Samantha M. Desmarais[1]
Henk P. Haagsman[2]
Annelise E. Barron[1]*

[1]Department of Bioengineering, Stanford University, Stanford, CA, USA
[2]Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands

Received April 1, 2012
Revised June 20, 2012
Accepted June 20, 2012

Review

Microfabricated devices for biomolecule encapsulation

Biomolecule encapsulation in droplets is important for miniaturizing biological assays to reduce reagent consumption, cost and time of analysis, and can be most effectively achieved by using microfabricated devices. Microfabricated fluidic devices can generate emulsified drops of uniform size with controlled dimensions and contents. Biological and chemical components such as cells, microgels, beads, hydrogel precursors, polymer initiators, and other droplets can be encapsulated within these drops. Encapsulated emulsions are appealing for a variety of applications since drops can be used as tiny reaction vessels to perform high-throughput reactions at fast rates, consuming minimal sample and solvent amounts due to the small size (micron diameters) of the emulsion drops. Facile mixing and droplet coalescence allow for a diversity of assays to be performed on-chip with tunable parameters. The simplicity of operation and speed of analysis with microencapsulated drops lends itself well to an array of quantitative biomolecular studies such as directed evolution, single-molecule DNA amplification, single-cell encapsulation, high-throughput sequencing, enzyme kinetics, and microfluidic cell culture. This review highlights recent advances in the field of microfabricated encapsulating devices, emphasizing the development of emulsifying encapsulations, device design, and current assays that are performed using encapsulating droplets.

Keywords:
Emulsion / Encapsulation / High throughput / Microdroplet / Microfluidics
DOI 10.1002/elps.201200189

1 Introduction

Microdevice encapsulation of biomolecules is a faster, simpler, and more efficient methodology for the analysis of biomolecules than traditional bench-top assays. Microfabricated encapsulating devices can create microenvironments that mimic larger-scale analyses, making these devices miniature analytical instruments. The development of new microfabricated device instrumentation based on biomolecule encapsulation enables previously uncharacterized (or poorly characterized) biologically active molecules to be analyzed, and launches a world of opportunity for small-scale experimentation in almost every scientific discipline. Microdevice encapsulation utilizes fine control of fluid flow to create emulsions in small-volume microbubbles, which enables precise management of biomolecule interactions. In general, microfluidic encapsulation implements the adage "oil and water do not mix"; due to immiscibility, aqueous droplets pinch off when collided with an oil stream, resulting in an encapsulation of the components present in the aqueous stream (Fig. 1). Oftentimes surfactants are added to the oil phase for enough miscibility with the aqueous phase in order to form microbubbles. This review highlights new platforms and processes to encapsulate biomolecules using microfabricated devices, as well as the assays that are possible to perform using this cutting-edge methodology. Due to the explosion of publications in this field in the last 3 years, only a subset of those innovations can be highlighted in this review. For a more complete accounting of droplet-based microfluidic devices, please see Han et al. [1] and Hahn et al. [2], and for overviews of specific aspects of droplet-based microfabricated devices, please see Baret [3] and Khademhosseini et al. [4].

Water-in-oil encapsulations, described above, are just one of a variety of encapsulations one can perform on-chip. We will highlight oil-in-water encapsulations, as well as multiple encapsulations that provide a way to deliver quantized amounts of reactants over time, mimicking multistep experiments, except with minimal researcher involvement. Droplets containing different reactants can be merged to allow "reactions" on-chip, fusing encapsulating membranes

Correspondence: Dr. Samantha M. Desmarais, Department of Bioengineering, Stanford University, Stanford, CA, USA
E-mail: sdesmar@stanford.edu
Fax: +1-650-723-9801

Abbreviations: CB[8], cucurbit[8]uril; **DMF**, digital microfluidics; **dPCR**, digital PCR; **FACS**, fluorescent-activated cell sorting; **FADS**, fluorescence-activated droplet sorting

*Additional corresponding author: Dr. Annelise Barron E-mail: aebarron@stanford.edu.

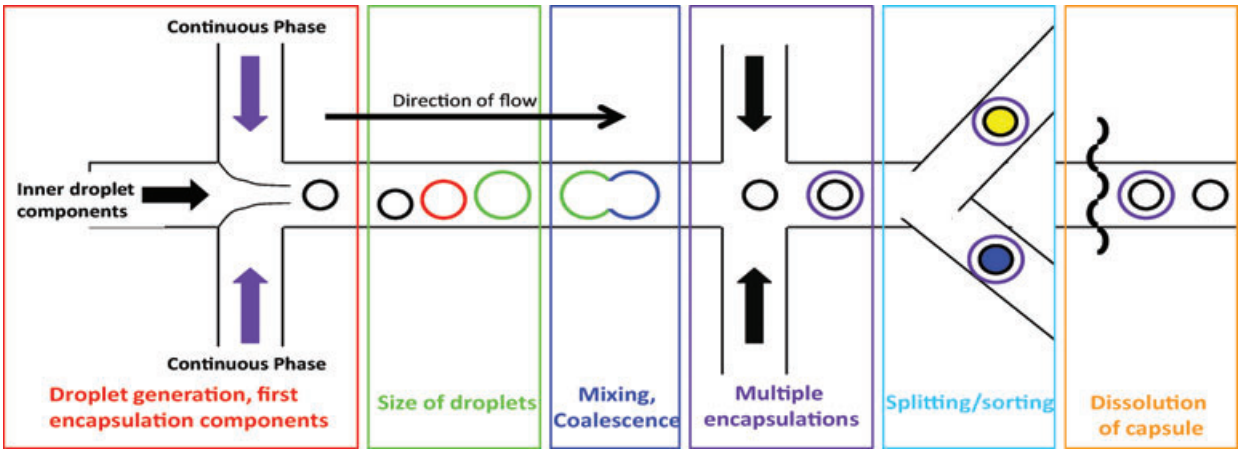

Figure 1. Points of variation in microfabricated devices for biomolecule encapsulations. Shown is a generic schematic of a device for droplet encapsulation, highlighting where researchers have invented new designs to produce enhanced encapsulation results.

to form a new reaction vessel. By adjusting pressures that control fluid flow and reagent concentration, the droplets can be calibrated to contain a known quantity of biomolecules. Microbubbles create a controlled microenvironment that facilitates very sensitive regulation of biological and chemical interactions. Droplet formation and size, droplet transportation and mixing, modes of detection, cell-based assays, solid-phase polymer production, droplet splitting, and capsule dissolution will be reviewed here to give an update on the recent progress researchers have made in this field. Refer to Table 1 for a guide to the different components of droplet emulsions covered in this review.

Encapsulating microfabricated devices are tools for performing microscale biological assays; therefore, the applications of this methodology are diverse. In vitro compartmentalization of genes or cells permits directed evolution through amplification, purification, selection, and expression of DNA and proteins, all on a self-contained microfabricated encapsulation device. Tissue constructs can be grown in vitro on chips using hydrogels for 3D encapsulation and a closer imitation of in vivo growth conditions. Droplet-based microfluidics are used to study biological and chemical reactions in a high-throughput manner, greatly enhancing the speed of analysis and drug discovery. Devices are portable and reduce consumption of reagents that are encapsulated in picoliter to nanoliter volume drops, reducing costs of analyses. Encapsulation of reagents results in minimal cross-contamination, negligible dispersion, and reduced evaporation in encapsulated droplets. For these and many other reasons that we will highlight in this review, microfabricated devices performing biomolecule encapsulations are currently on the cutting edge of instrumentation development, and promise to be a highly useful tool for the analysis of interbiomolecular reactions and interactions.

