Chem

CellPress REVIEWS

Review

Microfluidic Tools for Bottom-Up Synthetic Cellularity

Martina Ugrinic,[1] Andrew deMello,[1,*] and T.-Y. Dora Tang[2,*]

Microfluidic tools and technologies offer an engineering methodology for the bottom-up synthesis of artificial cells. Herein, we discuss how droplet-based microfluidic systems may be used for artificial cell and tissue synthesis. In addition, we point out some of the latest developments in the field of microfluidics that have potential use in bottom-up synthetic biology.

INTRODUCTION

The emerging field of bottom-up synthetic biology aims to construct "living" artificial cells through the combination and piecing together of basic functional components.[1] While the definition of "living" is a subject of much debate,[2] there are singular properties observed in living systems that are intriguing to capture within artificial cell-like compartments en route to living artificial cells. Such properties include, but are not limited to, replication, metabolism, motility, sensing, signaling, fusion, and growth.[3] A basic and primary feature of an artificial cell is compartmentalization. In living systems, cells are not only bound by a protein-rich lipid membrane but also contain membrane-bound and membrane-free sub-compartments. While the precise mechanism of how biological, multimolecular compartments form *in vivo* is still not entirely understood, appreciation of how one or two molecules might self-assemble to imitate biological compartments *in vitro* is well established. For example, amphiphilic molecules such as phospholipids, representing the basic building block of the cell membrane, will self-assemble in water to form a vesicle or liposome with an aqueous inner compartment delineated by a lipid bilayer. Similarly, intrinsically disordered proteins have been observed to phase separate *in vitro* via liquid-liquid phase-separation processes in the presence of polyethylene glycol and/or salt to form minimal membrane-free compartments. Here, phase separation is driven by a range of phenomena, including electrostatic interactions[4,5] and non-covalent cation-pi interactions.[6] The resulting dispersion comprises two coexisting liquid phases: a protein-rich phase in dynamic equilibrium with a protein-deprived phase.[7] Compartmentalization via the self-assembly of synthetic molecules into membrane-delineated water-oil emulsions or water-water droplets is also well established (Figure 1). Indeed, membranes comprising silica nanoparticles, microgel particles, amphiphilic block copolymers, or amphiphilic protein-polymer nanoconjugates have all been realized.[8–10] Moreover, synthetic mimics of membrane-free compartments can be formed from synthetic polymers or biologically relevant molecules via associative and dissociative liquid-liquid phase-separation processes, i.e., coacervates or aqueous two-phase systems, respectively. Such biological and synthetic compartments both act to spatially localize and isolate molecules and their reactions. Accordingly, an additional but important feature of an artificial cell is the ability to contain and support a biochemical or chemical reaction pathway, thus imparting specific functionalities to the compartment. Enclosing specific reactions increases the efficiency and controllability of the system by avoiding the potential side reactions that commonly occur in natural cells. While such a methodology could

The Bigger Picture

The inherent complexity of living cells often renders the direct study of complex biological phenomena very difficult, limiting the exploitation of such processes for engineering purposes, as is the goal in synthetic biology. Creating artificial, cell-like structures from a small number of molecular components represent an interesting alternative to engineering living cells because systems of desired complexity can be built with excellent control over all components. With the help of microfluidic platforms, the concept of building cells from scratch can be improved even further, engendering the production of millions of artificial cells in short periods of time and with excellent control over cell size and cell cargo. Such technological advances provide access to a variety of applications beyond the study of biological processes, such as targeted drug delivery, or the production of biological agents, such as drugs, proteins, and DNA.

CellPress REVIEWS

be exploited toward the aim of building "living" artificial cells, artificial cells with controllable properties can also be of utility in a broad range of applications, such as bioreactors for chemical synthesis, as platforms for *in vitro* reconstitution or as minimal physical models to probe complex biological processes where controlled functionality is desired.

Micron-sized compartments, the basic structural feature of an artificial cell, can be generated via bulk methodologies, where macroscale volumes, ranging from microliters to liters, of fluids are mixed in a test tube to form large numbers of microcompartments.[10–14] However, compartments generated in this way are almost always characterized by wide population size distributions, which are undesirable for quantitative experimentation or in studies where droplet reproducibility is key to the classification of specific behaviors. Droplet-based microfluidic methods, developed over the last 15 years, offer an alluring alternative to these bulk methodologies.[15] In simple terms, droplet-based microfluidic systems generate, manipulate, and process discrete droplets contained within an immiscible carrier fluid. They leverage immiscibility to create distinct and sequestered volumes that reside and advance within a continuous flow. Notably, these experimental platforms allow for the production of monodisperse droplets at kilohertz rates and provide independent control over each droplet in terms of its size, location, and chemical composition. The ability to handle fluids in such a manner enables ultra-high analytical throughput and generates data of exceptional precision while using a fraction of the reagents consumed in non-microfluidic systems. Importantly, droplet size can be readily tuned through variations in channel geometry and applied flow rates. (Although not the focus of the current discussion, droplets are recognized to be ideal vehicles for performing experiments on single cells. Individual cells can be encapsulated and manipulated within pL-volume droplets via passive techniques, such as co-flow, T-junction, step, and flow-focusing geometries. These platforms offer high-throughput routes to sequencing[16,17] and high-throughput antibody screening at the single-cell level.[18] The general approach can also be used in a range of applications in microbiology. For example, it is possible to manipulate and screen microbial populations at the single bacterium level, allowing the quantitation of variables, such as growth rate[19] and cell-to-cell variability.[20] Moreover, encapsulation of bacteria within droplets allows the dynamic monitoring of quorum-sensing processes between bacterial populations and the study of cellular communications across biofilms.[21]) The ultra-fast generation of droplets within such a well-controlled environment offers huge advantages when generating artificial cells. We now describe how microfluidic technologies have already been exploited for generating a variety of scaffolds that are used as basic building blocks in the synthesis of artificial cells and discuss advances and challenges associated with building more complex artificial cellular systems.

MICROFLUIDICS MEETS ARTIFICIAL CELLS

The most common material used in the fabrication of microfluidic devices is poly(dimethylsiloxane) or PDMS. PDMS is a silicone-based elastomer with excellent optical properties, a high tolerance to pressure and temperature, as well as high permeability to gas. All these features make it perfectly suited for cell culture and other biological assays. PDMS-based microfluidic devices are typically produced via a combination of conventional and soft lithographic methods, where the desired structural features are initially generated in an epoxy-based negative photoresist (the master) and then transferred into the PDMS via replica molding.[22] Compared to the fabrication of glass or silicon devices, only the initial photolithography step

[1]Department of Chemistry & Applied Biosciences, ETH Zurich, Vladimir Prelog Weg 1, 8093 Zurich, Switzerland

[2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany

*Correspondence:
andrew.demello@chem.ethz.ch (A.dM.),
tang@mpi-cbg.de (T.-Y.D.T.)

