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Droplet microfluidics: a tool for biology, chemistry and nanotechnology

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Abstract
The ability to perform laboratory operations on small scales using miniaturized devices provides numerous benefits, including reduced quantities of reagents and waste as well as increased portability and controllability of assays. These operations can involve reaction components in the solution phase and as a result, their miniaturization can be accomplished through microfluidic approaches. One such approach, droplet microfluidics, provides a high-throughput platform for a wide range of assays and approaches in chemistry, biology and nanotechnology. We highlight recent advances in the application of droplet microfluidics in chip-based technologies, such as single-cell analysis tools, small-scale cell cultures, in-droplet chemical synthesis, high-throughput drug screening, and nanodevice fabrication.

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Droplet microfluidics: A tool for biology, chemistry and nanotechnology

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ABSTRACT

The ability to perform laboratory operations on small scales using miniaturized devices provides numerous benefits, including reduced quantities of reagents and waste as well as increased portability and controllability of assays. These operations can involve reaction components in the solution phase and as a result, their miniaturization can be accomplished through microfluidic approaches. One such approach, droplet microfluidics, provides a high-throughput platform for a wide range of assays and approaches in chemistry, biology and nanotechnology. We highlight recent advances in the application of droplet microfluidics in chip-based technologies, such as single-cell analysis tools, small-scale cell cultures, in-droplet chemical synthesis, high-throughput drug screening, and nanodevice fabrication.

1. Introduction

More than a century ago, the transition from chemistry-in-a-tube to chemistry-in-a-tank approaches resulted in a revolution in the chemical industry. This transformation was a challenging one and chemists and engineers were faced with fundamental questions regarding plant design and scaling [1]. Their efforts were highly successful and chemical manufacturers gained the capability of generating drugs, textiles and other valuable chemicals at large scales. Today, we are witnessing a revolution in the opposite direction in length scale. This new trend is fueled by the need to manipulate precious and rare materials and fabricate portable measurement devices involving liquid-phase reactions. For example, detecting a biomolecule within a small sample from a patient, screening a large library of molecules for a certain function, and the need for mobile health devices has naturally led to the emergence of wet chemistry on a chip.

One can immediately identify fundamental differences between large-scale and small-scale chemistry. The former requires more reagents and generates more products; therefore, it is easy to have a sufficient amount of sample for analytical methods. For very large and inhomogeneous systems, in some cases, many samples might be needed for accurate characterization. It is also costly to set the reaction parameters, such as temperature and mixing [2]. At small scales, the cost of the reagents and the amount of toxic waste that must be handled is greatly reduced. In addition to the differences between large-scale and small-scale chemistry, some tasks that are trivially performed by chemists when working with test tubes are no longer trivial at small or large scales, e.g., mixing reagents and adding a new reagent pose challenges at both large or small scales.

Manipulation of liquids at small length scales is typically done with microfluidic devices. The behavior of liquids at the microscale is in a regime defined by the Reynolds number (*Re*). The Reynolds number, a term first coined by Arnold Sommerfeld [3], refers to the ratio of inertial forces to viscous forces, and in microfluidics, *Re* is equal to or less than unity. Experimental realization of flow in this regime dates to the early 1950s when efforts to dispense sub-nanoliter amounts of liquids were made, providing the basics of current ink-jet technology [4]. The invention of the ink jet was followed by the development of High-Performance Liquid Chromatography (HPLC) and the introduction of microvalves and micropumps during the 1980s. To generate small reaction volumes, the liquid can be split into micro/nano wells. Alternatively, a fluid stream can be split to make small droplets. The advantages of this approach are that it is

easy to transport the liquid drop and that there is a lack of Taylor dispersion, i.e., the absence of a dispersion in the fluid medium as it flows through a tube [4]. The breakup of a continuous flow into drops within a confined environment, such as a microfluidic system, has been realized, and efficient droplet generation protocols have been designed [5].

The physics behind droplet formation has been widely studied for a long time. For droplet formation at macroscopic length scales, the relative effect of viscous forces versus surface tension (defined as the capillary number *Ca*) governs the breakup. Droplet microfluidics is, however, governed by different physics. In microfluidic systems, confinement [6, 7] is a determining factor in droplet formation and has to be considered along with the capillary number [8]. Dominant interfacial and surface tension forces at small scales enable the precise generation and spatial stabilization of droplets. Droplet-based microfluidic systems can be fundamentally categorized into two basic designs: channel-based microfluidics in which the actuation occurs via liquid flows within microfabricated devices and the planar-surface approach where the actuation occurs through electrowetting or dielectrophoresis [4, 9]. The planar-surface approach, sometimes called digital microfluidics, enables manipulation of discrete droplets on an array of electrodes [10]. In this paper, we will focus exclusively on recent advancements in channel-based systems.

