Droplet microfluidics

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Droplet-based microfluidic systems have been shown to be compatible with many chemical and biological reagents and capable of performing a variety of "digital fluidic" operations that can be rendered programmable and reconfigurable. This platform has dimensional scaling benefits that have enabled controlled and rapid mixing of fluids in the droplet reactors, resulting in decreased reaction times. This, coupled with the precise generation and repeatability of droplet operations, has made the droplet-based microfluidic system a potent high throughput platform for biomedical research and applications. In addition to being used as microreactors ranging from the nano- to femtoliter range; droplet-based systems have also been used to directly synthesize particles and encapsulate many biological entities for biomedicine and biotechnology applications. This review will focus on the various droplet operations, as well as the numerous applications of the system. Due to advantages unique to droplet-based systems, this technology has the potential to provide novel solutions to today's biomedical engineering challenges for advanced diagnostics and therapeutics.

Introduction

Since the advent of microfluidics approximately two decades ago, there has been a steady increase in the interest and development of tools for fluid flow at the microscale.[1,2] This multidisciplinary technology involves fundamental concepts from a broad range of fields from biology to electrical engineering. This field generates an equally diverse array of applications that varies from drug delivery to point-of-care diagnostic chips to organic synthesis[3] and microreactors.[4–6] Microfluidic technology holds great promise as it can perform typical laboratory operations using a fraction of the volume of reagents in significantly less time. Reagents can be significantly reduced from milliliters and microliters to nanoliters and femtoliters whereas hours of reaction time could be decreased to mere seconds or less.

One subcatagory of microfluidics is droplet-based microfluidics.[7,8] Unlike continuous flow systems, droplet-based systems focus on creating discrete volumes with the use of immiscible phases. Microfluidic systems are characterized by the low-Reynolds number flow regime which dictates that all fluid flow is essentially laminar. Continuous-flow based systems have exploited this phenomenon to create many novel micro-environments.[9] For instance, a simple device has been created to study drosophila embryo development through local temperature control.[10] Laminar flow behavior also allows for the generation of precise concentration gradients that have been employed in the study of cell migration.[11] Although continuous flow devices offer fine control over flow characteristics, scaling up is a challenge as the size of devices scales almost linearly with the number of parallel experiments. Droplet microfluidics however, has the ability to perform a large number of reactions without increasing device size or complexity. In addition, recent discoveries have demonstrated that droplet microfluidic systems can perform simple Boolean logic functions, a critical step towards the realization of a microfluidic computer chip.[12–14]

Digital† or droplet-based microfluidics involves the generation and manipulation of discrete droplets inside microdevices.[15,16] This method produces highly monodisperse droplets in the nanometer to micrometer diameter range, at rates of up to twenty thousand per second.[17] Due to high surface area to volume ratios at the microscale, heat and mass transfer times and diffusion distances are shorter, facilitating faster reaction times. Unlike in continuous-flow systems, droplet-based microfluidics allows for independent control of each droplet, thus generating microreactors that can be individually transported, mixed, and analyzed.[18,19] Since multiple identical microreactor units can be formed in a short time, parallel processing and experimentation can easily be achieved, allowing large data sets to be aquired efficiently. Droplet microfluidics also offers greater potential for increased throughput and scalability than continuous-flow systems. In the past 5 years, several groups have used droplet microfluidics to form irregular particles,[20] double emulsions,[21] hollow microcapsules,[22] and microbubbles.[23] These particles can be used in a diverse range of applications, including the synthesis of biomolecules, drug delivery, and diagnostic testing.

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† Droplet-based microfluidic systems are sometimes referred to as "digital microfluidics." This term aims to emphasize the use of discrete and distinct volumes of fluids and to contrast with the continuous nature of other systems. However, as this may cause confusion among readers unfamiliar with the field, this review will use either "droplet microfluidics" or "droplet-based microfluidic systems" to refer to the field

This review aims to provide an overview of the operations that have been developed to manipulate droplets and how such techniques can be applied to the biomedical engineering field. The theory behind these operations will not be discussed in depth, and have been covered elsewhere in detail.[24–29] Recent reviews have focused on the theoretical basis[30] and the chemical reactions that can be performed in droplets.[31] We hope to illustrate the various methods and techniques that have been designed to provide precise control over parameters such as size, shape, and concentration inside the droplets. In addition, this review aims to illustrate the many uses of droplet-based microfluidic systems in real world biomedical applications.