https://doi.org/10.1016/j.chempr.2019.03.012

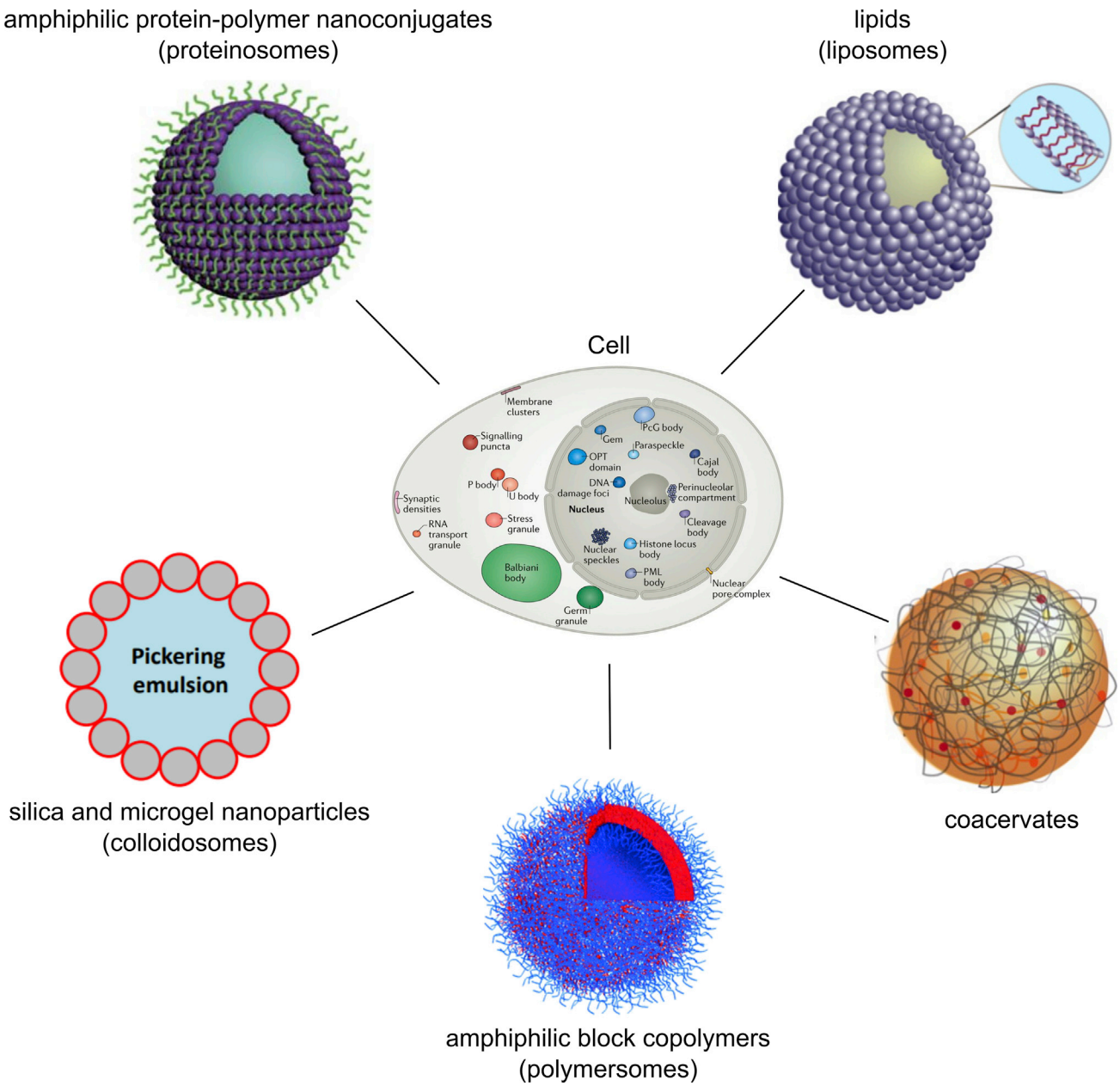

Figure 1. Schematic Representation of Artificial Cells

Membrane-bound systems can be stabilized by lipids, amphiphilic protein-polymer nanoconjugates, silica and microgel nanoparticles, and amphiphilic block copolymers, creating artificial cells called liposomes, proteinosomes, colloidosomes, and polymerosomes, respectively. Membrane-free systems are formed via associative or dissociative liquid-liquid phase-separation processes creating droplets held together by hydrophobic forces. These types of artificial cells are called coacervates. Adapted with permission from Huang et al.[10] (copyright 2013 Springer Nature) and reprinted with permission from Thompson et al.,[8] LoPresti et al.[57] (copyright 2009 Royal Society of Chemistry), Vieregg and Tang,[109] and Banani et al.[110] (copyright 2017 Springer Nature).

needs to be carried out in a cleanroom environment, with many copies of the original master being produced quickly and at low cost. PDMS-based microfluidic devices can be fabricated with excellent resolution and can incorporate complex fluidic networks. Both features permit the undertaking of complicated biological assays, such as deep sequencing[23] or stem cell reprogramming[24] within a single device. The usefulness of PDMS is really only limited by its low tolerance to organic solvents and

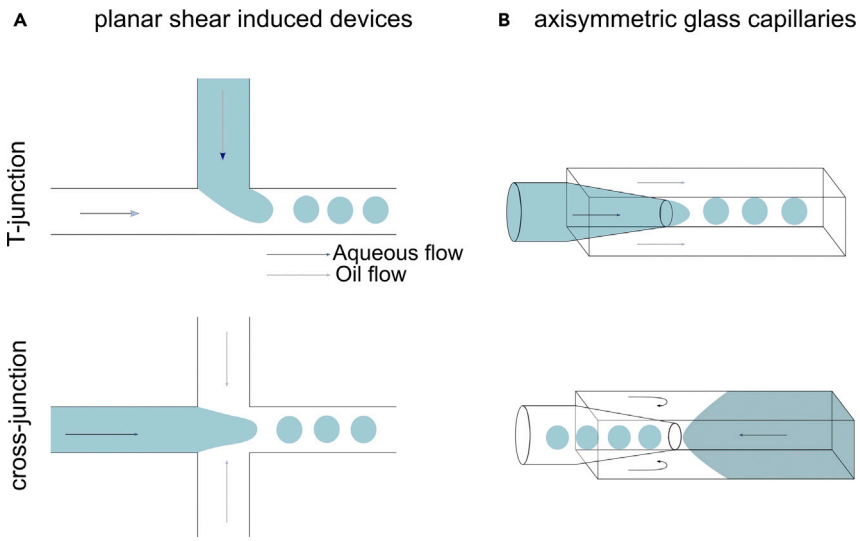

Figure 2. Structures of the Most Common Types of Microfluidic Droplet Generators
(A) T-junction and flow-focusing geometries are commonly used for the formation of water-in-oil droplets and coacervate droplets. For water-in-oil emulsions, droplets are formed at the intersection of channels carrying the immiscible carrier fluid and one or more channels carrying the disperse phase.
(B) A glass-capillary-based device used to generate single and multicompartmentalized double emulsions.

harsh chemicals,[25] although this issue may be ameliorated to a large extent through the use of a range of surface modifications based on parylene,[26] sol-gels,[27] or fluoropolymers,[28] for example.

When using microfluidic systems, droplets are typically generated at either "flow-focusing" geometries[29] or "T" junctions[30] (Figure 2A). These structures function to bring together streams of immiscible carrier fluid (usually a mineral or fluorinated oil) and one or more streams carrying the dispersed phase (usually an aqueous fluid). At the intersection of these flows, droplets are formed because of the differences in surface tension and sheer force of the flowing liquids.[31] The size of the formed droplets is largely determined by the channel dimensions, as well as liquid flow rates and their viscosities, and can be tuned in a precise manner.[32] Water-in-oil droplets can be stabilized by the addition of surfactants to the carrier fluid. Surfactants typically consist of at least one hydrophobic and one hydrophilic section, ensuring that it is energetically favorable for the surfactant to span the water-oil interface, with the hydrophilic region resting in the aqueous phase and the hydrophobic region in the oil phase. For effective stabilization, surfactants are chosen on the basis of the carrier oil, which often comprises hydrocarbon or (per)fluorinated carbon chains.[33] Common surfactants used for water-in-oil emulsions include Span 80,[34] Tween 20,[35] and non-ionic fluorosurfactants.[36,37] Surfactants can also be readily exchanged during experimentation, enabling stabilization while altering the physical and chemical properties of the droplet interface. For example, Dinsmore et al. reported the fabrication of solid capsules (colloidosomes) with controlled size, permeability, and mechanical strength via the self-assembly of colloidal particles at the interface of water-in-oil emulsion droplets.[38,39] Colloidosomes are typically generated in capillary reactors,[40] consisting of a tapered glass capillary placed inside another capillary (Figure 2B). Such a device is composed of two coaxially assembled glass capillaries, one with a square cross section and other with a round cross section.

The continuous phase fluid and the aqueous phase fluid enter from opposite ends of the square capillary. The oil phase focuses the aqueous phase into the tapered end of the round inner capillary, resulting in the formation of water-in-oil droplets.[41] Using such a configuration, the materials used to generate droplets can be readily interchanged; for example, silica nanoparticles can be exchanged for poly(*N*-isopropylacrylamide) (PNIPAAm) microgel particles, where the resulting water-in-oil droplets can be harvested, chemically crosslinked, and transferred into water. The sensitivity of such PNIPAAm microgel particles to temperature, pH, ionic strength, and electromagnetic fields[41] can be tuned, thus allowing the triggered release of species within the droplet.[42] Furthermore, capillary reactors can be easily scaled up to generate complex multiemulsions by the addition of more capillaries and alternating the supply of oil and water. Such systems are ideal for supporting hierarchical structures, which include reaction schemes as described later in this review.[43,44]

Surfactants and nanoparticles can be readily exchanged for lipids, which will stabilize water-in-oil emulsions via the same hydrophobic-hydrophilic principle as described previously, using either flow-focusing junctions or capillary-based microfluidic devices. Lipid vesicles are considered a minimal model for natural cells as they are made from the same elementary building block (lipids), which also make up biological cells.[45] Using the "emulsion" technique, lipid-stabilized water-in-oil droplets can be driven through a large interface between oil containing lipids and water. This results in the formation of a bilayer and thus lipid vesicle formation.[46,47] Off chip, this is achieved by centrifugation of water-in-oil emulsions generated microfluidically[48] or on-chip where droplets are driven through a lipid stabilized oil-water-interface.[45] Alternatively, liposomes can be formed by generating a water-in-oil-in-water double emulsion,[49,50] where oil leaves the double emulsion while droplets are under flow. However, lipid vesicles formed in this manner may have residual oil between the lipid layers, which will interfere with the integration of proteins and other more complex structures. In addition, lipid vesicles can be formed by microfluidic technologies via electroformation and hydration,[51] extrusion,[52] ice droplet hydration,[53] transient membrane ejection,[54] and droplet emulsion transfer.[46] A detailed discussion of these methods can be found elsewhere.[55] Because of the inherent biocompatibility of lipid vesicles, they make interesting carriers for therapeutic agents[56] and as artificial cellular chassis. Accordingly, microfluidic technologies are likely to have significant applications in the high-throughput generation of lipid-based compartments for applications in synthetic biology, including therapeutics.