Table 1. Components of droplet emulsions

Type of emulsion[a)]	Continuous phase	Encapsulant (shell)	Droplet components	Reference
W/O	Fluorinated oil	n/a	DNA, PCR mix, dyes, cells	[22, 43, 44]
W/O	HFE-7500 fluorinated oil	n/a	Drugs, enzymes, substrates, dyes, H_2O	[7, 9, 11, 13, 21, 23, 24]
W/O	Mineral oil	n/a	Amaranth or ink, curable monomer	[10, 20, 29]
W/O	Paraffin oil	n/a	PMBV, PVA	[30]
O/W	Aqueous phase (PVA)	n/a	PFO	[40]
O/W	Prepolymers	n/a	Heptane	[16]
W/O/W	2% PVA in H2O	1-octanol	0.25% Sodium alginate in H_2O	[5]
W/O/W	2–10% PVA in H2O	Isobornyl acrylate, PDMS, Suppocire AIM, hexane	2% wt. PVA, Hyrogel substrates, cells, dyes	[14, 37, 46, 47]
W/O/W, O/W/O	10% PVA or silicone oil	Hexadecane or 1% Pluronic	25% PEG or 2% silicone oil of DC749	[45]
O/W/O	HFE-7500 fluorocarbon oil	Water + 0.5 – 1% SDS	HFE-7500, octanol	[6, 8]
G/W/O	PDMS fluid 200	Glycerol	Nitrogen gas	[38]
O/W/O, W/O/W, O/W/O/W,	Soybean oil, benzyl benzoate	n/a	1% w/v pluronic F127 + 5% w/v glycerol	[18]
W/O/W/O, O/W/O/W, W/O/W/O/W	3% PVA	Hexadecane, 1% F108	Water	[17]

a) Abbreviations: W = water, O = oil, G = gas.

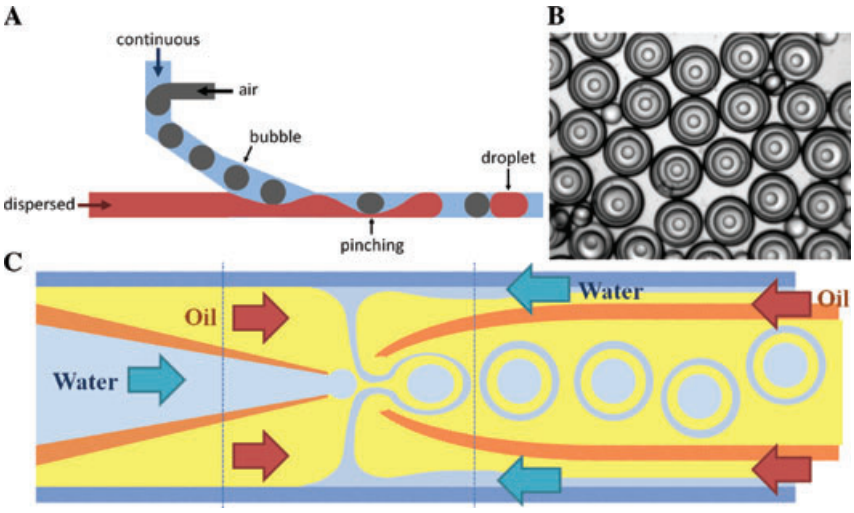

Figure 2. Creating encapsulations. (A) Droplet formation through air bubble percolation [8]. Air bubbles bend the jet of dispersed phase to pinch off droplets. Image adapted from [8], reproduced by permission of The Royal Society of Chemistry. (B) Quadruple emulsion droplets produced from device in C. that had been chemically modified to introduce a biphasic flow from the droplet generator. Onion droplets are approximately 400 μm in diameter [17]. (C) Capillary device for preparing triple emulsion droplets. B and C images adapted from [17], John Wiley and Sons.

2 Droplet generation

A traditional macroscale water-in-oil emulsion is made by the controlled dispensing of an aqueous solution into an oil bath while stirring; the resultant droplets are cooled and purified. This produces droplets that are large, contain high volumes, and are highly polydisperse, with little control over droplet content. Most droplet-based on-chip encapsulations are generated from the convergence of two streams of immiscible liquids in microchannels, where the pinching off of a droplet occurs for encapsulation. Single emulsions allow one encapsulation event (Fig. 1), whereas double emulsions consist of drops within drops, where the initial fluid is encapsulated in a droplet that has a "shell" of fluid that shields it from the continuous phase [5].

2.1 Modes of droplet generation

For microfluidic-scale droplet formation, precise control over converging liquid streams is important to dictate size, stoichiometry, and functionality of the droplets. Inlet fluid flow is traditionally controlled by applying pressure through pumps; however, the periodicity of the pumping mechanism can have significant unfavorable effects on the formation of small-volume emulsified droplets as a result of pressure fluctuations. Abate and coworkers addressed this problem by developing plug-triggered encapsulation in a PDMS microdevice, where a large enough object plugged flow to create a pressure rise that then pinched off the droplets to create a double emulsion [6]. This ensured that every droplet contained the intended reagent, eliminating empty droplets to increase efficiency [6]. This microfabricated device provides a way to create emulsions of uniform dimensions employing common pumps available to microfluidic researchers. Romanowsky and coworkers designed a high-throughput device using a single pump to create highly monodisperse double emulsions [5]. PDMS microchannels lend themselves well to parallel arrays, as they can be stacked two and three dimensionally. Romanowsky et al. designed this device with larger-than-usual distribution and collection channels to enable equal flow to all units, resulting in high-volume production of uniformly sized water/octanol/water double emulsions [5].

Abate and Weitz designed a low-tech fluid flow control system that circumvents the need to buy pumps or electronics to control the microdevice, facilitating device portability [7]. Syringes located at the device outlet provided the vacuum to direct fluid flow to create water drops in oil, termed syringe–vacuum microfluidics (SVM). The device design integrated hydrodynamic resistors and valves to allow for hand-controlled vacuum by clasping a 1-inch binder clip on the syringe. Tubing was used to connect the device to the syringe through a 16-gauge needle, generating a simple, elegant but functional design based entirely on common lab (and office) supplies. The drops created are monodisperse and can be collected in a syringe for future storage, incubation, or processing [7]. The picoliter volumes of reagents that can be produced and analyzed in the device provide the advantage of speed and sensitivity of analyses, in principle allowing for the development of field applications.

Air can be used as an alternative to syringes or pumps, to drive fluid flow and form droplets. Abate and Weitz produced both single and double emulsions using air bubbles to sever fluids into discrete drops [8]. The air bubbles were first forced into a stream of mobile phase; the stream was then merged into a restrictive channel with a second stream containing the droplet contents (Fig. 2A). The air bubble cut off the streams once they merged so that the fluid between air bubbles formed a drop, thus controlling drop size [8]. Because air bubbles formed the droplets, less mobile phase was required since air occupies most of the space in this microfabricated device. Using air to produce drops also allows for easy emulsion extraction, as air bubbles will float to the top in a collection device, naturally separating from the emulsion drops.

