bilayer with a different coefficient of thermal expansion, rather than a solder. Undesired folding was prevented by patterning additional polymer layers on the hinges. Above the temperature at which the polymer softened, the metal bilayer folded to form polyhedron carriers.  

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a second layer, copper, upon addition of H₂O₂ to the solution. The folding and unfolding of microstructures could be triggered by different chemicals to facilitate the customized selection of the target materials. This technique has also found utility in micro-robots. Gelatin and cellulose were used in a similar fashion to form a polymer layer that prevented folding. Dissolution of the gelatin or cellulose was triggered by collagenase or cellulase, respectively. The enzyme-based dissolution facilitated folding and unfolding in a specific biocompatible environment. The dissolution-based folding and unfolding steps are irreversible, and the carriers may not be reused. Reversible actuation of a microcarrier can be achieved through chemical oxidation and metal reduction. For example, when a microstructure is folded by dissolving a polymer layer from the nickel/copper/polymer trilayer hinge, the direction in which the nickel/copper bilayer folds is determined by environmental conditions. Oxidative environments induce folding to form converters with an inner copper layer and an outer nickel layer, whereas reductive environments induce the opposite configuration. Therefore, reversible encapsulation and release can be achieved through environment-triggered folding and unfolding. The size of the microcarriers could be reduced to 100 nm using e-beam lithography techniques, as shown in Figure 20c.

Biological applications were demonstrated by Kalinin et al., who used microcarriers to release chemo-attractants into living organisms. Patches of the microcarriers were selectively perforated to directionally release the chemo-attractants into a stationary medium, thereby creating 3D chemical patterns around the microcarrier. Release of the chemo-attractant, L-serine, from the microcarrier into the surrounding fluid induced E. coli to migrate toward the localized pores and form a 3D organizational structure through chemotaxis, as shown in Figure 20d.

This system is useful for in vitro studies of cell behavior.

4.2. Bilayer-Assisted Folding

As in the stress-driven self-folding of a metal bilayer at a hinge, polymer bilayers consisting of a stimulus-responsive hydrogel and an insensitive polymer could exhibit triggered folding. Hydrogels or polymers have several advantages over metals as microcarrier materials. First of all, hydrogels are more biocompatible and softer than metals. As discussed in the second section, microgels are widely employed as cell carriers due to their excellent biocompatibility. A variety of stimuli can be used to trigger the folding of hydrogel microcarriers. Hydrogels can exhibit a volume phase transition under various environmental conditions, such as temperature, pH, or in the presence of specific molecules and ions, depending on the material. Moreover, chemically or biologically degradable microcarriers can be designed to avoid the production of toxic byproducts. The fabrication of hydrogel bilayer microstructures is much easier than the fabrication of hinge-assisted metal microcarriers. Hinge-assisted carriers require multiple lithography and electrodeposition steps that are time-consuming and difficult to control. By contrast, hydrogel bilayers can be prepared simply using lithography without the need for complex procedures, such as deposition and lift-off of the materials. A hydrogel bilayer is composed of an active layer and a passive layer. The active layer expands or contracts, depending on the external conditions, whereas a passive layer maintains its volume to generate tensile stress. This stress forces the bilayer to fold, thereby forming enclosed capsules, as illustrated in Figure 20e.

A primitive form of such bilayer microstructures was prepared using a bilayer composed of two distinct hydrogels with different degrees of expansion. These bilayer microstructures folded in water. A hydrophobic bilayer composed of two degradable polymers, polysuccinimide and PCL, in place of a hydrogel, displayed spontaneous bilayer folding. This behavior was driven by the expansion of the polysuccinimide layer upon hydrolysis. Guan et al. triggered folding in a bilayer comprising a temperature-responsive active layer and an insensitive passive layer. The bilayer was prepared by dissolving poly(NIPAAm-4-acryloylbenzophenone(ABP)) in chloroform, followed by spin-coating onto a wafer to form an active layer. A toluene solution containing PCL and benzophenone was coated as an insensitive layer above an active layer as a passive layer. The bilayer was patterned into rectangular shapes by photolithography. A decrease in the temperature induced the rectangularly shaped bilayer to fold into tubes as the active layer expanded, thereby capturing microbeads. Unfolding occurred at higher temperatures, enabling reversible encapsulation and release. The same set of materials was used by Stoychev et al. to demonstrate the capture of yeast cells into self-folding microcarriers using star-shaped bilayer microstructures. A pH change provided a stimulus trigger to the pH-responsive hydrogel. He et al. prepared bilayer microstructures using an active layer of poly(methylacrylic acid) (PMAA) and a passive layer of poly(2-hydroxyethyl methacrylate) (pHEMA) using micromolding techniques. The passive layer surface had muco-adhesive patches containing drugs on the surface. The bilayer was attached to tissue, and the construct displayed curling at pH 6.5, leading to the directional release of drugs into the tissue. Although previous studies provided the triggered capture and release of materials, the size of target materials should be sufficiently large to avoid leakage through gaps between the patches. The size of the microcarrier gaps, produced by folding, were reduced by Shim et al. in a snowman-shaped bilayer microstructure consisting of an active layer of pHEMA-co-acrylic acid) and a passive layer of pHEMA, prepared by photolithography, as shown in the left image of Figure 20f.