Despite their biological significance, lipid vesicles or liposomes are not especially robust with vesicular stabilities on the order of days. In addition, the properties of the membrane will be limited by the chemistry and availability of lipid. Lipids are often difficult and expensive to synthesize from scratch, and direct extraction from cells may only produce small quantities of variable purity. More robust membrane-delineated water-in-water compartments can be readily produced by utilizing existing microfluidic technologies and by replacing the basic building block with other amphiphilic moieties. Diblock co-polymers are excellent candidates for the generation of highly stable and robust polymerosomes.[57] Indeed, synthetic methodologies have been developed to allow for the efficient synthesis of a diverse range of diblock co-polymers of varying length and composition. The diversity in available diblock co-polymer chemistry permits direct engineering of both physical and chemical membrane properties.[58] For example, tuning the ratio of polyethylene glycol-polylactic acid (PEG-PLA) diblock copolymers allows precise control of membrane thickness, mechanical response, and permeability.[59]

In addition to generating membrane-bound compartments, droplet-based microfluidic methods can be employed for the formation of membrane-free droplets. For example, coacervate droplets can be formed in a flow-focusing geometry from a polymer-rich bulk coacervate phase.[60] Contrary to normal droplet generation based on surface tension and shear force, coacervate droplets are generated through laminar flow breakup under low Reynolds number conditions. The resulting coacervate droplets exhibit significantly higher monodispersity compared to those formed using bulk methods and also display higher stability to coalescence. Membrane-free compartments are already utilized in a number of applications such as food processing and separation technologies,[61] and microfluidic generation of coacervate droplets could be further exploited in such applications. Coacervates as artificial cell-like structures are especially interesting because of their ability to selectively partition a large number of organic molecules,[62] as well as offer an alternative reaction spaces for biochemical reactions in comparison with membrane-bound compartments. Moreover, microfluidic technologies could be further exploited for the high-throughput generation and characterization of membrane-free droplets with controllable properties for studies in cell biology and origin-of-life studies.

INCORPORATING REACTIONS INTO DROPLETS

Another key criterion for building "living" artificial cells is the incorporation of guest molecules, such as enzymes and their substrates within a water-in-oil or water-in-water droplet. This permits the spatial localization of single or multistep enzyme cascades as observed in biology, where the membrane and the inner lumen of a compartment can be exploited to house different parts of a reaction cascade. The inclusion of guest molecules into micron-sized droplets is easily accomplished using microfluidic methods.[63] The molecular payloads of droplets can be controlled by mixing different aqueous streams on-chip prior to encapsulation. Moreover, different chemical conditions can be screened at high throughput within a single device. In this way, physicochemical effects such as surfactant concentration, droplet-to-droplet contact, and/or variation in salt concentration on compartmentalized enzyme reactions can be readily investigated.[64] For example, Nuti et al. successfully incorporated a two-step enzyme cascade based on glucose oxidase and horseradish peroxidase within lipid vesicles. Within the vesicle, glucose is converted to glucolactone and hydrogen peroxide by glucose oxidase, which is then reduced by peroxidase, simultaneously oxidizing Amplex Red to fluorescent Resorufin.[65] Compartmentalized multistep enzyme cascades can also be generated by using enzymes as the functional and structural element of the membrane. Here, chemically modified enzymes are used to stabilize water-in-oil emulsions. The membrane, which comprises PNIPAAM conjugated to an enzyme, is chemically crosslinked and then transferred into water. Production of water-in-oil emulsions, stabilized by enzyme conjugates, can be readily transferred to a microfluidic platform, allowing for high-throughput processing. Additionally, the generation of proteinosomes exhibiting a two-enzyme cascade has also been demonstrated with membrane-bound PNIPAAM-GOx encapsulating horseradish peroxidase. Importantly, the resulting proteinosomes have superior monodispersity and higher enzymatic activity when compared to droplets produced using bulk methodologies.[66]

The ability to produce proteins *in situ* via transcription and translation is a central challenge when generating biomimetic cells. To this end, micron-sized compartments can be activated by cell-free expression systems that permit the production of specific proteins *in vitro*.[67–70] Indeed, improved protein production has been

observed in dehydrated cell-free expression condensates[71] and in plasmid DNA condensates[72] within water-in-oil emulsions and within membrane-free coacervate droplets.[73] The observed increases in protein yield are almost certainly a result of higher achievable reagent concentrations within micron-sized droplets, as well as crowding effects from condensed DNA nanoparticles or dehydrated cell-free expression systems. Similar increases in protein yield have also been observed in gene expression of DNA hydrogels, where DNA hydrogels ligated with specific gene sequences could be read by cell-free gene expression.[74]

In all of the above examples, reagents are incorporated into the droplets during the generation process. However, additional reagents may also be incorporated into water-in-oil droplets at specific times after generation using a picoinjector.[75] Here, an electric field is applied to a moving droplet, destabilizing the membrane and allowing injection of soluble reagents in a serial or combinatorial manner. This capability allows the investigation of specific properties, such as the swelling of encapsulated hydrogels (within colloidosomes) by hydration.[76] In addition, picoinjector technology can be used to generate barcoded libraries of droplets. In such a process, pre-formed droplets are dosed with optical labels that impart a unique optical identity to each droplet. Barcoding is especially useful when processing large droplet populations, significantly increasing the multiplicity of an assay[77,78] and allowing high-throughput drug screens.[77]

HIGHER-ORDER STRUCTURES

The ability to produce multilayer compartments, inspired by the intracellular compartmentalization within natural cells, is critical for imparting spatial localization of biochemical reactions and building communication networks.[79] This is a basic engineering challenge that can be addressed through the use of microfluidic tools. For example, vesosomes (multivesicular vesicles) are either composed of multiple concentric lipid layers or multiple small lipid vesicles contained within a larger liposome.[80] Vesosomes can be activated by the incorporation of *in vitro* transcription and translation machinery within individual internal compartments, allowing real-time monitoring of RNA synthesis within the artificial cell (Figure 3A). Significantly, the ability to measure RNA synthesis as a function of time opens up possibilities of modeling transcriptional processes.[81] Recently, Deng et al. demonstrated a variation on this theme by encapsulating coacervates within lipid vesicles.[82] As coacervates contain no membrane, they mimic the membraneless compartments observed in natural cells and thus offer a physical model for investigating biological processes (Figure 3B), such as thermally responsive reversible compartmentalization, controlled storage, release of genetic molecules, and spatial organization of bioreactions.[82]

Microfluidic techniques can be used to generate systems with hierarchical structures from a broad range of starting molecules. For instance, Douliez et al. employed a flow-focusing device comprising three inlets to produce hydrogel droplets surrounded by a latex particle membrane (colloidosome) contained within individual water-in-oil droplets.[76] Such a colloidosome encapsulates modified latex particles, polyacrylic acid (PAA), polyethylene glycol (PEG), dextran, and a cross linking agent (carbodiimide). Upon mixing of the solutes within the emulsion, aqueous two-phase separation drives the formation of dextran-rich droplets, which can be chemically crosslinked by PAA and carbodiimide to form hydrogels coated with latex particles (Figure 4A). Here, the formation of hybrid cells composed of different classes of materials is driven by the self-assembly of the starting material. In addition, the