2.2 Size of droplets

An advantage of using microfabricated devices for biomolecule encapsulations is the small volume of the drops that are generated. This allows for sensitive detection assays with quantities on the order of picoliters. Recent work in the field focuses on the speed of generating micron-sized droplets, and on generating droplets in the nanometer range. Abate and Weitz have advanced the field by creating a device that makes small volume droplets at a fast rate, generating large quantities of these fine-tuned droplets by incorporating a splitting array that allowed for fast production of double emulsions [9]. Large droplets (88 μm diameter) were made at a fast rate for single emulsions and then sequentially split by a series of channels, resulting in 35 μm, monodisperse drops. Reliable splitting is controlled by a narrow constriction before each splitting fork and adjusted channel lengths for even drop flow. The result is a microfabricated device that improves the throughput of droplet making over 1000-fold compared to single drop makers [9].

Shemesh et al. have increased the applicability of droplet-based microfluidics to cell- and reagent-based assays by reducing the size of droplets in a device that creates nanoliter volume droplets reproducibly using piezoelectric droplet generators [10]. The piezoelectric actuators are externally mounted on the chip; microelectrodes do not need to be fabricated into the device body, allowing for chips to be disposable, eliminating costly and time-consuming steps. Reactant concentration can be rapidly switched, on demand, within single droplets with volumes down to 2 nanoliters [10]. Nanoliter volume droplets are appropriately small to accommodate single cells, advancing the technology of the field for potential applications such as high-throughput screening, digital PCR (dPCR) and sequencing.

A device that generates even smaller volume droplets (500 pL) at high speeds is a sample chopper developed by Deal and Easley [11]. These authors used opposing droplet generators to form the basis of a self-regulated microfluidic sample chopper, termed μChopper [11]. Extremely small droplets, with volumes in the hundreds of picoliters range and containing femtomolar quantities of UV absorbance dyes, were generated using the μChopper. This new device design overcomes path length limitations and large signal drift of absorbance detection in microfluidic devices to form the equivalent of an optical beam chopper, with practical applications in sensing pH and detecting absorbance [11]. The devices covered in this section highlight the trend during the past year toward smaller sized droplets and increased production rates that can only be achieved in microfluidic devices.

2.3 Droplet mixing

Creating reliable means to merge individual droplets is important for the construction of mini reaction vessels. Digital microfluidics (DMF) is an emerging field where microscale lab-on-a-chip devices maneuver droplets to perform traditional bench-top assays on-chip. Dielectrophoresis (DEP) is one method used to construct DMF, where reagent-filled droplets are precisely controlled temporally and spatially using electric fields to ensure coalescence.

Fan and coworkers used electrophoresis to make water-in-oil emulsions [12]. Electrowetting-on-dielectric (EWOD) and DEP were activated to form and move droplets. EWOD is utilized to move water droplets within a hexane bath, to enact a kind of scrubbing to fully remove the external layer of oil from the emulsion. There is flexibility in the system, with options for removal of the oil and varying droplet volume for accurate detection or other processes. Fan and coworkers showed the practical application of their device by measuring biofouling, demonstrating that encapsulation eliminated biofouling [12]. Based on this result, one can envision the use of encapsulants as an alternative to coating capillaries in CE or ME, prolonging the lifetimes of capillaries and microchips used in electrophoresis. In a similar manner, Mary and coworkers used dielectrophoresis to quantitatively merge reagent drops with solvent drops to perform dilution assays on-chip [13]. Dilutions were made within a dilution/splitting device by generating an electric field when a prefabricated drop reached the buffer stream, causing this drop to merge with the buffer stream and detach as a larger, more dilute drop. Mary et al. have created a device that allows for reaction and detection on the picoliter scale [13], while diluting reagents to enable multistep reactions in the future. Other advantages include less reagent consumption in these on-chip assays, which reduces overall cost, and reduction in background noise for more sensitive detection.

2.4 Droplet coalescence

Exciting applications for emulsion-based microfabricated devices include the synergistic delivery of chemicals for microscale reactions in droplet-sized reaction vessels. Included in this section are recent advancements that make multicomponent microscale reactions possible through the coalescence of emulsion droplets in microfabricated devices. Stone and coworkers created double emulsions containing two inner drops that coalesce on demand, creating a protected environment where reagents do not interact until triggered [14]. This emulsion droplet device was formed from three capillaries: one for injection, one for collection, and one to contain the flow of all phases. However, the injection tube was made up of four conjoined cylindrical capillaries, allowing for streams of different reagents to be encapsulated individually. Stone and coworkers used different oil and continuous phase flow rates to assess that higher flow induces core drops to coalesce, demonstrating that their device can induce internal coalescence of drops based on fluid flow alone [14], obviating the need for external fields and thermal treatments.

Fletcher and coworkers built a microfluidic device that coalesced cell membranes [15] to replicate vesicle fusion to cell membranes, viral entry, and other cell membrane events.

Lipid composition, membrane proteins, and the contents of vesicles could be varied to perform experiments that mimic two components (either cells or vesicles) communicating. The device Fletcher and coworkers built first constructs planar bilayers between two aqueous droplets surrounded by lipids dissolved in oil and divided by a thin acrylic membrane. Oil-insoluble lipid and transmembrane proteins fused to the oil/water interface at the acrylic membrane to form a lipid monolayer. The acrylic divider was removed to free the bubbles to merge together, thus constructing a double lipid membrane [15]. By regulating and synthesizing cellular membranes with tunable components, Fletcher and coworkers have created a platform for probing cellular membrane-dependent functions such as endocytosis and exocytosis.

Another aspect of programmed droplet fusion is the sequestering of incompatible reagents and chemicals for controlled release reactions. Richtering and coworkers created a microscale analytical instrument by preparing emulsions consisting of oppositely charged droplets that do not coalesce unless triggered through neutralization of a pH-sensitive hydrogel [16]. Using a microfabricated device, Richtering and coworkers prepared oil-in-water emulsions and mixed the resultant droplets immediately after formation to detect differences in emulsifying behaviors that allowed droplets to stay separated. When pH was changed from 7 to either 4 or 9, droplets coalesced immediately upon contact. This device provides a way to study real-life emulsions in close detail that is not possible in bulk-scale preparations, such as in detergents where anionic and cationic surfactants are in close contact [16]. In addition, this device offers a way to package materials that otherwise have short reaction times, to be able to produce the desired reaction on demand.

2.5 Multicompartment encapsulations

In addition to single emulsion encapsulation, devices have been microfabricated to enable a multiplicity of emulsions, such as triple and quadruple emulsions and encapsulations containing up to six core particles. Kim and Weitz created a microfluidic device to fabricate multiple concentric shells in emulsion droplets in one step (Fig. 2B) [17]. Triple and quadruple emulsions were created by injecting a drop into the center of an emulsion, which triggered droplet breakoff (Fig. 2C). By chemically treating the inner surfaces of glass capillaries to produce either hydrophobic or hydrophilic regions, multiple emulsions with aqueous inner phases or oil inner phases were created [17], showcasing the versatility of this microfluidic device. The shell structures surrounding the droplets are a significant step in the field, enabling 3D controlled release of reagents and coordination of independent sequential reaction steps. These self-contained reaction vessels could prevent contamination from internal and external sources resulting in cleaner, more accurate results.