Droplet manipulation

As interests grow in the field of droplet-based microfluidics, more technologies are being developed to control, manipulate, and functionalize droplets. Beyond the various methods of droplet generation, operations performed on droplets include fission, fusion, sorting, and mixing of the droplet contents. In addition to these manipulations, the phase of the droplets can be changed through polymerization. Encapsulation of cells, proteins, and DNA and the synthesis of micro/nano particles using droplets have also been demonstrated.

Droplet device considerations

When creating droplet microfluidic systems, some of the most critical matters to address are the materials used for the device and the fluids used for droplet generation. A large number of microfluidic devices are fabricated using poly(dimethyl) siloxane (PDMS), which is a relatively inexpensive and easily moldable elastomeric polymer. However, since PDMS undergoes swelling and deformation in the presence of strong organic solvents, other materials with greater solvent resistance such as glass,[22] silicon,[32] and thiolene have also been used.

Although the size of the orifice of the T-junction or flow-focusing nozzle strongly influences the size of droplets formed, other factors such as the viscosity of the immiscible phases, use of surfactants, and hydrophilicity or hydrophobicity of the channel surface can also be used to augment the size ranges of droplets and particles formed.

The dimensionless capillary number, Ca, plays a key role in determining droplet dynamics, such as fission or droplet break off. The capillary number is defined as: $Ca = \eta v/\gamma$, where η is the viscosity of the continuous phase, v is the velocity of the continuous phase, and γ is the interfacial tension between the oil and water phases. Above a certain critical capillary number, droplet break off occurs. It is important to note that the critical capillary number is system dependent as different values have been reported by various groups using different geometries.[21,26,33] To realize this number, it is important to consider the relative viscosity between the discrete and continuous phases. Selection of a more viscous continuous phase will facilitate formation of droplets. For the formation of water-in-oil (W/O) emulsions, the continuous phase commonly consists of oils or water-immiscible organic solvents, which tends to be naturally more viscous than water. In the case of oil-in-water (O/W) emulsions, the addition of viscous water-miscible fluids such as glycerol into the aqueous continuous phase improves shearing of the more viscous oily discrete phase.[34]

At the microscale level, the high surface area to volume ratio places increasing importance on the interfacial effects which can be found between the immiscible phases in the channel or between the continuous phase and the channel walls. Controlling wetting of the channel walls by the continuous phase is important when switching between the generation of W/O and O/W emulsions or for the formation of double emulsions. To prevent the discrete phase from adhering to the channel walls, W/O droplets are formed in hydrophobic channels, whereas O/W emulsions require hydrophilic channels. Hydrophobic treatments such as silanization and siliconization are used to make a hydrophilic surface hydrophobic.[35] Surface treatments such as oxygen plasma and polyvinyl acetate (PVA) coating can be implemented to temporarily convert the naturally hydrophobic surface of PDMS into a hydrophilic surface.[36] Channel coating with PEG has also been used as a hydrophilic surface treatment and a means to prevent protein adsorption.[37] This is a concern for biodetection devices which aim to enable low working concentrations of material.

The surface wettability can also be altered with the addition of surfactants. Xu *et al.* demonstrated that the addition of Span 80 to water acting as the immiscible phase can change a partially hydrophilic polymethyl methacrylate (PMMA) surface into a completely hydrophobic surface, and addition of Tween 20 to the aqueous phase converts the PMMA surface into an oleophobic surface.[38] Surfactants are also used to prevent unwanted coalescence between droplets by reducing the surface tension between the continuous and discrete phases. Particles and emulsions can be stabilized with the addition of a small concentration of lipophilic surfactant to a water-immiscible continuous phase, or hydrophilic surfactant to an aqueous continuous phase.