2. Technical aspects of droplet engineering

Chemical reactions in nature often occur in an aqueous environment. Thus, setting up biologically relevant reactions at a small scale requires the generation of aqueous microdroplets. Standard methods exist to generate droplets, either in a hydrodynamically driven manner (e.g., flow focusing, co-axial and T-junction geometries) or in a manner driven by an external force (e.g., droplet generation based on an electromagnetic valve that provides the ability to produce a droplet on demand [11] or droplet generation by pneumatic micropumps) [12]. Recently, new methods have been introduced to increase the rate of generating and producing droplets, for example, by introducing gradient confinement [13] or by parallelizing conventional structures [14]. Figure 1 shows key examples of droplet-generating methods. In a parallelized system, structures are ideally subjected to identical flow conditions in each droplet generator. Such a level of uniformity is however challenging because of the feedback between parallel channels. In contrast, droplet generation in the gradient-confinement method does not depend on flow conditions. Recently, a combined strategy was introduced which includes parallelized 3D

generators and principles based on gradient confinement [15]. Developments in droplet-generation technologies are fundamental to the development and application of droplet-based approaches.

Surface chemistry is an important factor in the ability to generate, control and manipulate droplets in microfluidics chips. For full control of the generation, size and movement of aqueous droplets in an organic continuous phase, the surface must be wetted by the continuous phase under a large variety of conditions. A number of materials have been used to build microfluidic structures. These include cyclo-olefin copolymer (COC), para-methoxymethamphetamine (PMMA), fluorinated ethylene propylene (FEP), polycarbonate (PC), and polydimethylsiloxane (PDMS). The choice of the materials is made based on the desired properties. Cyclic-olefin copolymer is optically transparent and has low background fluorescence [16]; PMMA is biologically compatible and gas impermeable [17]; FEP has good solvent resistance [18].

Depending on the type of disperse/continuous phase, surface modifications might be necessary before the structures can be used. Examples include modification by silane (e.g. PEG-silane or silane with fluorinated groups) and polyelectrolyte multilayers (PEMs) that can be used to modulate hydrophobicity, charge and to add biological specificity to surfaces [19]. For multiphase droplet generation, channel surface properties differ at different stages of droplet synthesis, depending on the continuous phase at different positions in the chip, while for single-phase droplet systems typically the continuous phase does not change and a uniform surface chemistry is used along the channel [20, 21].

Surface functionalization of channels and surfactant concentration in the continuous phase affect droplet stability. Too little surfactant may lead to crosstalk between droplets, droplet merging and leakage of materials from droplets [22], while too much surfactant might cause droplet splitting due to low surface tension, or lead to micelle formation and loss of droplet content [23, 24]. Furthermore, one should note that surface functionalization of channels, and surfactant concentration, are related: surfactants can get adsorbed to the channel surface and functional layers desorbed from the surface. This dynamics has not been systematically described in the literature but can profoundly affect the stability of the system.

In organic chemistry, reactions are typically performed in non-aqueous environments. Recently, a simple technique that enables precise loading of droplets of both wetting and non-

wetting liquids was proposed. This technique relies on the use of a combination of compressed inert gas and gravity to exert driving and retracting forces on the liquid [25]. Performing biological reactions, particularly enzymatic reactions, in organic solvents is of interest to industry. Efforts have been made to increase the stability and catalytic activity of certain enzymes by dissolving them in a non-aqueous solvent [26, 27].