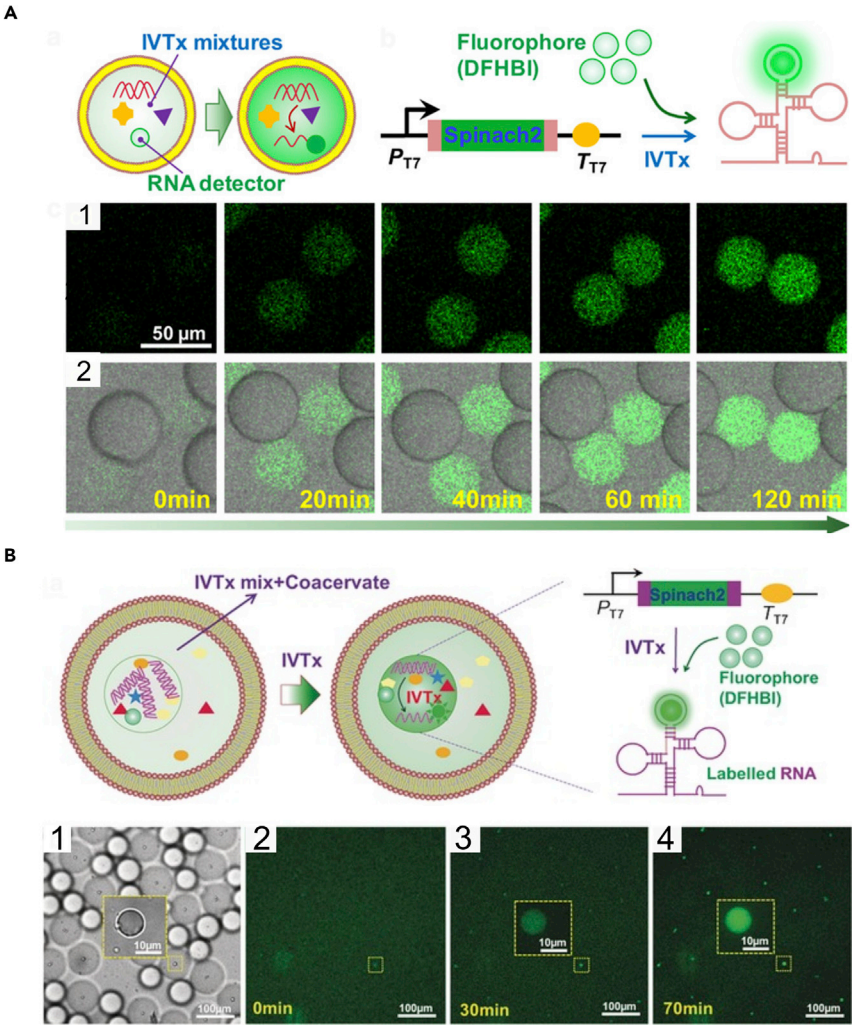

Figure 3. Cell-free Expression System Encapsulation within Artificial Cells

(A) Top: cartoons showing *in situ* detection of *in vitro* transcription (IVTx) inside liposomes and the working principle of the RNA aptamer Spinach2. These are short aptamer tags that exhibit fluorescence upon binding otherwise non-fluorescent fluorophores, with fluorescence intensity increasing with increasing RNA production. Bottom: (1 and 2) sequence of images showing the synthesis of RNA in liposomes. Reprinted with permission from Deng et al.[80] Copyright 2017 American Chemical Society.

(B) Top: illustrations of IVTx in coacervate droplets in liposomes and the working principle of the detection of generated RNA using the Spinach2 aptamer and 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI). Bottom: (1) optical image of liposomes containing a coacervate droplet (polycation, spermidine; polyanion, polyU RNA) and IVTxmix; (2–4) confocal images showing RNA generation in coacervates over time. Reprinted with permission from Deng and Huck.[82] Copyright 2017 Wiley-VCH Verlag GmbH & Co. KGaA.

generation of hybrid cells based on different building blocks can lead to different physical properties, such as an increase in the mechanical strength of the membrane. For example, a droplet-based microfluidic system has been used to generate droplet-stabilized giant unilamellar vesicles, where a lipid membrane is stabilized by a polymer shell on its exterior.[83] Significantly, studies demonstrated that these multilayered compartments could be loaded with a variety of biomolecules via picoinjection, thus enabling increased molecular complexity in both a rational and hierarchical manner (Figure 4B).

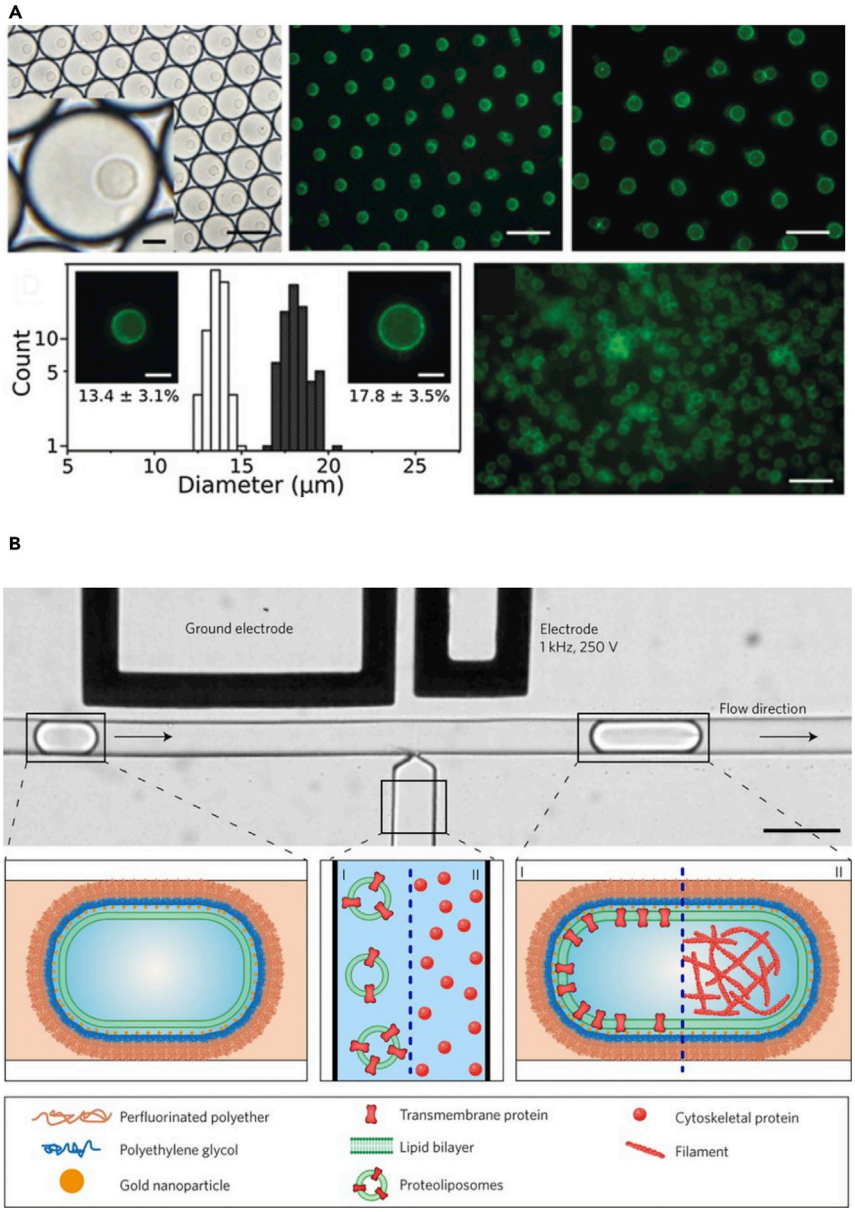

Figure 4. Picoinjection Can Be Used to Create Higher-Order Artificial Cells in Microfluidic Devices
(A) Microfluidic fabrication of monodisperse hybrid PAA/dextran hydrogel colloidosomes. Top: white-light and epifluorescence images of 40 pL water-in-oil (w/o) droplets containing single hydrogelled colloidosomes. Bottom left: epifluorescence image after picoinjection of water (3-fold dilution) with size distributions of both pristine (white) and swollen (black) colloidosomes. Bottom right: an epifluorescence image of colloidosomes extracted from the w/o emulsion, centrifuged and resuspended in PEG (7%). Scale bars, 50 μm (10 μm in all insets). Reprinted with permission from Douliez et al.[76] Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA.
(B) Top: image of a picoinjection device. Bottom: schematic representation of the process for incorporating transmembrane and cytoskeletal proteins into droplet-stabilized giant unilamellar vesicles (dsGUVs) by means of high-throughput droplet-based picoinjection technology. Scale bar, 50 μm. Reprinted with permission from Weiss et al.[83] Copyright 2018 Springer Nature.

As noted, micron-sized compartments are basic building blocks needed for the synthesis of artificial cells and may also be integrated to construct communication networks, where one compartment, for instance, might trigger a response in another

compartment via chemical cues. An elegant strategy to achieve such communication involves the encapsulation of biological cells within an artificial compartment and utilization of the cells' machinery as part of the biochemical cascade. To this end, Elani et al. encapsulated BE colon carcinoma cells within lipid vesicles and showed that the carcinoma cells were able to communicate with the external environment.[84] In brief, carcinoma cells were engineered to express the enzyme β-galactosidase, which in turn hydrolyzed lactose into galactose and glucose. Once produced, glucose was observed to permeate out of the cells into the aqueous phase contained within the vesicle where glucose oxidase and horseradish peroxidase reagents were confined. Glucose oxidation by glucose oxidase and horseradish peroxidase produces a fluorescence signal, which can be measured and quantified. Significantly, carcinoma cells were protected by the vesicle from external assault, with the encapsulated cells remaining viable for several days. This elegant study showcases the potential of interfacing living and artificial cells to create new functional devices and materials. It is likely that similar systems could find utility in applications such as cell therapy,[85] chemo-enzymatic hybrids cascades,[86] or cell-based sensors. Villar et al. showed that a more complex compartmentalized enzymatic network could also be built with water-in-oil emulsions stabilized by lipids, which are tightly connected in an array to generate multisomes (i.e., a network of small water droplets contained within a larger drop of oil that is itself suspended in an external aqueous environment).[87] Such a multisomal network was assembled by bringing monolayered water-in-oil droplets into close contact, generating droplet interface bilayers and effectively binding the emulsion droplets to one another (Figure 5A). This structure allowed the exchange of small molecules between different compartments, connecting different enzymatic reactions between droplets such that one droplet produces the substrate for a reaction occurring in the adjacent droplet.[45] Complex multicompartment systems of this ilk may provide a new way of building soft-tissue-like materials with advanced functionalities. Indeed, more recently additive printing techniques have been used to produce 3D-patterned, interconnected networks of lipid-bounded structures functionalized with transmembrane proteins, with compartment communication being driven by solute exchange, osmotic pressure, or membrane proteins (Figure 5B).[88] 3D patterning is also an emerging technique used to print aqueous droplets containing mammalian cells. Such droplets can be used to produce robust, patterned constructs in oil, which can then be transferred to culture medium without any loss in (high) viability. This type of printed tissue has been used to generate cartilage-like structures containing type 2 collagen.[89] Furthermore, 3D culture formats can mimic the native cellular microenvironment in the form of hydrogel matrices[90] or through the generation of functional aggregates such as spheroids[91,92] or organoids.[93] This type of 3D cell culture has been discussed in detail elsewhere.[94]