Multicompartment emulsions have been created within a modular microfluidic device for control over final reagent concentrations. Wang et al. developed an emulsion microfluidic device with three hardware components; a drop maker, a connector, and a liquid extractor [18]. By varying the quantities of these different parts, quadruple- and quintuple-component double emulsions, and quintuple- and sextuple-component triple emulsions were created in this device. The ratio of different components was adjusted such that more droplets of one reagent were encapsulated with fewer droplets of other components. Because the multicomponent structure dictated fluid flow, both oil emulsions and aqueous emulsions were possible without chemical modification. Another benefit of using this microdevice is the ability to control the emulsions within a single encapsulation, such that both double and single emulsions can be co-encapsulated in a triple emulsion [18]. One can envision this as useful for timing delivery of reagents for a multistep reaction.

2.6 Splitting droplets

Droplet splitting is a notable facet of miniaturizing assays into microdroplet emulsions, as it can be used for reactant extraction and further analysis. Droplet splitting is important to fully manipulate and process droplets in multistep reactions, yet it is a difficult task as droplets are very small and typically covered in oil. Managing droplet division can be accomplished on-chip, though. Sinz and Darhuber investigated self-propulsion of droplets in chemically defined open-surface microfluidic chips [19]. A surfactant droplet was first deposited on the end of a hydrophilic pattern, then sub-phase liquid was deposited along the length of the hydrophilic geometry. Through capillary action, the droplet converged with the tip of the sub-phase liquid and slid onto it, effectively entering the chemical highway. The surfactant droplet then self-propelled along the sub-phase liquid. A splitting junction was incorporated into the device design, for partitioning into two smaller drops controlled by thermal steering. Heating one branch of the splitting junction directed droplets into the room temperature branch [19]. The authors have created a microfabricated device that is easily manipulated due to its open-air construction, self-propelled movement, and thermally triggered splitting, precluding the need for externally powered actuation for any part of the chip.

Much like many electrophoresis researchers, Zeng and coworkers used an electrical-based method with electroosmotic flow to break open droplets and select how many internal components were siphoned away for further analysis or simple product collection [20]. Droplets were drained by creating an EOF that disturbed the water–oil interface without preventing droplet reformation, to allow potential further analysis of the intact droplet, sans the amount extracted upstream. The extraction of substances could be controlled by varying the electric field strength and channel height [20]. Zeng and coworkers have developed a technology that enables microfabricated devices to become full analysis

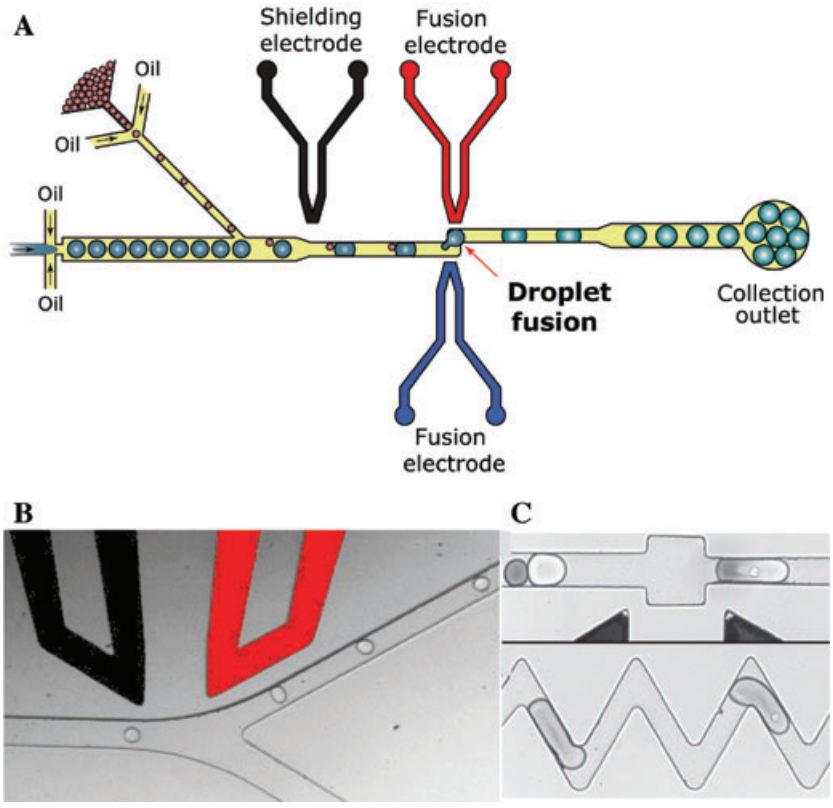

Figure 3. Dielectrophoresis facilitates droplet mixing and detection. (A) A droplet fusion device coalesced droplets between the fusion electrodes, and used a shielding electrode to prevent premature coalescence [21]. Reprinted (adapted) from [21] with permission. Copyright 2009 American Chemical Society (B) Sorting is initiated by applying an AC electric field across the electrodes to direct droplets into the upper arm [43]. Image adapted from [43], reproduced by permission of The Royal Society of Chemistry. (C) Fusion (top) of droplets between electrodes using an AC field and mixing (bottom) of droplets using a delay line to provide time for encapsulated cells to fully stain before detection [44]. Image adapted from [44], Copyright 2009 National Academy of Sciences of the United States of America.

microreactors, with an elegant extraction process from emulsified droplets.

3 Applications/state-of-the-art

The potential applications of droplet-based microfluidic devices are diverse, but work must be done to modify macroscale assays to microfluidic dimensions. This involves not only reducing sample and reagent input required with most macroscale assays, but it requires microfluidic devices to be fine-tuned to collect reliable data that can improve upon macroscale readouts. This section of the review will cover detection of low-sample concentrations, droplet dissolution and sorting to enhance detection, polymer synthesis, and cell-based assays in droplets.

3.1 Detection within encapsulations

The development of new methodologies to measure reaction endpoints in small-volume encapsulated droplets is necessary, as limits of detection are challenged in microdroplets due to low-chemical concentrations, or even single molecules, that are encapsulated. Most droplet-based microfluidic devices utilize fluorescence for detection, but improve upon the sensitivity of fluorescence measurements to achieve accurate analysis from droplets. Mazutis and coworkers created new DNA-based applications using dPCR, where single DNAs were encapsulated, amplified, quantitated, translated, and finally measured for enzymatic activity [21]. dPCR amplified single molecules of DNA in many separate reactions emulsified within a fluorinated oil mobile phase. Droplets were collected and loaded into a final device that electrocoalesced, yielding 2000 droplet fusions per second, and detected fluorescence to measure enzymatic activity of the encoded protein (Fig. 3A). A shielding electrode in this droplet fusion device prevented unwanted droplet merging upstream of the droplet coalescence region [21]. Zhong et al. measured fluorescence intensities to detect multiplexed PCR reactions in single emulsions [22]. Their microfabricated device multiplexed dPCR within picoliter emulsion droplets, with over a million single-molecule DNA encapsulation reactions detected fluorescently on-chip. The same fluorophore was used for both reference and target DNA reactions so that the difference in signal strength identified one from the other, establishing an intensity profile assay to detect PCR products [22]. Zhong and coworkers have designed a device that provides a sensitive method for nucleic acid analysis in conjunction with multiplexing for high throughput.