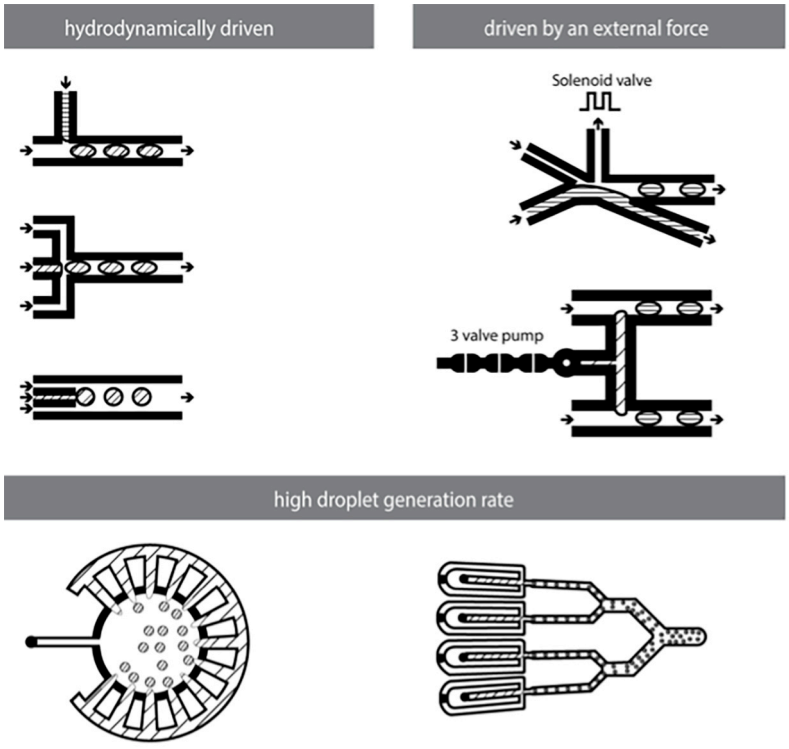

Figure 1. *Schematics of droplet generators. Top panel depicts examples of generators that are driven hydrodynamicaly (top to bottom: T-junction, flow-focusing, co-flow) or based on an external force (top to bottom: generators based on electromagnetic valves, pneumatic micropumps). The bottom panel illustrates examples of generators with high droplet-generation rates: gradient confinement (left) and parallelized conventional structures (right).*

3. Droplet-based cell studies: cell and synthetic biology

Droplet microfluidics opens up new opportunities in biology. More specifically, it facilitates screening and allows for dramatically increased throughput. While bioassays can be miniaturized by using microwells with a picoliter volume, such approaches are technically challenging due to capillary effects and liquid evaporation. Droplet-based cell assays in picoliter volumes have the

advantage that they prevent dilution and evaporation. Cell-based assays include single-cell assays, assays with microculture and assays with synthetic cells.

3.1. Cell biology and tissue engineering

Engineering droplets that contain single or multiple cells with precise control of the cell count is an emerging technology. To incorporate cells within droplets, a bulk liquid containing cells is typically dispersed into droplets. Here, the probability of finding a certain number of cells inside a droplet follows the Poisson distribution [28]. To avoid droplets that lack cells, one should typically have 10^6 cells/ml at the start for a droplet size of approximately 100 micrometer [29]. Prior removal of multi-cellular aggregates leads to a better encapsulation efficiency [30]. In some applications, the encapsulation of two cells is desirable. Such a level of control cannot be precisely accomplished through the Poisson distribution. Recently, through a combination of droplet microfluidics with inertial microfluidics, a more uniform co-encapsulation of cells within droplets was demonstrated [31].

Cells trapped in droplets can be subjected to analysis or manipulation [32, 33]. If the droplet contains enough nutrients, cells can be cultured within droplets [34] (Figure 2). Interestingly, the gas exchange needed for culturing can be readily provided, typically by the use of fluorinated oils with high oxygen permeability or through the device itself. These small cultures can be sorted based on the expression of a certain fluorescent protein or based on the number of cells within a droplet in a label-free manner. The latter has been recently achieved using real-time image-based droplet classification [35]. Electrical impedance measurements allow for fast (> 100 Hz), label-free detection of cells within a droplet with single-cell sensitivity [36]. This approach also allows for discrimination between viable and non-viable cells. Moreover, using this method, rare cell types may be sorted from a large pool of other cells, a strategy that is particularly relevant to medical oncology, where finding low-frequency cancer cells at the very early stage of cancer or after chemotherapy/radiotherapy is important for therapeutic decisions.

Analysis of cells often requires some preparation and handling, such as lysing the cell to expose intracellular biomolecules to the detection assay. On-chip lysis of cells within droplets can be chemically achieved using a pulsed laser microbeam, using electroporation or using the addition of lysis buffer to the droplets during encapsulation [37].