DROPLET CHARACTERIZATION FACILITATED BY MICROFLUIDICS

As described, microfluidic tools allow for the controlled generation and activation of individual droplets and hierarchical structures. In addition, the same tools can be tuned and engineered to handle and characterize artificial cells in a high-content and high-throughput manner. Indeed, a diversity of functional droplet operations is possible within microfluidic formats. For example, large numbers of droplets may be stored and incubated on- or off-chip for analysis over extended periods of time[95] (Figure 6A). Additionally, and as previously noted, droplets can be formed and their internal contents mixed at high throughput.[96] This may then be followed by processes such as droplet merging, sorting, splitting, and dilution, all performed in a bespoke manner.[97–100] Finally, analytes can be added to droplets post formation

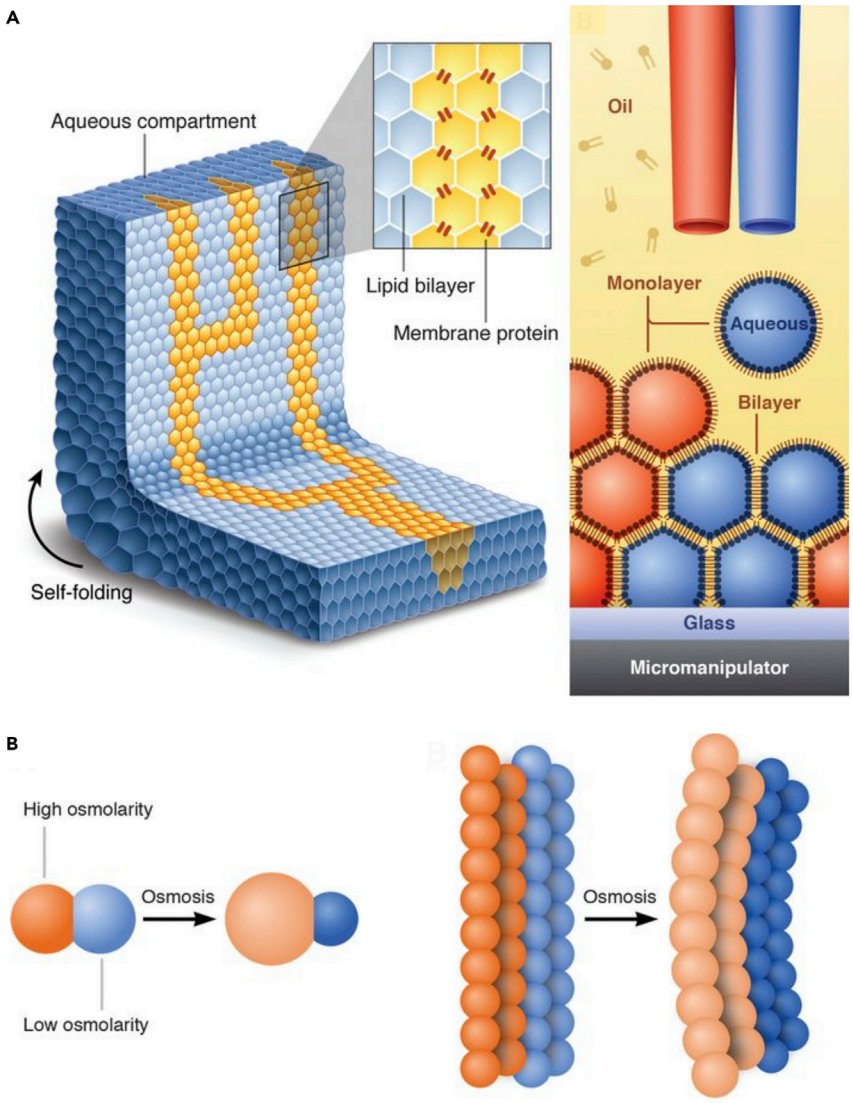

Figure 5. Printed Droplet Networks

(A) Top: illustration of a printed droplet network and schematic of the printing process.

(B) Self-folding droplet networks. Two droplets of different osmolarities are joined by a lipid bilayer. The flow of water through the bilayer causes the droplets to swell or shrink, and a droplet network comprises two strips of droplets of different osmolarities. Reprinted with permission from Villar et al.[88] Copyright 2013 AAAS.

using picoinjectors[75] (Figure 6B). All of the above operations ensure that droplet-based platforms are proficient at executing a range of biological and chemical experiments in an efficient, integrated, and rapid manner. It should also be remembered that valves[101] can be incorporated into elastomeric devices to allow the immobilization and isolation of individual lipid vesicles. For example, by combining a trapping system[102] and valve that switches between "flow" and "stationary" states, single liposomes can be isolated from other parts of a system,[103] providing an elegant and quick solution to buffer exchange and the incorporation of reactants within liposomes. Moreover, analytical techniques such as fluorescence-activated cell sorting (FACS) can be transferred to chip-based formats to allow for fluorescence-activated droplet sorting (FADS).[104] Indeed, droplets produced by

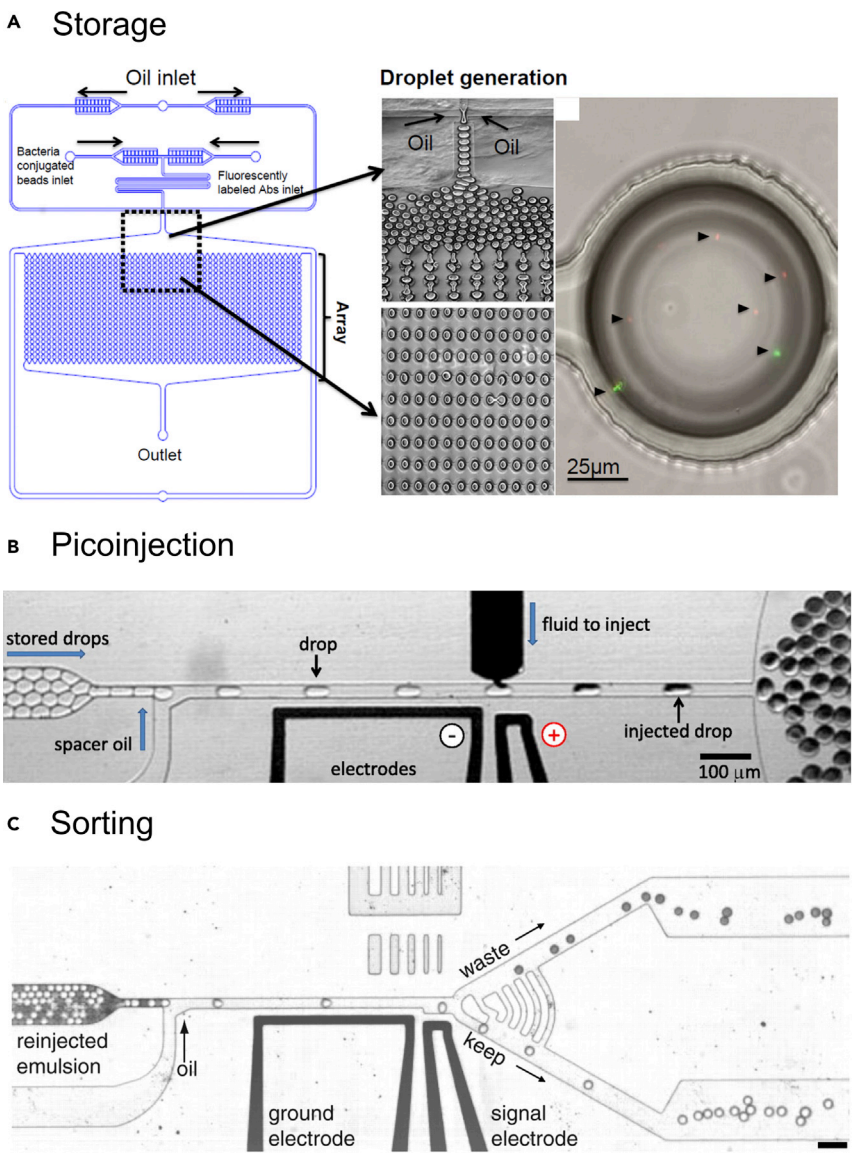

Figure 6. Droplet Unit Operations

(A) On-chip droplet incubation using arrays. Reprinted from Golberg et al.[95]

(B) Electric field triggered picoinjection. Reprinted with permission from Abate et al.[75] Copyright 2010 National Academy of Sciences.