Droplet generation

The power of droplet-based microfluidic systems lies in the formation of uniform droplets and particles, thus fine control over the size, shape, and monodispersity of droplets is of the utmost importance. Although the same basic principles and materials are used, a variety of techniques have been developed for droplet generation. One method of forming droplets is in the form of an emulsion created using two immiscible fluids such as water and oil. However air–liquid droplet systems have also been studied extensively.[39] In addition to the viscous forces used to create droplets from streams, surface/interfacial chemistry and channel geometry also play important roles in droplet generation.[40]

Droplets and particles have the potential to become important tools for drug delivery and biosensing. In order for them to function properly, correct dosing and manufacturing must be ensured. Since biological and chemical properties of microparticles are strongly affected by both the size and morphology, it is essential to be able to generate these structures at well-defined volumes and composition. Traditional top-down methods of emulsion and particle formation, such as

direct agitation of immiscible fluids and grinding of polymeric material, respectively, result in broad size distributions.[41] On the otherhand, droplet microfluidics has been shown to generate highly monodisperse droplets with smaller than 1% size variations.[42]

Researchers in the field have developed a variety of different droplet formation techniques, of which four will be covered in this section. T-junction and flow-focusing are two methods that depend on channel geometry to control the generation of droplets. In addition, emerging technologies have enabled electrodes to be integrated into microdevices to provide electrical control over droplet formation. Two examples of these electrohydrodynamic (EHD) methods are dielectrophoresis (DEP) and electrowetting on dielectric (EWOD).

T-junction. In the T-junction configuration, the inlet channel containing the dispersed phase perpendicularly intersects the main channel which contains the continuous phase.[43–46] The two phases form an interface at the junction, and as fluid flow continues, the tip of the dispersed phase enters the main channel. The shear forces generated by the continuous phase and the subsequent pressure gradient cause the head of the dispersed phase to elongate into the main channel until the neck of the dispersed phase thins and eventually breaks the stream into a droplet (Fig. 1a). The sizes of the droplets can be changed by altering the fluid flow rates, the channel widths, or by changing the relative viscosity between the two phases. T-Junctions are not limited to single inlets as more complicated schemes have been used for performing chemical reactions,[47] and forming gas plugs[48] and droplets of alternating composition.[49]

Flow-focusing. In the flow-focusing configuration, the dispersed and continuous phases are forced through a narrow region in the microfluidic device [50–54] (Fig. 2a). The design employs symmetric shearing by the continuous phase on the dispersed phase which enables more controlled and stable generation of droplets. An extension of flow-focusing is

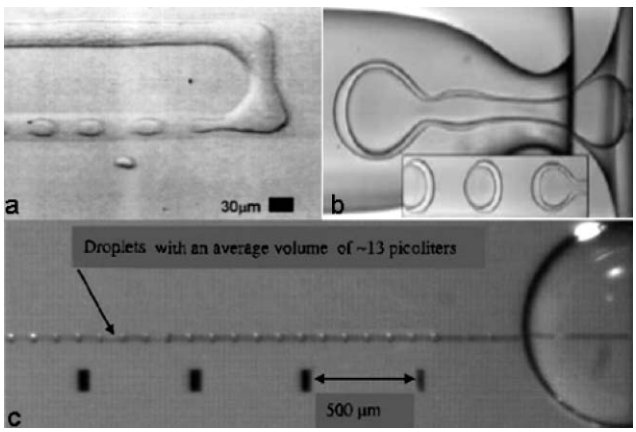

Fig. 1 Droplet formation with different mechanisms: (a) T junction (reproduced with permission from ref. 44), (b) capillary focusing generation of oil/water/oil emulsion (reproduced with permission from ref. 22), and (c) dielectrophoresis-based generation (reproduced with permission from ref. 62).

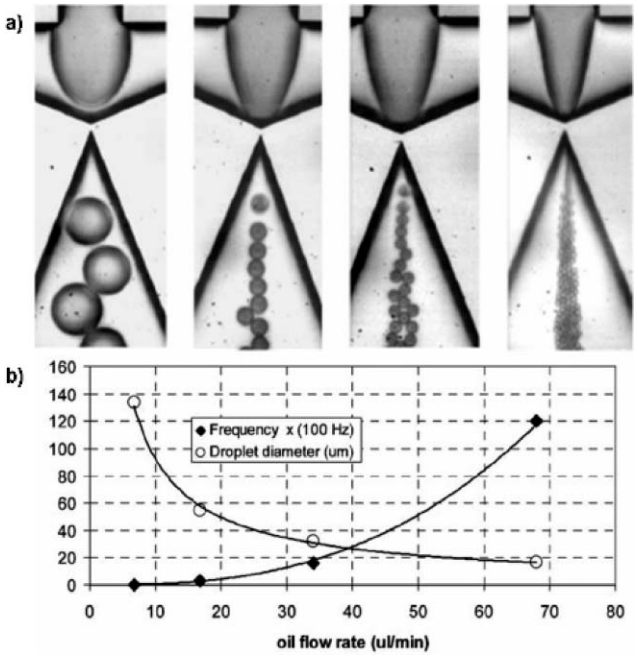

Fig. 2 (a) Formation of water-in-silicone oil droplets using a flow focusing design with an embedded circular orifice. (b) Graph showing decreasing droplet size and increasing frequency of formation with increasing oil flow rate (reproduced with permission from ref. 54).