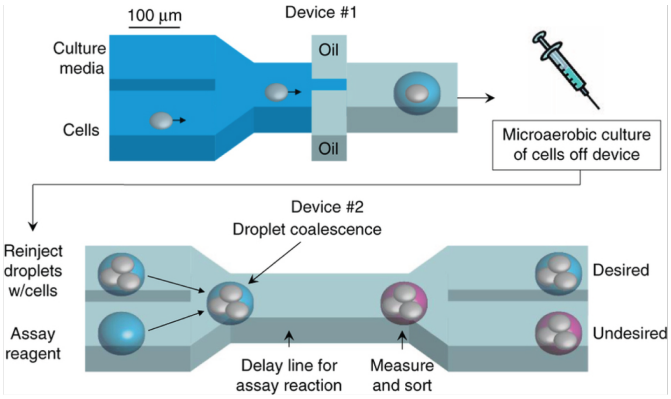

Figure 2. *Droplet-based microfluidics and cell biology. Initially, droplets containing cells and culture media are generated and collected in a syringe that provides a micro-aerobic environment when capped. The syringe is placed in an incubator for cell culturing. Subsequently, the droplets from the syringe are reinjected into another microchannel structure, where they are merged with another set of droplets containing fluorescent enzymatic assay reagents. After a delay, the reaction begins in the merged droplets. The droplet fluorescence is measured using a laser/detector system. Consequently, the extracellular concentration of the metabolite of interest is quantified. This system can screen one to two clones per second. The figure is reproduced, with permission, from Wang et al. [34].*

Preservation of biological materials and medical samples after they are processed in a droplet-based microfluidic platform remains a relatively unexplored area. Various methods, including cryopreservation, chemical fixation, and freeze drying, have been developed to preserve biological samples after they are processed with bulk methods. These techniques can likely be adapted to preserve droplets containing biological materials. For live cells within droplets, it will be important to design protocols for freezing droplets with cells inside and recovering the cells.

In tissue engineering and regenerative medicine, microfluidics has attracted interest due to its use in designing and fabricating organ-like structures. Cellular organizations can be implemented in microfluidic devices in a manner that captures the complexity of real tissues. Microfluidics has also been used to make minimal model systems for certain organs; examples include simple lung-on-chip, kidney–on-chip and eye-on-chip platforms. These structures capture certain mechanical and organizational properties of respective tissues, a capability that was lacking with regular 2D cell culture systems. Most applications of microfluidics use the channel structures as scaffold. In

certain applications, droplets were used as scaffold instead [38-41]; cells can be encapsulated in droplets and allowed to proliferate to form spheroids. Usage of droplets opens interesting opportunities for cell-biology studies. For example, the spheroid size can be used to direct stem-cell behavior [42, 43]. When mesenchymal stem cells are entrapped within droplets, smaller droplet sizes promote homogeneous chondrogenic differentiation towards hyaline chondrocytes whereas larger spheroids producing more heterogeneous tissue [44].

3.2. Synthetic cells

Droplet microfluidics is rapidly becoming an important tool in studies that aim to develop synthetic cells. Various approaches exist to arrive at synthetic cells. Some researchers have explored the possibility of incorporating cellular components into giant lipid vesicles [45]. Others have tried to reconstitute cellular machinery into microfabricated solid wells. For example, microtubule asters and cellular divisomes have been assembled in droplets, and their dynamics visualized (Figure 3a) [46, 47]. Alternatively, one could also use a droplet as a platform in synthetic biology. Several approaches could be considered: 1) Water in an oil droplet. Phospholipids are incorporated in the water-oil interface to mimic the cytoplasmic leaflet of cellular membranes. Cellular components can be added to the droplet interior. 2) Multiphase droplets containing an internal water phase surrounded by a thin layer of the oil phase and residing in an aqueous carrier fluid. By studying the interaction of two droplets, one can study the interaction of synthetic cells [48, 49].

4. Droplet-based biochemistry and chemical biology

Microfluidics platforms are well suited for biochemistry. Enzymatic reactions in particular have been studied in microchannels, and both detection and analysis techniques have been adapted from bulk biochemistry to microchannel biochemistry. For example, Stone *et al*. adapted the Michaelis-Menten kinetic theory to model the kinetics of an enzymatic reaction in a system made of two merging channels; one channel carried the enzyme, and the other carried the substrate [50]. Diffusion and flow rates are included in the new model. Detection techniques, particularly fluorescence methods [51, 52] and more recently vibrational spectroscopy (e.g., ATR-FTIR [53]), have been adapted to extract chemical information from the contents of microfluidic channels in the presence of flow.