(C) Fluorescence-activated droplet sorting. Reprinted with permission from Agresti et al.[111] Copyright 2010 National Academy of Sciences.

microfluidic methods can be sorted on-chip at extremely high rates (up to 30 kHz).[105] Droplet sorting, as with picoinjection, is accomplished by applying an external electromagnetic field to passing droplets, resulting in a dielectrophoretic force, which maneuvers selected droplets away from the main population. Combining microfluidic sorting with activated artificial cells, such as those described using cell-free expression systems, allows the efficient and high-throughput selection of droplets with specific properties. Although the most common readout methods are based on fluorescence, Gielen et al. have recently shown that sorting can also be achieved using absorbance signals (Figure 6C).[98] This significantly extends the capabilities of

microfluidic sorting platforms, although it should be noted that absorbance-based sorting schemes typically operate at rates below 300 Hz.

Finally, compartment studies are not only restricted to droplet-based systems; compartment simulation is achievable through the fabrication of micron-sized wells.[106] For example, Tayar et al. fabricated arrays of patterned gene circuits within 6 pL compartments. These were connected through a capillary channel to each other and to a perpendicular flow channel, allowing spatially localized protein synthesis to be initiated by diffusion of cell extract into given compartments. This is a beautiful example of activated compartments devoid of droplets and offers a lateral route to probe reaction-diffusion effects on compartmentalized cell-free expression.

CONCLUSIONS

Droplet-based microfluidic tools permit the controllable generation of micron-sized compartments from a range of different molecules, characterization and analysis of large numbers of such compartments, alteration of chemical and biological parameters, and the generation of network-based systems where cellular entities can interact with each other. The modular aspect of microfluidic components allows the performance of complex operations that would be difficult or impossible to achieve using conventional tools. For example, droplet generation, picoinjection, and sorting can be easily combined within a monolithic substrate. While these engineering methodologies are clearly advantageous for bottom-up synthetic biology, the artificial cells generated with these techniques are not only restricted to mimicking natural cells but also can be utilized for other purposes in modern biology, pharmaceutical science, biotechnology, and origin-of-life studies. Furthermore, microfluidic tools have enabled strategies toward whole organ-on-the-chip systems,[107,108] which in the future may transform the process of drug screening. This will not only help in preclinical testing of drug substances and toxicological studies but also help in producing more reliable preclinical pharmacokinetic and pharmacodynamic data. It is therefore important that engineers and natural scientists work together in an interdisciplinary way to realize the full potential of microfluidics for artificial cell synthesis and beyond.

REFERENCES AND NOTES

1. Buddingh', B.C., and Van Hest, J.C.M. (2017). Artificial cells: synthetic compartments with life-like functionality and adaptivity. Acc. Chem. Res. *50*, 769–777.
2. Luisi, P.L. (1998). About various definitions of life. Orig. Life Evol. Biosph. *28*, 613–622.
3. Ganti, T. (2003). The Principles of Life (Oxford University Press).
4. Pak, C.W., Kosno, M., Holehouse, A.S., Padrick, S.B., Mittal, A., Ali, R., Yunus, A.A., Liu, D.R., Pappu, R.V., and Rosen, M.K. (2016). Sequence determinants of intracellular phase separation by complex coacervation of a disordered protein. Mol. Cell *63*, 72–85.
5. Protter, D.S.W., Rao, B.S., Van Treeck, B., Lin, Y., Mizoue, L., Rosen, M.K., and Parker, R. (2018). Intrinsically disordered regions can contribute promiscuous interactions to RNP granule assembly. Cell Rep. *22*, 1401–1412.
6. Friedland, A.E., Lu, T.K., Wang, X., Shi, D., Church, G., and Collins, J.J. (2009). Synthetic gene networks that count. Science *324*, 1199–1202.
7. Alberti, S. (2017). Phase separation in biology. Curr. Biol. *27*, R1097–R1102.
8. Thompson, K.L., Williams, M., and Armes, S.P. (2015). Colloidosomes: synthesis, properties and applications. J. Colloid Interface Sci. *447*, 217–228.
9. Martino, C., Lee, T.Y., Kim, S.-H., and Demello, A.J. (2015). Microfluidic generation of PEG-b-PLA polymersomes containing alginate-based core hydrogel. Biomicrofluidics *9*, 024101.
10. Huang, X., Li, M., Green, D.C., Williams, D.S., Patil, A.J., and Mann, S. (2013). Interfacial assembly of protein–polymer nano-conjugates into stimulus-responsive biomimetic protocells. Nat. Commun. *4*, 1–9.
11. Dora Tang, T.Y., Rohaida Che Hak, C., Thompson, A.J., Kuimova, M.K., Williams, D.S., Perriman, A.W., and Mann, S. (2014). Fatty acid membrane assembly on coacervate microdroplets as a step towards a hybrid protocell model. Nat. Chem. *6*, 527–533.
12. Li, M., Green, D.C., Anderson, J.L.R., Binks, B.P., and Mann, S. (2011). In vitro gene expression and enzyme catalysis in bio-inorganic protocells. Chem. Sci. *2*, 1739.
13. Qiao, Y., Li, M., Booth, R., and Mann, S. (2017). Predatory behaviour in synthetic protocell communities. Nat. Chem. *9*, 110–119.
14. Liu, X., Zhou, P., Huang, Y., Li, M., Huang, X., and Mann, S. (2016). Hierarchical proteinosomes for programmed release of multiple components. Angew. Chem. Int. Ed. *55*, 7095–7100.
15. Elvira, K.S., i Solvas, X.C., Wootton, R.C.R., and deMello, A.J. (2013). The past, present and potential for microfluidic reactor technology in chemical synthesis. Nat. Chem. *5*, 905–915.
16. Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell *161*, 1202–1214.

17. Klein, A.M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D.A., and Kirschner, M.W. (2015). Droplet barcoding for single-cell. transcriptomics applied to embryonic stem cells. Cell *161*, 1187–1201.

18. Shembekar, N., Hu, H., Eustace, D., and Merten, C.A. (2018). Single-cell droplet microfluidic screening for antibodies specifically binding to target cells. Cell Rep. *22*, 2206–2215.

19. Baraban, L., Bertholle, F., Salverda, M.L.M., Bremond, N., Panizza, P., Baudry, J., de Visser, J.A.G.M., and Bibette, J. (2011). Millifluidic droplet analyser for microbiology. Lab Chip *11*, 4057–4062.

20. Damodaran, S.P., Eberhard, S., Boitard, L., Rodriguez, J.G., Wang, Y., Bremond, N., Baudry, J., Bibette, J., and Wollman, F.A. (2015). A millifluidic study of cell-to-cell heterogeneity in growth-rate and cell-division capability in populations of isogenic cells of Chlamydomonas reinhardtii. PLoS One *10*, e0118987.

21. Weitz, M., Mückl, A., Kapsner, K., Berg, R., Meyer, A., and Simmel, F.C. (2014). Communication and computation by bacteria compartmentalized within microemulsion droplets. J. Am. Chem. Soc. *136*, 72–75.

22. Micro Chem. SU-8 2000 Permanent Epoxy Negative Photoresist Processing GUIDELINES FOR SU-8 2025, SU-8 2035, SU-8 2050 and SU-8 2075. http://microchem.com/pdf/SU-82000DataSheet2025thru2075Ver4.pdf.

23. Lan, F., Haliburton, J.R., Yuan, A., and Abate, A.R. (2016). Droplet barcoding for massively parallel single-molecule deep sequencing. Nat. Commun. *7*, 11784.

24. Skelley, A.M., Kirak, O., Suh, H., Jaenisch, R., and Voldman, J. (2009). Microfluidic control of cell pairing and fusion. Nat. Methods *6*, 147–152.

25. Lee, J.N., Park, C., and Whitesides, G.M. (2003). Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices. Anal. Chem. *75*, 6544–6554.

26. Flueckiger, J., Bazargan, V., Stoeber, B., and Cheung, K.C. (2011). Characterization of postfabricated parylene C coatings inside PDMS microdevices. Sens. Actuators B. Chem. *160*, 864–874.

27. Abate, A.R., Lee, D., Do, T., Holtze, C., and Weitz, D.A. (2008). Glass coating for PDMS microfluidic channels by sol-gel methods. Lab Chip *8*, 516–518.

28. Yang, T., Choo, J., Stavrakis, S., and de Mello, A. (2018). Fluoropolymer-coated PDMS microfluidic devices for application in organic synthesis. Chem. Eur. J. *24*, 12078–12083.

29. Anna, S.L., Bontoux, N., and Stone, H.A. (2003). Formation of dispersions using "flow focusing" in microchannels. Appl. Phys. Lett. *82*, 364–366.

30. Thorsen, T., Roberts, R.W., Arnold, F.H., and Quake, S.R. (2001). Dynamic pattern formation in a vesicle-generating microfluidic device. Phys. Rev. Lett. *86*, 4163–4166.