Highly sensitive fluorogenic assays on microdroplet emulsion devices have been used to improve the drug discovery process. Building upon the foundations of Paracelsus, Miller and coworkers developed a dose-response analysis that measured 10 000 data points per compound to provide a highly accurate drug efficacy profile [23]. A concentration

gradient was created over thousands of droplets by injecting drugs premixed with fluorescent dyes into a stream of buffer that constantly flowed into a capillary where drug samples were dispersed. Drug samples were mixed from the capillary with a target enzyme and a fluorogenic substrate on a microfluidic device, then segregated into 140-pL droplets. This created microemulsions with fixed concentrations of assay reagents but varying concentrations of the drug of interest. Results from this microfluidic device uncovered new modes of action and increased potency of currently known inhibitors. Advantages of using this device include high throughput, small reagent volumes and flexibility to be applied to different assays due to the encapsulation technology [23].

3.2 Cell-based assays in droplets

Encapsulation in microdroplet emulsions facilitates the analysis of complex cell-derived samples by protecting them from shear stress, dehydration, and harsh reactants. Mary and coworkers controlled incubation times in microdroplet devices [24], so that a multitude of analyses, such as enzymatic assays that require time to react enzymes with substrate, can potentially be performed on chip. They generated a plug to prevent Poiseuille flow, causing drops to be in contact, enabling uniform movement that created a constant incubation time. Device efficacy was evaluated by measuring the reaction kinetics of an enzyme and showing that the control over incubation time generated a more accurate measurement of the enzyme's activity [24].

Microfluidic cell-based assays are being advanced with the replication of a natural 3D environment to encase cells for prolonged viability and further manipulation. Hydrogels have emerged as the method of choice to extend cell life in culture plates or microdevices. For example, our lab has developed enzymatically crosslinkable protein polymers to create tunable hydrogels that can serve as cellular scaffolding [25, 26]. Gelation kinetics lends these materials well to synthesis in microfabricated devices. Doyle and coworkers created hydrogel building blocks within flow-focused microfluidic devices using the principles of microfluidic gradients [27] to attain microscale formation of these three-dimensional bioarchitectures [28, 29]. The Janus hydrogel particles that Doyle and coworkers manufactured are biocompatible and multifunctional with an eye toward future clinical uses [29]. A potential use of particles was showcased through DNA hybridization experiments by binding a DNA probe to one hemisphere of the particles and incubating with fluorescently labeled complementary DNA. Selective capture of the probes was attained on one-half of the particles. Creation of this device expands future hydrogel applications by creating hydrogels with tunable surface functionalities.

Hydrogel syntheses were tested by Aikawa and coworkers when they used a flow-focusing droplet-based microfluidic device for continual encapsulation of cells. Two biocompatible polymers first encountered each other on-chip in the presence of a cell, and formed a stable hydrogel to encapsulate cells that maintained viability up to 8 days on the hydrogels [30]. Intercellular communication, cell migration, and cell adhesion can be tuned by regulating fluid flow to adjust the size and permeability of hydrogels. The encapsulating hydrogel was dissolved with D-sorbitol, a biocompatible reagent, within 10 min to release the cells for tissue implants or other medical uses. One can envision applications of this device in developing nations in conjunction with WHO, where hydrogel-encapsulated cells could be transported out of these nations for high-tech disease analysis.

Work in the lab of Ali Khademhosseini focuses on hydrogel micromolding [31, 32]. Much akin to previous work encapsulating nanoparticles for drug delivery applications [33], Khademhosseini and coworkers used PDMS molds to define the physical shape of hyaluronic acid (HA) [31], then photopolymerized the HA hydrogels with collagen components to create a more mechanically stable hydrogel [34]. Alginate hydrogels allowing enhanced biomolecule diffusion were created by incorporating micropores into the 3D cell network [35]. Methacrylated gelatin hydrogels were micromolded with carbon nanotubes for structural reinforcement [36]. Using native elements of the extracellular matrix like collagen and other natural components [37] imbues these hydrogels with important cell signaling properties that result in good cell encapsulation and faster proliferation of cells. Khademhosseini and coworkers have incorporated microchannels into their hydrogels to enhance cell viability by allowing adhesion and migration [27], demonstrating the usefulness of this biofabrication technique in the 3D culturing of cells. These hydrogels are tunable for stiffness, making it possible for them to mimic different cellular niches in native tissues for enhanced cell viability and potential use in biomedical tissue engineering applications.

3.3 Synthesis of solid-phase polymers

Solid polymer beads are used in many facets of science, so synthesis of solid-phase polymers is an important area to study. Polymer particles have a wide range of applications, such as drug delivery, release of materials in make-up, and fluorescent bead-based assays. Due to the spherical shape, solid-phase polymers lend themselves easily to synthesis on droplet-based microfluidic devices. Droplet-based microfabricated devices can be used to synthesize solid-phase polymers with fine control over bead dimensions, compositions, and polydispersity. The work of Wan and Stone showcased how hollow inorganic particles can be created from a microbubble template in their microfabricated device [38]. Different from typical microdevices that encapsulate using all liquid phases, Wan and Stone encapsulated gas bubbles within an aqueous layer to form micron-diameter droplets. The liquid aqueous shell was the template for a sol-gel reaction that formed the solid particle barrier. Adjusting flow rates of the different phases controlled the size of the bubbles, the number of bubbles generated, and the thickness of the shell, showing that double-emulsion gas-liquid microstructures can be

fabricated from a "microbubble emulsion" device. Advantages of this microdevice synthesis include limited availability of the aqueous phase to naturally control the extent of sol-gel reaction, quick synthesis on the order of tens of seconds, and in situ formation of hollow particles with minimal reaction steps [38].

Chen and coworkers used tapered glass capillaries inside a square tube to generate hollow particles [39]. Chen and coworkers photopolymerized the middle oil phase of double emulsions instead of hydrolyzing a sol-gel reaction, to demonstrate that hollow capsules can be made with tunable mechanical properties (elastic or brittle) and permeability. Combinations of polymers and colloidal particles were used in the middle phase to create well-defined microcapsules with tuned shell structures on the order of 5 μm thickness [39]. Monodisperse solid particles between 150 nm and 2 μm in diameter were synthesized in a PDMS microfluidic chip by Kuehne et al. [40]. Polyfluorene drops were created in a continuous aqueous phase containing PVA as a stabilizer, then the toluene solvent was evaporated and particles were centrifuged to remove excess PVA. A new coating for PDMS was used, Parylene-C, to allow toluene to be flowed within the microfluidic device without swelling the PDMS and blocking the microchannels. Approximately 8000 drops per second were produced [40], demonstrating the high throughput and great versatility of this device.

3.4 Sorting droplets

Sorting is an effective method for separation of droplets. For mammalian and bacterial cell sorting, cytometry is typically used to characterize cells, either chemically [41] or based on fluorescent identity (fluorescent-activated cell sorting, FACS) [42]. In traditional optical cell-sorting methods, emitted fluorescence is measured for each cell in order to sort it, so the speed of analysis is limited to detecting one cell at a time. Additionally, FACS analysis can induce high shearing forces that damage or kill cells. Microfluidic cell sorters and magnetic bead-based assays are examples of droplet-based microemulsions that have incorporated droplet sorting on-chip, to enact fast, gentle sorting of biomolecules.