Biochemistry in microdroplets is an emerging field with promising applications, including digital PCR, directed evolution approaches, and new drug-screening strategies. High-throughput droplet-based microfluidic platforms have, for example, been used to study the directed evolution of CotA laccase. This technique allows for the evaluation of the distribution of enzymatic activity within a large library, rapid enrichment of a library in active variants at a high-throughput rate, and precise selection of variants depending on their enzymatic activity [54]. Similar to the case of biochemistry in microfluidics, there is a need to demonstrate the applicability of detection and analysis techniques for monitoring the reactions that occur in dynamic droplets.

A combination of droplet-based microfluidics and a reductionist biochemistry approach can be applied to study both virus-host cell interactions and the interaction of a virus with intracellular organelles and may lead to the development of high-throughput antiviral drug screening assays. The virus-host interaction can be studied within each droplet by encapsulating host-like liposomes in droplets and then merging the droplet with a second droplet containing fluorescently labeled viruses and a fusion inhibitor at a range of doses. Viral fusion within the merged droplet can be triggered after the appropriate incubation time. The fusion phenomenon can be studied by fluorescence microscopy, and information regarding the mechanism and kinetics of the fusion in addition to the efficiency of fusion can be extracted by tracking each droplet. Moreover, one can learn about the mechanism of action of an inhibitor by determining the stage at which the fusion process is arrested [55].

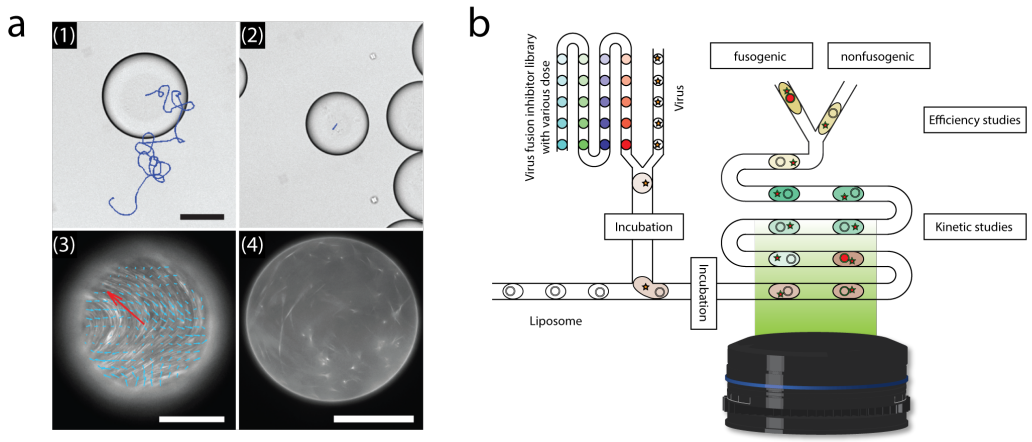

Figure 3. *Droplet-based microfluidics and a synthetic cell. (a) 1. Droplets containing extensible microtubule bundles exhibit spontaneous autonomous motility when partially*

compressed between chamber surfaces. A droplet trajectory recorded over a time interval of 33 min is overlaid on a bright-field droplet image. The scale bar for 1 and 2 is 80 µm. (2) In the absence of ATP, passive droplets exert no internal forces, and the only contribution to their movement is minor drift. (3) Fluorescence image of active microtubule bundles that spontaneously adsorb onto the oil–water interface. The resulting active liquid crystalline phase exhibits streaming flows, indicated with blue arrows. The red arrow indicates the direction of the instantaneous droplet velocity. The image is focused on the droplet surface that is in contact with the coverslip. Scale bar, 100 µm. (4) The image of a droplet taken at a midplane indicates that the droplet interior is largely devoid of microtubule bundles. Scale bar, 100 µm. The figure is reproduced, with permission, from Sanchez et al. [47]. (b) Reconstruction of the interaction of viruses with a host cell within a microdroplet. Microaliquots of a library of drugs and the corresponding titrations are assembled in a droplet that contains the virus. After incubation, the droplets form a complex with the droplets that contain a synthetic host membrane. This step is followed by a second incubation period. The droplets are then sent to the detection module, where the kinetics of binding, hemi-fusion and pore formation are monitored. Finally, the droplets are sorted based on fusogenicity (Figure 3b).