31. Gu, H., Duits, M.H.G., and Mugele, F. (2011). Droplets formation and merging in two-phase flow microfluidics. Int. J. Mol. Sci. *12*, 2572–2597.

32. Günther, A., and Jensen, K.F. (2006). Multiphase microfluidics: from flow characteristics to chemical and materials synthesis. Lab Chip *6*, 1487–1503.

33. Shinoda, K., Hato, M., and Hayashi, T. (1972). Physicochemical properties of aqueous solutions of fluorinated surfactants. J. Phys. Chem. *76*, 909–914.

34. Wang, K., Lu, Y.C., Xu, J.H., and Luo, G.S. (2009). Determination of dynamic interfacial tension and its effect on droplet formation in the T-shaped microdispersion process. Langmuir *25*, 2153–2158.

35. Shui, L., van den Berg, A., and Eijkel, J.C.T. (2009). Interfacial tension controlled W/O and O/W 2-phase flows in microchannel. Lab Chip *9*, 795–801.

36. RAN Biotechnologies. Flurosurfactants for microfluidics. https://www.ranbiotechnologies.com.

37. RainDance Technologies. |Liquid biopsy| Digital PCR| Sequencing. http://raindancetech.com.

38. Dinsmore, A.D., Hsu, M.F., Nikolaides, M.G., Marquez, M., Bausch, A.R., and Weitz, D.A. (2002). Colloidosomes: selectively permeable capsules composed of colloidal particles. Science *298*, 1006–1009.

39. Sander, J.S., and Studart, A.R. (2011). Monodisperse functional colloidosomes with tailored nanoparticle shells. Langmuir *27*, 3301–3307.

40. Umbanhowar, P.B., Prasad, V., and Weitz, D.A. (2000). Monodisperse emulsion generation via drop break off in a coflowing stream. Langmuir *16*, 347–351.

41. Shah, R.K., Kim, J.W., and Weitz, D.A. (2010). Monodisperse stimuli-responsive colloidosomes by self-assembly of microgels in droplets. Langmuir *26*, 1561–1565.

42. Sander, J.S., and Studart, A.R. (2013). Nanoparticle-filled complex colloidosomes for tunable cargo release. Langmuir *29*, 15168–15173.

43. Chu, L.Y., Utada, A.S., Shah, R.K., Kim, J.W., and Weitz, D.A. (2007). Controllable monodisperse multiple emulsions. Angew. Chem. Int. Ed. *46*, 8970–8974.

44. Wang, W., Zhang, M.J., Xie, R., Ju, X.J., Yang, C., Mou, C.L., Weitz, D.A., and Chu, L.Y. (2013). Hole-shell microparticles from controllably evolved double emulsions. Angew. Chem. Int. Ed. *52*, 8084–8087.

45. Elani, Y., Law, R.V., and Ces, O. (2014). Vesicle-based artificial cells as chemical microreactors with spatially segregated reaction pathways. Nat. Commun. *5*, 5305.

46. Ota, S., Yoshizawa, S., and Takeuchi, S. (2009). Microfluidic formation of monodisperse, cell-sized, and unilamellar vesicles. Angew. Chem. Int. Ed. *48*, 6533–6537.

47. Deshpande, S., Caspi, Y., Meijering, A.E.C., and Dekker, C. (2016). Octanol-assisted liposome assembly on chip. Nat. Commun. *7*, 10447.

48. Pautot, S., Frisken, B.J., and Weitz, D.A. (2003). Production of unilamellar vesicles using an inverted emulsion. Langmuir *19*, 2870–2879.

49. Deshpande, S., and Dekker, C. (2018). On-chip microfluidic production of cell-sized liposomes. Nat. Protoc. *13*, 856–874.

50. Shum, H.C., Lee, D., Yoon, I., Kodger, T., and Weitz, D.A. (2008). Double emulsion templated monodisperse phospholipid vesicles. Langmuir *24*, 7651–7653.

51. Kuribayashi, K., Tresset, G., Coquet, P., Fujita, H., and Takeuchi, S. (2006). Electroformation of giant liposomes in microfluidic channels. Meas. Sci. Technol. *17*, 3121–3126.

52. Dittrich, P.S., Heule, M., Renaud, P., and Manz, A. (2006). On-chip extrusion of lipid vesicles and tubes through microsized apertures. Lab Chip *6*, 488–493.

53. Sugiura, S., Kuroiwa, T., Kagota, T., Nakajima, M., Sato, S., Mukataka, S., Walde, P., and Ichikawa, S. (2008). Novel method for obtaining homogeneous giant vesicles from a monodisperse water-in-oil emulsion prepared with a microfluidic device. Langmuir *24*, 4581–4588.

54. Pautot, S., Frisken, B.J., and Weitz, D.A. (2003). Engineering asymmetric vesicles. Proc. Natl. Acad. Sci. USA *100*, 10718–10721.

55. Van Swaay, D., and Demello, A. (2013). Microfluidic methods for forming liposomes. Lab Chip *13*, 752–767.

56. Chemocare. Doxil. Drug information. http://chemocare.com/chemotherapy/drug-info/doxil.aspx.

57. LoPresti, C., Lomas, H., Massignani, M., Smart, T., and Battaglia, G. (2009). Polymersomes: Nature inspired nanometer sized compartments. J. Mater. Chem. *19*, 3576.

58. Gaitzsch, J., Appelhans, D., Wang, L., Battaglia, G., and Voit, B. (2012). Synthetic bio-nanoreactor: mechanical and chemical control of polymersome membrane permeability. Angew. Chem. Int. Ed. *51*, 4448–4451.

59. Ho, C.S., Kim, J.W., and Weitz, D.A. (2008). Microfluidic fabrication of monodisperse biocompatible and biodegradable polymersomes with controlled permeability. J. Am. Chem. Soc. *130*, 9543–9549.

60. van Swaay, D., Tang, T.Y.D., Mann, S., and DeMello, A. (2015). Microfluidic formation of membrane-free aqueous coacervate droplets in water. Angew. Chem. Int. Ed. *54*, 8398–8401.

61. Zuidam, N.J., and Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them. In Encapsulation Technologies for Active Food Ingredients and Food Processing (Springer), pp. 3–29.

62. Douliez, J.P., Martin, N., Gaillard, C., Beneyton, T., Baret, J.C., Mann, S., and Beven, L. (2017). Catanionic coacervate

droplets as a surfactant-based membrane-free protocell model. Angew. Chem. Int. Ed. *56*, 13689–13693.

63. Teh, S.Y., Lin, R., Hung, L.H., and Lee, A.P. (2008). Droplet microfluidics. Lab Chip *8*, 198–220.

64. Gruner, P., Riechers, B., Semin, B., Lim, J., Johnston, A., Short, K., and Baret, J.C. (2016). Controlling molecular transport in minimal emulsions. Nat. Commun. *7*, 10392.

65. Nuti, N., Verboket, P.E., and Dittrich, P.S. (2017). Multivesicular droplets: a cell model system to study compartmentalised biochemical reactions. Lab Chip *17*, 3112–3119.

66. Ugrinic, M., Zambrano, A., Berger, S., Mann, S., Tang, T.D., and Demello, A. (2018). Microfluidic formation of proteinosomes. Chem. Commun. *54*, 287–290.

67. Noireaux, V., Maeda, Y.T., and Libchaber, A. (2011). Development of an artificial cell, from self-organization to computation and self-reproduction. Proc. Natl. Acad. Sci. USA *108*, 3473–3480.

68. Chalmeau, J., Monina, N., Shin, J., Vieu, C., and Noireaux, V. (2011). α-Hemolysin pore formation into a supported phospholipid bilayer using cell-free expression. Biochim. Biophys. Acta *1808*, 271–278.

69. Noireaux, V., and Libchaber, A. (2004). A vesicle bioreactor as a step toward an artificial cell assembly. Proc. Natl. Acad. Sci. USA *101*, 17669–17674.

70. Noireaux, V., Bar-Ziv, R., and Libchaber, A. (2003). Principles of cell-free genetic circuit assembly. Proc. Natl. Acad. Sci. USA *100*, 12672–12677.

71. Sokolova, E., Spruijt, E., Hansen, M.M.K., Dubuc, E., Groen, J., Chokkalingam, V., Piruska, A., Heus, H.A., and Huck, W.T.S. (2013). Enhanced transcription rates in membrane-free protocells formed by coacervation of cell lysate. Proc. Natl. Acad. Sci. USA *110*, 11692–11697.

72. Galinis, R., Stonyte, G., Kiseliovas, V., Zilionis, R., Studer, S., Hilvert, D., Janulaitis, A., and Mazutis, L. (2016). DNA nanoparticles for improved protein synthesis in vitro. Angew. Chem. Int. Ed. *55*, 3120–3123.

73. Dora Tang, T.-Y., van Swaay, D., deMello, A., Ross Anderson, J.L., and Mann, S. (2015). *In vitro* gene expression within membrane-free coacervate protocells. Chem. Commun. *51*, 11429–11432.