shear-focusing, which aims to create a singular point of highest shear, which exists at the narrowest region of the nozzle.[52] This singular point ensures that the break-off of droplets from the fluid stream occurs consistently at that point thus forming uniform droplets. The sizes of the droplets can decreased by increasing flow rates of the continuous phase. Additionally, as shown in Fig. 2b, an increase in oil flowrates also increases the frequency of droplet generation.

Since both designs operate based on shearing of fluids, a similar set of paramters govern generation in flow-focusing designs. Properties such as channel geometry, flow rate, and viscosity all play critical roles in controlling droplet generation. Many variations of the basic flow focusing design have been developed to facilitate more complex applications.[34] Flow focusing channels may be fabricated using different methods including soft lithography or the insertion of capillary sheathes into microdevices. In the capillary design, the dispersed phase is injected through the capillary needle, and the continuous phase forms an outer shell around the central capillary.[22] Both phases are forced through the orifice and the droplets are broken off downstream of the orifice (Fig. 1b). Lee *et al.* broadened the size range of droplets that can be generated by incorporating pneumatically controlled walls to widen or narrow the flow focusing region.[55] Malloggi *et al.* was able to control electrowetting of the channel surface and enable electrical control of flow-focusing droplet generation.[56] Flow focusing methods have also been used to generate microbubbles,[57] multifunctional particles,[58,59] ionic fluid emulsions,[60] and double emulsions.[61]

DEP-driven droplet generation. Dielectrophoresis or DEP, can be used to generate uniform droplets by pulling the

droplets from a fluid reservoir[62–66] (Fig. 1c). It is different from electro-osmosis and other EHD processes because the fluid can be electrically neutral, and the force exerted on the uncharged fluid is caused by a nonuniform electric field. The operation principle behind DEP-driven droplet formation is based on the phenomenon that polarizable fluids will be attracted to areas of higher electric field intensity. Whereas EWOD-based droplet platforms utilize a 'wetting' force, DEP functions through the contribution of three main forces: a wetting force on the interfacial line between the droplet, its surrounding medium, and the surface it contacts; a force on the interface of the two fluids; and a body force due to pressure gradients in the fluid. The size and uniformity of the droplets depend on the magnitute and the frequency of the applied voltage. The droplets do not need to be in contact with a surface, but it is necessary that the droplet is composed of a liquid of higher dielectric permittivity than its surrounding fluid.

EWOD-driven droplet generation. EWOD is one form of EHD which has been implemented in the formation of microliter sized droplets.[67–70] A comparison between DEP and EWOD droplet generation is given by Zeng and Korsmeyer[71] and Jones.[72] The mechanism of EWOD-based generation relies on the observation that an electric field can change the interfacial energy between a fluid and the surface it is in contact with.[73] Since interfacial energy directly affects the contact angle, an electrical field can be used to reduce the contact angle and cause the fluid to 'wet' the surface. In essence, the hydrophilicity of an area can be temporarily increased around the fluid stream. EWOD devices can be fabricated as either a one or two plane device. In a two plane device the ground electrode is often placed on the top layer with the control electrodes on the bottom (Fig. 3). Both layers include an insulating layer separating the droplets from the electrodes. Activation of the electrodes initiates fluid wetting of the channel and within tens of microseconds, the fluid begins to form a short liquid finger between the electrodes. The electrodes are then switched off, reverting the surface back to being hydrophobic. This causes the finger to break off from the reservoir, and form a droplet. The size of the droplet is dependent on the electric field strength, frequency of the applied field, and width of the channel opening. For example,