Baret and coworkers developed microfluidic fluorescence-activated droplet sorting (FADS) [43] as an alternative to FACS. In FADS, cell sorting is based on fluorescence intensity correlating to cellular enzyme reactions encapsulated in picoliter emulsion drops. Positive hits were sensed through fluorescence intensity, which triggered a pulse of alternating current across the electrodes at the sorting junction, directing the positive hit droplets into the narrow arm of the Y-junction (Fig. 3B). Dielectrophoresis sorted drops at up to 2000 droplets per second, and allowed viable *Escherichia coli* cells to be recovered. This FADS device is less expensive than traditional FACS, as it consumes less reagent, and offers other advantages such as sterility, sorting at high speeds, short analysis time, and increased sensitivity for detection of small quantities of cells [43].

Brouzes and coworkers at Raindance Technologies (RDT) created a new cell viability assay on a microfabricated device, where cells and live/dead fluorescent dyes were encapsulated separately, an AC field was used to electrocoalesce one cell droplet with one dye droplet to form a reaction vessel, and the reaction drop flowed through a mixing section of the device to ensure thorough mixing of the contents (Fig. 3C) [44]. This work is the basis of RainStorm™ technology, which generates picoliter drops containing single molecules, reagents, or cells to form individual reaction vessels that can be processed at ultra high speeds. Drops are combined and positioned through the use of electrophoresis, with sorting decisions based on enzymatic, cellular, or chemical reaction products in the drops (www.raindancetech.com). Brouzes and coworkers crafted a device design that is modular and allows for additional capability to be built into it, and enabled 100-fold faster throughput over conventional high-throughput screening techniques [44]. CytonomeST has developed an optical cell sorter, the Gigasort™, that performs faster than traditional FACS methods by operating 72 microfluidic sorters simultaneously (www.cytonome.com), which enables sorting at rates of 144 000 cells per second, all within a 2" × 3" glass microchip. This device significantly advances the process of cell sorting, an integral aspect to high profile and high-speed discovery disciplines such as regenerative and personalized medicine.

3.5 Dissolution of capsule

In order for microfabricated devices for biomolecule encapsulation to translate most macroscale assays and reactions to a microscale, modes of droplet dissolving must be developed to analyze droplet content. Microfluidic droplet devices offer significant improvements in cost, consumption, and ease-of-use over macroscale assays, so studies investigating droplet dissolution are indispensible. Below are some examples of dissolving emulsions in drops using microfluidic technologies that have been employed for microscale reactions.

Kim and coworkers produced double emulsions with a solidified shell to protect them [45], much like the encasings on M+M'S® candies. Both water-in-oil-in-water and oil-in-water-in-oil double emulsions were generated within a capillary microfluidic device similar to that shown in Fig. 2C. Microcapsules ruptured within approximately 2 months' time for a type of slow release of internal components, and rupturing was also selectively induced within days by creating a large osmolarity difference when submerged in water (Fig. 4A). One can envision an application such as a miniature Morse code that could be created in this device, where droplets are collected linearly in tubing and sent to the recipient within a set amount of time before the message disintegrates due to the microcapsule shell content. More immediate uses are for drug delivery, mammalian cell mimics, and cosmetic encapsulations, as the solidified shell can be made on the order of tens to hundreds of nanometers to ensure dissolving and/or permeability in biologically relevant media [45].

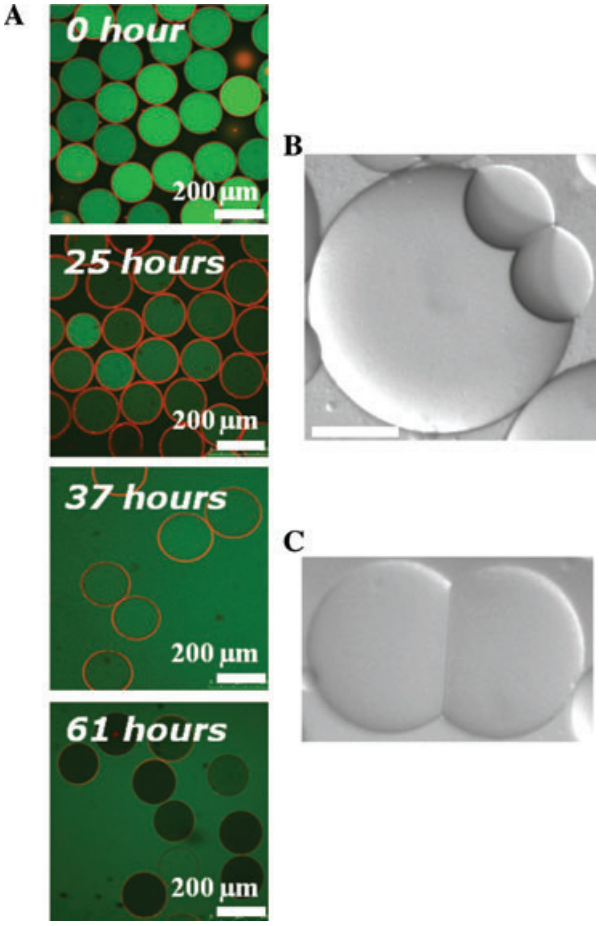

Figure 4. Dissolution of Capsules. (A) Microcapsules are made with biodegradable shells composed of poly(lactic acid) that lead to degradation through exposed esters. The inside of the microcapsules are composed of 10 wt percent poly(vinyl alcohol) containing a green dye. Indicated are the time intervals after capsule preparation [45]. Image adapted from [45], reproduced by permission of The Royal Society of Chemistry. (B) Image showing a dewetted double-emulsion drop with two inner droplets. Scale bar is 50 μm. (C) Multicompartment polymersomes created by removing oil from double emulsion drops shown in B. Polymersomes are approximately 50 μm in diameter [46]. B and C images adapted from [46], John Wiley and Sons.

Evaporation-induced solidification of the shell structure has also been used to make polymersomes in a microfluidic device [46]. Water-in-oil-in-water double emulsions were created in a capillary microfluidic device by adding diblock copolymers to the middle phase so that polymersome walls formed after evaporation of the oil and hardening of the shell (Fig. 4B). The diblock copolymers were mixed with both a good (and volatile) solvent and a poor (and nonvolatile) solvent such that as the volatile solvent evaporated, diblock copolymers became less soluble in the poor solvent, causing inner droplets to stick to one another to form multicompartment polymersomes (Fig. 4C). The variation of solvent flow rates controlled the number of inner drops in the double emulsion, developing another device to compartmentalize multiple reactants for prevention of cross-contamination in reactions, and control the amounts of reactants based on numbers of inner drops [46]. Zhao and coworkers developed a phase-transition method for encapsulating active reagents such that leakage out of microcapsules was better controlled [47]. Calcium carbonate formed precipitates close to the interior of the droplet shell to physically block pores, preventing diffusion out of the shell. The microcapsules were collected, cooled (solidified), and stored off-chip, with minimal leakage of approximately 5% over a month's time. Reagents were actively released by heating microcapsules above the melting temperature of the shell, triggering reagent release within 2 min [47]. Zhao and coworkers have demonstrated that, much like the hardening of the exterior shell of the microcapsule, forming solid precipitates within the microcapsule can be used to precisely control reaction parameters such as reagent release and permeability.