74. Park, N., Um, S.H., Funabashi, H., Xu, J., and Luo, D. (2009). A cell-free protein-producing gel. Nat. Mater. *8*, 432–437.

75. Abate, A.R., Hung, T., Mary, P., Agresti, J.J., and Weitz, D.A. (2010). High-throughput injection with microfluidics using picoinjectors. Proc. Natl. Acad. Sci. USA *107*, 19163–19166.

76. Douliez, J.P., Martin, N., Beneyton, T., Eloi, J.C., Chapel, J.P., Navailles, L., Baret, J.C., Mann, S., and Béven, L. (2018). Preparation of swellable hydrogel-containing colloidosomes from aqueous two-phase pickering emulsion droplets. Angew. Chem. Int. Ed. *57*, 7780–7784.

77. Ng, E.X., Miller, M.A., Jing, T., Lauffenburger, D.A., and Chen, C.H. (2015). Low-volume multiplexed proteolytic activity assay and inhibitor analysis through a pico-injector array. Lab Chip *15*, 1153–1159.

78. Beneyton, T., Thomas, S., Griffiths, A.D., Nicaud, J.M., Drevelle, A., and Rossignol, T. (2017). Droplet-based microfluidic high-throughput screening of heterologous enzymes secreted by the yeast Yarrowia lipolytica. Microb. Cell Fact. *16*, 18.

79. Chen, A.H., and Silver, P.A. (2012). Designing biological compartmentalization. Trends Cell Biol. *22*, 662–670.

80. Deng, N.N., Yelleswarapu, M., Zheng, L., and Huck, W.T.S. (2017). Microfluidic assembly of monodisperse vesosomes as artificial cell models. J. Am. Chem. Soc. *139*, 587–590.

81. Greive, S.J., Weitzel, S.E., Goodarzi, J.P., Main, L.J., Pasman, Z., and von Hippel, P.H. (2008). Monitoring RNA transcription in real time by using surface plasmon resonance. Proc. Natl. Acad. Sci. USA *105*, 3315–3320.

82. Deng, N.N., and Huck, W.T.S. (2017). Microfluidic formation of monodisperse coacervate organelles in liposomes. Angew. Chem. Int. Ed. *56*, 9736–9740.

83. Weiss, M., Frohnmayer, J.P., Benk, L.T., Haller, B., Janiesch, J.W., Heitkamp, T., Börsch, M., Lira, R.B., Dimova, R., Lipowsky, R., et al. (2018). Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. Nat. Mater. *17*, 89–96.

84. Elani, Y., Trantidou, T., Wylie, D., Dekker, L., Polizzi, K., Law, R.V., and Ces, O. (2018). Constructing vesicle-based artificial cells with embedded living cells as organelle-like modules. Sci. Rep. *8*.

85. Rabanel, J.M., Banquy, X., Zouaoui, H., Mokhtar, M., and Hildgen, P. (2009). Progress technology in microencapsulation methods for cell therapy. Biotechnol. Prog. *25*, 946–963.

86. Schmidt-Dannert, C., and Lopez-Gallego, F. (2016). A roadmap for biocatalysis – functional and spatial orchestration of enzyme cascades. Microb. Biotechnol. *9*, 601–609.

87. Villar, G., Heron, A.J., and Bayley, H. (2011). Formation of droplet networks that function in aqueous environments. Nat. Nanotechnol. *6*, 803–808.

88. Villar, G., Graham, A.D., and Bayley, H. (2013). A tissue-like printed material. Science *340*, 48–52.

89. Graham, A.D., Olof, S.N., Burke, M.J., Armstrong, J.P.K., Mikhailova, E.A., Nicholson, J.G., Box, S.J., Szele, F.G., Perriman, A.W., and Bayley, H. (2017). High-resolution patterned cellular constructs by droplet-based 3D printing. Sci. Rep. *7*, 7004.

90. Ranga, A., Gobaa, S., Okawa, Y., Mosiewicz, K., Negro, A., and Lutolf, M.P. (2014). 3D niche microarrays for systems-level analyses of cell fate. Nat. Commun. *5*, 4324.

91. Bartosh, T.J., Ylöstalo, J.H., Mohammadipoor, A., Bazhanov, N., Coble, K., Claypool, K., Lee, R.H., Choi, H., and Prockop, D.J. (2010). Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc. Natl. Acad. Sci. USA *107*, 13724–13729.

92. Tomasi, R., Sart, S.S., Champetier, T., and Baroud, C.N. (2018). Studying 3D cell cultures in a microfluidic droplet array under multiple time-resolved conditions. bioRxiv. https://doi.org/10.1101/407759.

93. Lancaster, M.A., Corsini, N.S., Wolfinger, S., Gustafson, E.H., Phillips, A.W., Burkard, T.R., Otani, T., Livesey, F.J., and Knoblich, J.A. (2017). Guided self-organization and cortical plate formation in human brain organoids. Nat. Biotechnol. *35*, 659–666.

94. Moshksayan, K., Kashaninejad, N., Warkiani, M.E., Lock, J.G., Moghadas, H., Firoozabadi, B., Saidi, M.S., and Nguyen, N.-T. (2018). Spheroids-on-a-chip: recent advances and design considerations in microfluidic platforms for spheroid formation and culture. Sens. Actuators B Chem. *263*, 151–176.

95. Golberg, A., Linshiz, G., Kravets, I., Stawski, N., Hillson, N.J., Yarmush, M.L., Marks, R.S., and Konry, T. (2014). Cloud-enabled microscopy and droplet microfluidic platform for specific detection of Escherichia coli in water. PLoS One *9*, e86341.

96. Bringer, M.R., Gerdts, C.J., Song, H., Tice, J.D., and Ismagilov, R.F. (2004). Microfluidic systems for chemical kinetics that rely on chaotic mixing in droplets. Philos. Trans. A Math. Phys. Eng. Sci. *362*, 1087–1104.

97. Varma, V.B., Ray, A., Wang, Z.M., Wang, Z.P., and Ramanujan, R.V. (2016). Droplet merging on a lab-on-a-chip platform by uniform magnetic fields. Sci. Rep. *6*, 37671.

98. Gielen, F., Hours, R., Emond, S., Fischlechner, M., Schell, U., and Hollfelder, F. (2016). Ultrahigh-throughput–directed enzyme evolution by absorbance-activated droplet sorting (AADS). Proc. Natl. Acad. Sci. USA *113*, E7383–E7389.

99. Abate, A.R., and Weitz, D.A. (2011). Faster multiple emulsification with drop splitting. Lab Chip *11*, 1911.

100. Niu, X., Gielen, F., Edel, J.B., and deMello, A.J. (2011). A microdroplet dilutor for high-throughput screening. Nat. Chem. *3*, 437–442.

101. Unger, M.A., Chou, H.P., Thorsen, T., Scherer, A., and Quake, S.R. (2000). Monolithic microfabricated valves and pumps by multilayer soft lithography. Science *288*, 113–116.

102. Tan, W.H., and Takeuchi, S. (2007). A TRAP-and-release integrated microfluidic system for dynamic microarray applications. Proc. Natl. Acad. Sci. USA *104*, 1146–1151.

103. Robinson, T., Kuhn, P., Eyer, K., and Dittrich, P.S. (2013). Microfluidic trapping of giant unilamellar vesicles to study transport through a membrane pore. Biomicrofluidics *7*, 044105.

104. Baret, J.C., Miller, O.J., Taly, V., Ryckelynck, M., El-Harrak, A., Frenz, L., Rick, C., Samuels, M.L., Hutchison, J.B., Agresti, J.J., et al. (2009). Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. Lab Chip *9*, 1850–1858.

105. Sciambi, A., and Abate, A.R. (2015). Accurate microfluidic sorting of droplets at 30 kHz. Lab Chip *15*, 47–51.

106. Tayar, A.M., Karzbrun, E., Noireaux, V., and Bar-Ziv, R.H. (2015). Propagating gene expression fronts in a one-dimensional coupled system of artificial cells. Nat. Phys. *11*, 1037–1041.

107. Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., and Ingber, D.E. (2010). Reconstituting organ-level lung functions on a chip. Science *328*, 1662–1668.

108. Musah, S., Dimitrakakis, N., Camacho, D.M., Church, G.M., and Ingber, D.E. (2018). Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a glomerulus chip. Nat. Protoc. *13*, 1662–1685.

109. Vieregg, J.R., and Tang, T.D. (2016). Polynucleotides in cellular mimics: coacervates and lipid vesicles. Curr. Opin. Colloid Interface Sci. *26*, 50–57.

110. Banani, S.F., Lee, H.O., Hyman, A.A., and Rosen, M.K. (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. *18*, 285–298.

111. Agresti, J.J., Antipov, E., Abate, A.R., Ahn, K., Rowat, A.C., Baret, J.C., Marquez, M., Klibanov, A.M., Griffiths, A.D., and Weitz, D.A. (2010). Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. Proc. Natl. Acad. Sci. USA *107*, 4004–4009.