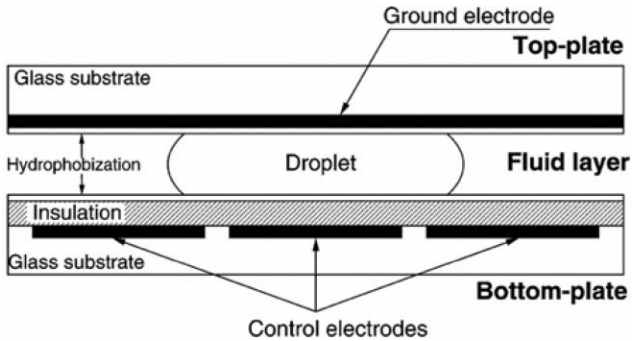

Fig. 3 Cross sectional schematic of EWOD-based droplet system. In most cases, indium tin oxide serves as the electrodes, a teflon coating provides hydrophobization, and parylene C provides insulation (reproduced with permission from ref. 32).

higher frequencies produce small droplets whereas lower frequencies generate larger droplets.

Picoliter to femtoliter sized aqueous droplets have also been produced using EHD generation methods.[74,75] One advantage of EHD generation is that no external pumps are required, allowing the system to become more compact and appealing for use in point-of-care devices. In addition, the number of droplets produced can be controlled by the applied electric field strength. Groups have used increased field strengths with the EHD droplet generation techniques for preparing samples by electrospray for mass spectrometry experiments, or lower field strengths for controlled dispensing of samples onto chips for protein or DNA arrays.[76,77]

Microbubble generation. In addtion to liquid–liquid phase emulsions, gas-liquid dispersions have also been reported in microfluidic systems. Control over the size and volume fraction of microbubbles are critical for their applications. In a monodisperse microbubble system, the Laplace pressure, the driving force behind gas diffusion into a liquid, is considerably reduced and results in more stable microbubble formation. Microfluidic systems, with the ability to handle liquids in the microscale, provide effective methods for the generation of monodisperse microbubbles. Designs of microfluidic devices for microbubble generation include T-junction,[24,57] capillary,[80,81] and flow focusing.[82–86] The size, generation frequency, and gas fraction of microbubbles depend on the flow rates of the gas and continuous phase, viscosities of fluids, and channel geometry.

A large number of methods have been developed for droplet generation, but due to the formatting of this review they can not all be covered in detail presently. Droplet generation systems have been created using a variety of different methods of generation and control mechanisms including pressure,[87] flowrate,[88] viscosity,[89] electrical,[19,90] and centrifugal force.[91] In addition, generation components have been parallelized to scale up droplet generation.[92,93] Table 1 provides a cursory summary of the droplet size and generation frequency ranges of various droplet-based microfluidic systems. It is critical to note that this is a very limited set of results and should be in no way considered as limits to these systems. The table however does demonstrate the wide range of capabilities of droplet microfluidics.

Droplet fission

The advantages of using droplet microfluidics over continuous flow systems are its throughput, scalability, and its ability to run parallel experiments. Since each droplet can serve as a vessel for reagents, by splitting the single droplet into two or more droplets, the experimental capacity can be easily scaled up. Therefore droplet fission or splitting is a critical operation that can enhance the effectiveness of droplet-based microfluidic systems. In addition to increasing experimental throughput, droplet fission can also be used as a method to control the droplet content concentration.[94]

Passive fission. Passive methods do not rely on peripheral power sources or components to perform fission. Instead, they

Table 1 Size and frequency distributions for various droplet generation systems

	Geometry and material	Continuous phase	Size/μm	Frequency/Hz
Water in oil	Channel array in silicon[78]	Kerosene with monolaurate	21	~5300 (est.)
	T-junction in acrylated urethane[44]	Decane, tetradecane, and hexadecane with Span 80	10 to 35	20 to 80
	T-junction in PMMA[45]	High oleic sunflower oil	100 to 350	10 to 2500
	T-junction in PDMS[260]	$C_{14}F_{12}$ with $C_6F_{13}(CH_2)_2OH$	7.5 nl (plug flow)	2
	Shear-focusing in PDMS[52]	Oleic acid	13 to 35 (satellites <100 nm)	15–100
Oil in water	Channel array in silicon[78]	Water with SDS	22.5	~5300 (est.)
	Sheath flow in glass capillary[79]	Water with SDS	2 to 200	100 to 10000
Gas in liquid	Flow-focusing in PDMS[83]	Water with Tween 20	10 to 1000	>100000
	Shear-focusing in PDMS[86]	Water with phospholipids	5 to 50	>1000000
Liquid in air	DEP on hydrophobic insulator[62]	Air	10 pl	~8 (est.)
	EWOD on hydrophobic insulator[32]	Air	~700 nl	~1 (est.)