Lastly, the double emulsions created from microfabricated devices can provide a structural mold for microcapsules. Abell and coworkers developed cargo carrying microcapsules molded on microfluidic droplets by casting cucurbit uril (CB[8]) simultaneously with methyl viologen functionalized gold nanoparticles and a naphthol-copolymer to form hollow microcapsules with diameters ranging from 10 to 24 μm [48]. To produce hollow microcapsules, evaporation depleted the inner aqueous phase, resulting in a shriveled spherical structure. Abell and coworkers demonstrated the practical use of these hollow microcapsules by creating CB [8] cages encapsulating *E. coli* cells, and by selectively releasing cargo. Advantages of this on-chip method are numerous: microcapsules are stable, resistant to heat (up to 100°C), created in one step, and are multifunctional due to the use of CB [8] and gold nanoparticles [48]. Potential applications include drug delivery, cell encapsulation, and controlled microreactions in the fields of health care, cosmetics, agriculture, and human consumables.

4 Concluding remarks/future directions

Microfluidic devices are a relatively new platform for encapsulation; significant advances in microfabricated device design have been achieved in the last decade to make miniaturizing bench-top assays on-chip a feasible venture. Microfluidic junctions provide new insights into analyte transport in fabricated devices, where the natural aversion of oil for water is utilized to create miniature reaction vessels in droplets [49], and also to break up reaction droplets to deliver chemical products [20]. Droplet-based microfabricated devices create monodisperse emulsions through droplet coalescence and splitting to add or dilute reagents in a specific order for a complex reaction [50]. Two converging streams can be turned into a cell lysis reaction on-chip, where a double emulsion is created with two core drops, one containing cells and one containing cell membrane-dissolving chemicals [14]. Reagents may be titrated in real-time for ultra-sensitive kinetic assays [51]. Researchers in the microfluidic emulsion

droplet field have focused on designing devices for maximum applicability by creating multiple steps of reagent addition, manipulating droplet size for fluidic transport [52] and detection [53], or mimicking the cellular environment through creation of more membrane-like encapsulations [46].

Emulsions are by nature difficult to handle due to multiple liquid encapsulating layers and small dimensions. But recent biomolecule encapsulations can be formulated into hollow or solid spheres or beads [54], where the external layer of encapsulant can be dewetted or dissolved from encapsulated drops to form spheres or beads molded on the form of the droplet shape. Stable dynamic microcapsules can be made based on the high affinity of macrocyclic molecules for multiple aqueous compounds through noncovalent interactions [48]. Magneto-responsive particles can be encapsulated within microcapsules and purified with magnetic fields to make improved magnetic active inks with increased resolution [17]. Innovative ways of extracting contents from droplets or analyzing on-chip include breaking emulsified droplets based on EOF [20], or retaining pores in the shell for future chemical delivery [47] to allow for multistep processing postemulsion in complex assays.

Recent advances presented herein include electrophoretically based microfabricated devices, where piezoelectric actuators are used to fuse together drops for reagent mixing. On-chip dielectrophoresis progress in the last few years includes lower viscosity of oils for lower driving voltages [12], electrocoalescence for enhanced read-out [55], reduction of background noise [13], efficient cell sorting [43], high-throughput DNA amplification [21], and tissue engineering [56]. Electrophoresis is integral to the field of DMF by electrocoalescing droplets to form diverse reactions and assays. Electrophoresis plays a significant role in microdroplet-based emulsifications by initiating droplet mixing, and its role will only be augmented as the push for more complex assays will necessitate finer control over droplet coalescence.

The microfabricated devices presented herein have applications not only in advancing the rate of discoveries in basic research, but also in decreasing costs in commercial ventures such as biocide development and cosmetics. Also, microfluidic devices with cheap costs of production and easy set-ups, made possible by self-propelled droplets and hand-operated flows, can have real-world applications in developing countries. Microscale encapsulation devices reduce chemical consumption, thereby reducing money spent on reagents, allowing for poorly funded laboratories to perform reaction assays at significantly reduced costs. With the development of recycling technologies that would recirculate continuous phase, much like a water fountain recirculates water, reduced initial investment can be achieved to allow sensitive, reliable, and informative experimentation regardless of financial circumstances.

Most devices presented in this review are made using soft lithography of PDMS, which is an attractive material given its biocompatibility and easy bonding to glass [57]. Creating the molds to pattern the PDMS stills involves work in clean rooms, which entails expensive processes and are not widely available. Work in the future should focus on improved ways of creating devices to fashion microemulsions without the need to visit a fabrication facility. As it stands now, fields such as analytical chemistry, biology, organic synthesis, and toxicology all can benefit with implementation of microfabricated devices for biomolecule encapsulation, without much optimization necessary based on current literature. We look forward to new advances in miniaturizing science made possible by encapsulating on-chip.

This work was supported by HFSP grant number RGP0016/2009-C. The authors thank Dr. Nicolynn Davis and Dr. Russell Monds for helpful scientific discussion and feedback on the manuscript.

The authors have declared no conflict of interest.

5 References

[1] Wang, J. T., Wang, J., Han, J. J., *Small* 2011, *7*, 1728–1754.

[2] Hardt, S., Hahn, T., *Lab. Chip* 2012, *12*, 434–442.

[3] Baret, J. C. *Lab. Chip* 2012, *12*, 422–433.

[4] Gauvin, R., Parenteau-Bareil, R., Dokmeci, M. R., Merryman, W. D., Khademhosseini, A., *Wiley Interdiscip. Rev. Nanomed Nanobiotechnol* 2012, *4*, 235–246.

[5] Romanowsky, M. B., Abate, A. R., Rotem, A., Holtze, C., Weitz, D. A., *Lab. Chip* 2012, *12*, 802–807.

[6] Abate, A. R., Rotem, A., Thiele, J., Weitz, D. A., *Phys. Rev. E. Stat. Nonlin Soft Matter Phys.* 2011, *84*, 031502.

[7] Abate, A. R., Weitz, D. A., *Biomicrofluidics* 2011, *5*, 014107.

[8] Abate, A. R., Weitz, D. A., *Lab. Chip* 2011, *11*, 1713–1716.

[9] Abate, A. R., Weitz, D. A., *Lab. Chip* 2011, *11*, 1911–1915.

[10] Shemesh, J., Nir, A., Bransky, A., Levenberg, S., *Lab. Chip* 2011, *11*, 3225–3230.

[11] Deal, K. S., Easley, C. J., *Anal. Chem.* 2012, *84*, 1510–1516.

[12] Fan, S. K., Hsu, Y. W., Chen, C. H., *Lab. Chip* 2011, *11*, 2500–2508.

[13] Mary, P., Chen, A., Chen, I., Abate, A. R., Weitz, D. A., *Lab. Chip* 2011, *11*, 2066–2070.

[14] Chen, H., Zhao, Y., Li, J., Guo, M., Wan, J., Weitz, D., Stone, H., *Lab. Chip* 2011, *11*, 2312–2315.

[15] Richmond, D. L., Schmid, E. M., Martens, S., Stachowiak, J. C., Liska, N., Fletcher, D., *Proc. Natl. Acad. Sci. USA* 2011, *108*, 9431–9436.

[16] Liu, T., Seiffert, S., Thiele, J., Abate, A. R., Weitz, D., Richtering, W., *Proc. Natl. Acad. Sci. USA* 2012, *109*, 384–389.