One major application of droplet-microfluidics is in drug screening. Libraries of drugs often contain millions of compounds to be screened. Both continuous-flow and droplet-based flows have been used for drug screening, each having their own advantages and drawbacks. Droplet-based flows offer precise control over sample dispersion and residence time, although gradients are significantly more difficult to establish (for performing dose-response analyses). Droplet-based flows have been increasingly used to assay drugs such as antiviral antibodies [55] and antibiotics [56]. In droplet assays, drugs are often added along with their targets to the dispersed phase before droplet generation occurs. Drug action may start before droplets are formed, or after in triggered systems. The latter has been demonstrated in viral fusion studies in which antibodies and peptides are screened for inhibiting fusion and the reaction is pH triggered [57].

5. Droplet-based chemical synthesis

The development of droplet microfluidics has significantly influenced chemical synthesis, micro/nano fabrication and synthetic biology. Compared to continuous-flow chemistry where

channel fouling could lead to poor product control and reactor failure, droplet chemistry prevents fouling by isolating the reaction from the channel walls [58]. Droplets can be used as chambers within which synthesis occurs; they can be used as a structure that directs agents to generate complex nanostructures [59] and they can be used to generate cell-like structures. Performing reactions within droplets can have a profound impact on the reaction kinetics. For example, when imine synthesis was performed in emulsion droplets, the apparent equilibrium constant and the forward rate constant were found to be inversely proportional to the droplet radius [60]. Compartmentalization affects the reaction thermodynamics at the mesoscale, although there exists no confinement on the molecular scale.

Most synthesis reactions that have been performed within droplets are single-step synthesis reactions [61-65]. One major issue in complex synthesis is that multiple reaction steps are involved. New reagents often must be added to the droplet; this requirement can be met by the fusion of droplets containing the new reagents with the reaction droplet or by the injection of the new reagent into the reaction droplet [58] (Figure 4). However, in many cases material must be removed from the droplets, a step that cannot be achieved by washing but can only be done by splitting off part of the droplet.

An essential requirement for droplet-based chemistry and chemical synthesis is the ability to monitor the content of the reaction chamber over time. Quantitative and qualitative analysis of the droplet content is crucial in the development and application of droplet microfluidics. Various analytical detection techniques have been used to analyze droplet content, including imaging-based methods, laser-based molecular spectroscopy, electrochemical detection, capillary electrophoresis, mass spectrometry, NMR, absorption and chemiluminescence detection [66].

For many analytical methods, preparatory steps are needed before the content of the droplets can be subjected to analysis. For instance, when introducing droplets to analysis by mass spectrometry, a common approach is to transfer the component of interest from the droplet into a carrier phase via diffusive exchange. The carrier phase will then be directed to the mass spectrometer. Alternatively, the droplet can be extracted and introduced directly into an electrospray ionization-mass spectrometry detection system [67]. For infrared spectroscopy, the droplet medium is often IR active and will not allow for detection of the desired signal. The component of interest is then transferred to an IR-transparent liquid before spectroscopic analysis is conducted [68].