depend on shear forces created by channel design to split the droplets at precise locations into controlled volumes. Droplet splitting has been performed with several channel designs including a T junction,[94,95] branching channels,[96,97] and with channel obstructions[98] (Fig. 4a–b). Theoretical investigation of passive droplet break up has been studied thoroughly.[99] Passive droplet splitting can be controlled by varying the flow rate of the continuous phase and resistances in the channels. When the flow applied to either half of a droplet is equal at the bifurcating junction, the two outlet channels will pull at the droplet halves symmetrically, causing the droplet to break up into two equal-sized daughter droplets. Droplet splitting was also demonstrated by Link *et al.* using a PDMS block obstruction in the channel.[98] Placement of the obstacle along the vertical axis determined the degree of asymmetrical break up of the resulting droplets. Sato *et al.* fabricated a three dimensional SU-8 microfluidic chip which incorporates a T-junction for droplet generation and micromesh structure for droplet splitting.[100]

Active fission. In contrast to passive fission methods, active fission may rely on external power or electrical control of the splitting mechanism. EWOD is a technology several groups have used to actively divide a droplet into smaller droplets. This method has also been used for transport, fusion, mixing, and other fundamental fluidic operations that will be discussed later in this review.[101]

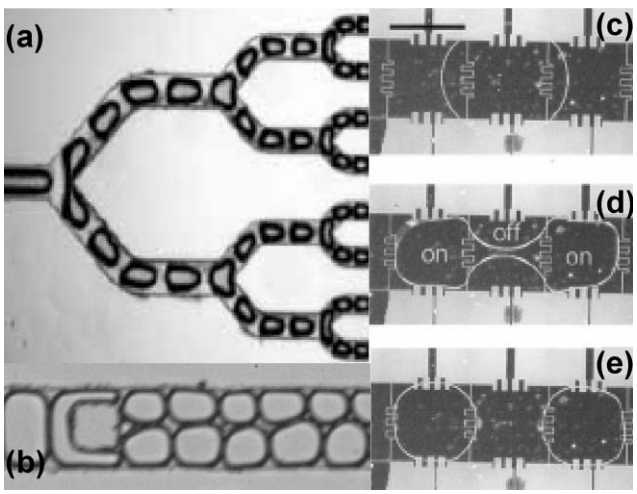

Fig. 4 (a) Bifurcating channel geometry used to halve droplets at each junction. (reproduced with permission from ref. 97). (b) Pillar in channels demonstrates asymmetric fission of water-in-oil droplets (reproduced with permission from ref. 98). (c–e) Active fission of droplets using DEP through surface electrodes in EWOD system (reproduced with permission from ref. 67).

Transport and splitting of droplets by EWOD are not done in closed channels nor on open surfaces, but between electrically addressable parallel plates. There are no pre-defined channels to guide the droplets since the path of travel can be dynamically addressed through the electrode pads. The droplets lie on a dielectric surface which sandwiches the electrodes with a non-conductive substrate. Splitting is achieved when the surfaces near the opposite ends of a droplet are activated, and the surface central to the droplet is grounded. The activated regions will pull the droplet towards its respective ends, causing the droplet to pinch and divide in the middle. The controllable splitting of a droplet into two equal volumes has been demonstrated with EWOD[67] (Figs. 4c–e). Thermally induced surface gradients were also utilized to manipulate droplet fission and sorting in micro-channels.[102] Higher temperature lowers the viscosity and interfacial tension of liquids, therefore this concept can be applied to selectively attract an aqueous plug into a heated branch channel much the same way an activated electrode can attract an aqueous droplet.

Droplet fusion

Controlled coalescence of droplets is an important means of performing reactions within droplets. Reactions in droplets can be used for a number of applications, including the formation of particles, chemical synthesis, kinetics studies, or for the synthesis of biomolecules. For some reactions, it is critical for reagents to be kept separate until the proper conditions are available. Containment inside droplets provides an effective method to achieve this goal in a microfluidic device. Because premature fusion of the droplets will result in unreliable data, or deformed particles, it is important for droplet fusion to be a highly controlled process. As with previous fluidic operations, there are passive and active methods of controlling droplet fusion. Channel geometry and electrodes were implemented for passive and active fusion, respectively.