The snowman structures transformed into microcapsules through folding in the presence of an aqueous solution at pH 9, as shown in the middle and right images of Figure 20g. Macro-gap between two patches is completely sealed as shown in Figure 20g; this is attributed to softness of hydrogel which facilitate conformal contact between the patches. Encapsulation of 1 µm PS particles at pH 9 and the subsequent release of the PS particles at pH 4 was demonstrated, as shown in Figure 20h. No particle leakage was observed at pH 9, demonstrating that small gap sizes, less than 1 µm, arose from tight contacts between the soft hydrogel patches. When the microcarrier encapsulated dextran with molecular weight of 2 000 000, it took approximately 1 h that half of the dextran is released from the microcapsule at pH 9, while approximately 2 min for open capsules at pH 4; this prove the size of gap is comparable to size of the dextran, tens of nanometers.
5. Conclusions

Over the past decade, a variety of microcarriers and capsules have been designed and produced through creative combinations of emerging technologies, soft matter physics, and chemistry. These new approaches have provided tremendous control over the size, shape, structure, and materials. The resultant microcarriers have exhibited new functionalities or enhanced performances that are difficult to achieve using conventional approaches. The future biomedical applications of such microcarriers depend on their size, shape and microstructure. For example, nanoparticles smaller than 200 nm potentially provide efficient delivery of cancer drugs into tumor cells; such nanoparticles can be prepared by nanoprecipitation technique. Microcarriers smaller than a few micrometers can be efficiently internalized within cells when the carriers are designed to have specific size and shape; this provides direct release of drugs into cell interiors for their termination or growth factors for fast tissue repairing. Microgels larger than cells, a few tens of micrometers, enable proliferation of cells within them, providing useful building blocks for tissue construct and isolated compartments from immune system for transplantation. Microcapsules or vesicles with similar size to cells can be employed as artificial cells for fundamental studies of cell functions and production of natural ingredients. In addition, many stimuli-responsive microcarriers can provide triggered release of encapsulants in desired time and location, thereby useful for drug delivery while maximizing effect of the drug. Despite such significant advances and new strategies for producing novel microcarriers, most microcarriers developed thus far have yet met the requirements for practical biological applications; this is attributed that new technologies or combination of conventional technologies, rather than market demand, drive the production of such microcarriers. Above all, the productivity of a microcarrier prepared by a new route is usually low, and the cost of production is too high to replace conventional carriers made through bulk methods. Advanced microcarriers receive limited usage only as high-end products. In addition, many procedures used to produce novel microcarriers involve toxic solvents or precursors that are not appropriate for in vivo usage, although the final products include only small amounts of the residual precursors. Moreover, long periods of time are required to evaluate the toxicity and effects of a microcarrier, thereby delaying their in vivo application and commercial use. We expect that these problems can be addressed through continued research efforts in the field of soft matter. The parallelized production of microcarriers will enhance the productivity and reduce the costs of production. Modifications to the systems currently in use will enable the use of only biocompatible materials during microcapsule preparations. Other important issues still need to be addressed in the fields of soft matter and biomedical science. Biological systems are very complex, and no panacea microcarrier is available that is useful for all purposes with optimal performances. Each application is associated with specific optimal conditions under which a microcarrier application should function, such as the release profiles of one or multiple drugs. Therefore, researchers who design microcarriers should recognize the needs from biomedical researchers or patients and requirements of specific biological applications, and engage in interdisciplinary studies through communications and collaborations with researchers in the fields of biomedical science and engineering.

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