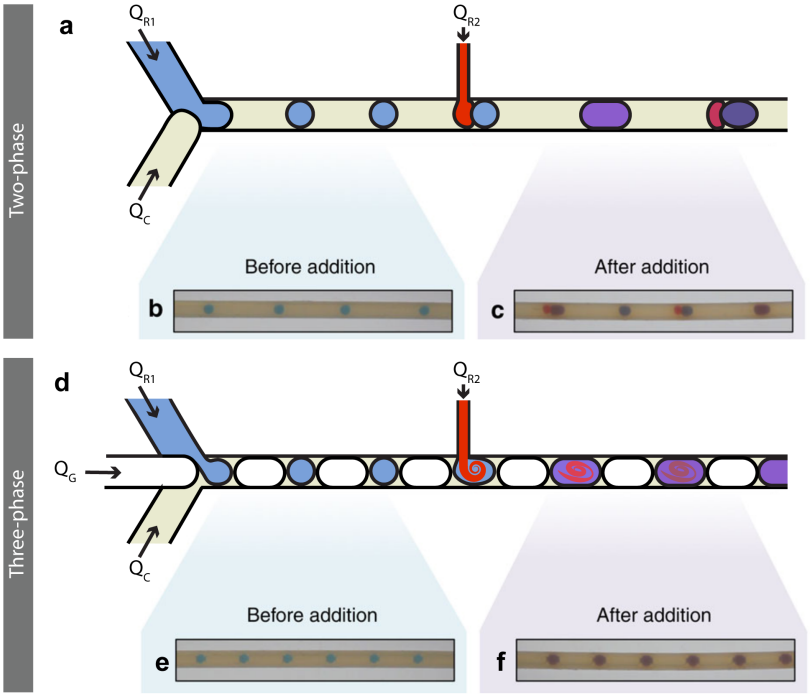

Figure 4. *Droplet-based microfluidics and chemical synthesis. (a) and (d) depict the experimental setups used to compare the efficiency of the direct addition of a reagent (red-dyed solvent) into a two- and three-phase droplet flow (blue droplets). A two- or three-phase stream of blue-dyed ODE droplets is first produced by flowing ODE/PFPE (Q_{R1}/ Q_c) or ODE/PFPE/Ar (Q_{R1}/ Q_c/ Q_g) through a junction. Then, a T-junction is used to inject a stream of red-dyed ODE (Q_{R2}) into the flowing droplets. (b-c) and (e-f) are images of the droplets before and after the addition of the red-dyed solvent for the two- and three-phase flows respectively. The figure is reproduced, with permission, from Nightingale et al. [58].*

6. Droplet-based micro and nanotechnology

Droplets are used in micro and nanotechnology for different reasons. Droplets can be used as (i) containers for material transport, (ii) reaction chambers to fabricate nanomaterials, and (iii) building blocks to make structures [69].

One major area of droplet-based nanotechnology involves the transport of liquid cargo in the form of oil-in-water or water-in-oil emulsion droplets. These platforms move the droplets along various types of tracks, including microfluidic channels, nanotubes and organic planar tracks, such as graphene strips. Microtubules are biological nanotubes that act as the skeleton of cells and

play the role of linear tracks for the movement of molecular motors and the associated cargos. These rigid biological polymers have been widely used in bio-nanotechnology and microfluidics. In microfluidics, the system was employed to sort, transport and concentrate molecules [70, 71]. However, droplet technology and microtubule-based technologies have only been recently combined to generate lab-on-a-chip devices. Bottier *et al*. used a hybrid platform in which kinesin motors actively carry oil-in-water droplets along microtubules [72]. The transport of droplets along organic tracks has recently attracted attention. Yin *et al*. discovered that voltages on the order of a few millivolts can be produced by moving a droplet of seawater or ionic solution over a strip of monolayer graphene under ambient conditions [73].

When aqueous droplets come in contact with a hot surface, the Leidenfrost effect produces an insulating vapor layer that keeps that water from boiling rapidly. This effect has been recently employed to use droplets as platforms for green chemistry and nanoparticle fabrication [74]. For example, the technology was used to fabricate nanoporous black gold, which acts as a plasmonic wideband superabsorber. Another demonstrated application of this technology is the synthesis of superhydrophilic and thermally resistant metal–polymer hybrid foams.

Water-in-oil droplets can be assembled to form networks. These networks can be designed to perform useful tasks. Lipids and surfactants are typically used to prevent the merging of the contacting droplets [75]. Despite being thin, bilayers form robust interfaces, allowing for flexible network architectures. One can readily excise a droplet from a network and replace it with another one without disrupting the integrity and functioning of the rest of the network [76]. Droplet networks have been designed to act as light sensors, batteries and electrical systems [76, 77]. Networks of aqueous droplets with distinct chemical compositions have also been realized [48]. At the contact sites, pores can be engineered to allow chemical communication between droplets [76]. Inserting electrodes into droplets can modulate the current through these pores via the application of desired voltages across the pores [76]. These pores can be engineered for networks with specific uses; interesting applications include the fabrication of devices such as current limiters, half-wave rectifiers and full-wave rectifiers [76, 77].

An important droplet-based technology is optofluidic lasers, in which biochemical or biological molecules are incorporated into the gain medium [78] (Figure 5). An optofluidic laser consists of three main components: a gain medium in the fluidic environment, an optical cavity and an pumping system. The photons emitted from the gain medium are trapped by the cavity, and the

optical feedback induces stimulated emission. When a sufficiently large number of gain molecules in the cavity are excited by optical pumping, the available gain becomes greater than the total loss in the cavity, and laser oscillation builds up. Optofluidic lasers have enormous potential as biosensors. Driven by a variety of biochemical processes, the number of molecules in the gain medium can be altered. The output laser characteristics depend on the composition of the gain medium and can thus be used as probes for the reaction. A number of published reports describe the use of microfabricated lasers, including a microfluidic dye laser [79] and a laser that could be tuned using digital microfluidics [80].