[17] Kim, S. H., Weitz, D. A., *Angew. Chem. Int. Ed Engl.* 2011, *50*, 8731–8734.

[18] Wang, W., Xie, R., Ju, X. J., Luo, T., Liu, L., Weitz, D., Chu, L., *Lab. Chip* 2011, *11*, 1587–1592.

[19] Sinz, D. K., Darhuber, A. A., *Lab. Chip* 2012, *12*, 705–707.

[20] Zeng, S., Pan, X., Zhang, Q., Lin, B., Qin, J., *Anal. Chem.* 2011, *83*, 2083–2089.

[21] Mazutis, L., Araghi, A. F., Miller, O. J., Baret, J. C., Frenz, L., Janoshazi, A., Taly, V., Miller, B., Hutchison, J., Link, D., Griffiths, A., Ryckelynck, M., *Anal. Chem.* 2009, *81*, 4813–4821.

[22] Zhong, Q., Bhattacharya, S., Kotsopoulos, S., Olson, J., Taly, V., Griffiths, A., Link, D., Larson, J., *Lab. Chip* 2011, *11*, 2167–2174.

[23] Miller, O. J., El Harrak, A., Mangeat, T., Baret, J. C., Frenz, L., El Debs, B., Mayot, E., Samuels, M., Rooney, E., Dieu, P., Galvan, M., Link, D., Griffiths, A., *Proc. Natl. Acad. Sci. USA* 2012, *109*, 378–383.

[24] Mary, P., Abate, A. R., Agresti, J. J., Weitz, D. A., *Biomicrofluidics* 2011, *5*, 024101.

[25] Davis, N. E., Karfeld-Sulzer, L. S., Ding, S., Barron, A. E., *Biomacromolecules* 2009, *10*, 1125–1134.

[26] Davis, N. E., Ding, S., Forster, R. E., Pinkas, D. M., Barron, A. E., *Biomaterials* 2010, *31*, 7288–7297.

[27] Chung, B. G., Choo, J., *Electrophoresis* 2010, *31*, 3014–3027.

[28] Suh, S. K., Bong, K. W., Hatton, T. A., Doyle, P. S., *Langmuir* 2011, *27*, 13813–13819.

[29] Yuet, K. P., Hwang, D. K., Haghgooie, R., Doyle, P. S., *Langmuir* 2010, *26*, 4281–4287.

[30] Aikawa, T., Konno, T., Takai, M., Ishihara, K., *Langmuir* 2012, *28*, 2145–2150.

[31] Khademhosseini, A., Eng, G., Yeh, J., Fukuda, J., Blumling, J., Langer, R., Burdick, J. A., *J. Biomed. Mater. Res. A.* 2006, *79*, 522–532.

[32] Ling, Y., Rubin, J., Deng, Y., Huang, C., Demirci, U., Karp, J. M., Khademhosseini, A., *Lab. Chip* 2007, *7*, 756–762.

[33] Acharya, G., Shin, C. S., McDermott, M., Mishra, H., Park, H., Know, I. C., Park, K., *J. Control. Release* 2010, *141*, 314–319.

[34] Brigham, M. D., Bick, A., Lo, E., Bendali, A., Burdick, J., Khademhosseini, A., *Tissue Eng. Part A.* 2009, *15*, 1645–1653.

[35] Hwang, C. M., Sant, S., Masaeli, M., Kachouie, N. N., Zamanian, B., Lee, S., Khademhosseini, A., *Biofabrication* 2010, *2*, 035003.

[36] Shin, S. R., Bae, H., Cha, J. M., Mun, J. Y., Chen, Y., Tekin, H., Shin, H., Farshchi, S., Dokmeci, M., Tang, S., Khademhosseini, A., *ACS Nano* 2012, *6*, 362–372.

[37] Rahfoth, B., Weisser, J., Sternkopf, F., Aigner, T., von der Mark, K., Brauer, R. *Osteoarthr. Cart.*, 1998, *6*, 50–65.

[38] Wan, J., Stone, H. A., *Langmuir* 2012, *28*, 37–41.

[39] Chen, P. W., Erb, R. M., Studart, A. R., *Langmuir* 2012, *28*, 144–152.

[40] Kuehne, A. J., Weitz, D. A., *Chem. Commun. (Camb)* 2011, *47*, 12379–12381.

[41] Boardman, A., Chang, T., Folch, A., Dovichi, N. J., *Anal. Chem.* 2010, *82*, 9959–9961.

[42] Tung, J. W., Heydari, K., Tirouvanziam, R., Sahaf, B., Parks, D., Herzenberg, L. *Clin. Lab. Med.* 2007, *27*, 453–68.

[43] Baret, J. C., Miller, O. J., Taly, V., Ryckelynck, M., El-Harrak, A., Frenz, L., Rick, C., Samuels, M., Hutchison, J., Agresti, J., Link, D., Weitz, D., Griffiths, A., *Lab. Chip* 2009, *9*, 1850–1858.

[44] Brouzes, E., Medkova, M., Savenelli, N., Marran, D., Twardowski, M., Hutchison, J., Rothberg, J., Link, D., Perrimon, N., Samuels, M., *Proc. Natl. Acad. Sci. USA* 2009, *106*, 14195–14200.

[45] Kim, S. H., Kim, J. W., Cho, J. C., Weitz, D. A., *Lab. Chip* 2011, *11*, 3162–3166.

[46] Shum, H. C., Zhao, Y. J., Kim, S. H., Weitz, D. A., *Angew. Chem. Int. Ed Engl.* 2011, *50*, 1648–1651.

[47] Zhao, Y., Shum, H. C., Adams, L. L., Sun, B., Holtze, C., Gu, Z., Weitz, D., *Langmuir* 2011, *27*, 13988–13991.

[48] Zhang, J., Coulston, R. J., Jones, S. T., Geng, J., Scherman, O., Abell, C., *Science* 2012, *335*, 690–694.

[49] Zhang, W. Y., Zhang, W., Liu, Z., Li, C., Zhu, Z., Yang, C., *Anal. Chem.* 2012, *84*, 350–355.

[50] Theberge, A. B., Mayot, E., El Harrak, A., Kleinschmidt, F., Huck, W., Griffiths, A. *Lab. Chip*, 2012, *12*, 1320–1326.

[51] Cai, L. F., Zhu, Y., Du, G. S., Fang, Q., *Anal. Chem.* 2012, *84*, 446–452.

[52] Tostado, C. P., Xu, J. H., Du, A. W., Luo, G. S., *Langmuir* 2012, *28*, 3120–3128.

[53] Hofmann, T. W., Hanselmann, S., Janiesch, J. W., Rademacher, A., Bohm, C. H., *Lab. Chip* 2012, *12*, 916–922.

[54] Ogonczyk, D., Siek, M., Garstecki, P., *Biomicrofluidics* 2011, *5*, 013405.

[55] Fallah-Araghi, A., Baret, J. C., Ryckelynck, M., Griffiths, A. D., *Lab. Chip* 2012, *12*, 882–891.

[56] Lin, Y. H., Yang, Y. W., Chen, Y. D., Wang, S. S., Chang, Y., Wu, M., *Lab. Chip* 2012, *12*, 1164–1173.

[57] Zhou, J., Ellis, A. V., Voelcker, N. H., *Electrophoresis* 2010, *31*, 2–16.