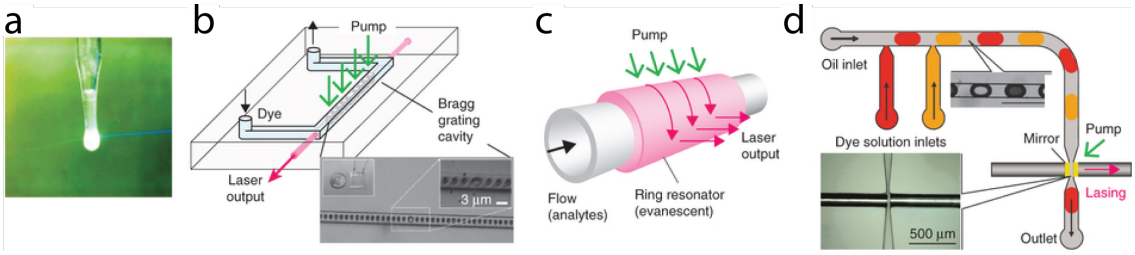

Figure 5. *Droplet-based microfluidics and nanotechnology. (a) Stimulated emission from a droplet of fluorescein disodium salt in gelatin. (b) Optofluidic laser based on a distributed feedback grating embedded in a microfluidic channel. The periodic structures form a pair of virtual mirrors for resonant light to bounce back and forth to provide optical feedback. (c) Optofluidic laser using an evanescently coupled ring resonator. The resonant light circulates along the circumference to provide optical feedback. (d) Optofluidic laser using dye microdroplets and an integrated Fabry-Pérot cavity formed by two reflectors coated on optical fiber tips. The resonant light bounces back and forth between the two reflectors to provide optical feedback. The figure is reproduced, with permission, from Fan et al. and references therein [78].*

7. Conclusions

Much of the world's technology requires fluid manipulation. Micro- and nanotechnologies are aimed at miniaturizing current technologies and developing new ones. The development of these new technologies involve extending those manipulations to small volumes, i.e. microdroplets, while maintaining precise dynamic control over the droplet properties, such as the position, concentration, temperature and stability. Over the last several years, we have witnessed much progress in this direction with many new methodologies developed. Currently, we are able to

conveniently generate small droplets on chips, merge and break them, and under certain conditions, manipulate droplet geometry, chemical contents, and internal flow profiles, while monitoring all these processes *in situ*. Current efforts are primarily focused on translating these proof-of-concept technologies to real applications in biology, chemistry and nanotechnology.

Future steps in developing droplet-based technologies depend on the final applications. For biological and pharmaceutical applications, one major step to be taken is related to compound delivery into droplets at high throughput; millions of droplet-based assays can be performed on one chip per day but materials exchange with drops has not been scaled up yet. Promising developments are underway: we have recently learned how to change the acidity of microdroplets using acids and bases that are soluble both in water and oil [81]. A natural extension of this technology would allow for exchanging redox agents and even enzymes. For organic chemistry applications, a major advance will likely come from adapting microfluidic approaches to organic solvents; for such applications PDMS, even after surface modification, is not suitable for high-throughput industrial use. Although a recent study found that PDMS functionalized with poly-p-xylylene polymers (parylenes) remains functional for several hours [82], glass, poly(methyl methacrylate) (PMMA) or cyclic olefin copolymer (COC), teflon and FEP will likely turn out to be more suitable materials for long-timescale applications with organic solvents [83]. Among emerging areas in droplet-based nanotechnology is the development of methods to assemble nano-particles in fluid flow. Self-assembly reactions could be modulated and programmed by flow in a manner that controls the final products, the kinetics of self-assembly, and the final yield of the reactions. Studying the self-assembly of nano-rods, nano-wires, and nano-spheres in microdroplets may results in not only useful products but also interesting physics.

As droplet-based lab-on-chip technologies become widespread, new practical challenges will emerge. How can we recover droplets without loss? How can droplets be preserved and stored? How can droplets be sent to other laboratories? Perhaps one could freeze droplets containing live cells using similar protocols to those used for freezing/thawing large volumes of cells. Related to logistical challenges, there is a need to develop standardized ways to barcode and package large numbers of droplets. In addition to standardized handling of droplets, there is a need for standard approaches to instrumentation. Chip designs need to be standardized so that one could combine modules and components from different vendors; this is not currently possible in most cases and products from different vendors are often incompatible. In computer hardware technology, a

motherboard interconnects components; future developments can be directed to generate fluidic "motherboards" that support easy assembly of modules from any vendor. Overcoming these challenges in the field promises much-improved platforms for laboratory operations and environment-friendly practices.

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