Passive fusion. In passive droplet fusion, the design of the channel is used to control the location of droplet fusion. Proper fusion can be challenging since it depends on droplet

frequency matching under high flow conditions. These challenges can be overcome since droplet generation frequency can be controlled by flowrate and channel geometry.[103] If the frequencies of two droplet generators are well synchronized, then fusion can occur at a channel junction. Fusion has also been demonstrated with a channel obstruction that is large enough to increase channel resistance but small enough to allow the merged droplets to pass through.[104] Fidalgo *et al.* demonstrated droplet fusion with selective hydrophilic treatment of a portion of the microchannel.[105] A sequence of alternating droplets are flowed through the channel and as pairs of droplets of different aqueous compositions approach the hydrophilic patch, they become trapped and fuse with each other.

Droplet fusion initiates when two or more droplets are brought close to each other by draining the continuous phase between the droplets until a thin film of fluid forms connecting the interfaces.[106] Increased pressure on the film and imbalance in the surface tension will cause the film to rupture and the droplets to coalesce (Fig. 5a–b). Several different channel configurations have been designed, but the primary method of passive fusion is the incorporation of an expanded portion in the microchannel.[107] This expanded region promotes continuous phase drainage by reducing the droplet flow rate. The droplets then enter a segment of narrower channel that increases the flow rate and causes the film to rupture thus allowing droplet fusion. Tan *et al.* demonstrated droplet fusion with various channel expansion designs.[94] The flow rectifying design was found to offer the greatest control over droplet fusion and had the capabilities to fuse three or more droplets simultaneously. The design works by removing fluid from the expansion region at equal volumes by pulling fluid through the top and bottom channels. This ensures that the droplets will not deviate from the intended path. The volume between droplets can be drained at precise flow rates, providing control over the number of droplets fused. Hung *et al.* demonstrated the generation and pair-wise fusion of uniform alternating droplets using an expanded channel configuration[108] (Fig. 5c).

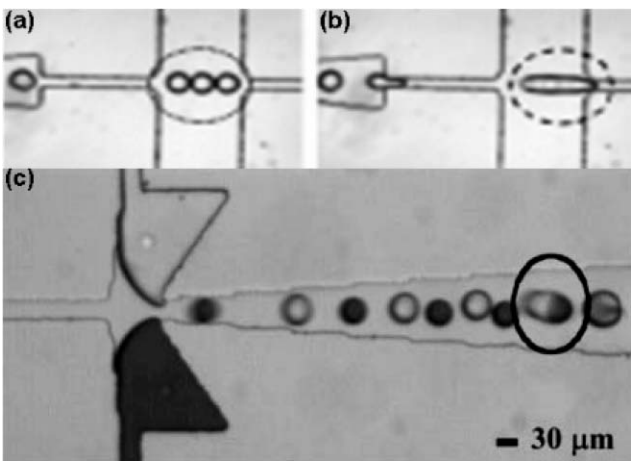

Fig. 5 Passive fusion of droplets using channel geometry. (a–b) Fusion of a series of three droplets with continuous phase drainage (reproduced with permission from ref. 94). (c) Fusion of alternately generated droplets in a widening nozzle channel (reproduced with permission from ref. 108).

By controllably fusing two droplets containing different reagent solutions, CdS nanoparticles were generated on chip.

A method of introducing additional reagents into droplets without using fusion of droplets was demonstrated by Li *et al.*[109] Instead of containing different reagents in separate droplets, the additional reagent was added to a droplet by a set of three narrow side channels. As the droplets pass by, materials from the side channels are pushed into the droplets. The group was able to demonstrate that by using three narrow channels, contamination of side channels is significantly reduced when compared to using a single side channel. This method allows serial addition of reagents without the need to generate and synchronize a large number of different droplets. The side channel also can be used to generate immiscible third liquid droplets as spacers to prevent droplet fusion.[110]

Active fusion. Active fusion of droplets has been achieved using EWOD and other electric-controlled methods.[111] Priest *et al.* performed electrocoalescence of tightly packed droplets within 100 μs with voltage as low as 1 V DC[112] (Fig. 6a–b). Electrodes were placed parallel to the droplet channels and a range of AC and DC voltages were used to fuse droplets or cells.[113] DEP has also been used as a method of droplet fusion.[114] As long as the droplet composition is dielectrically distinct from its carrier fluid, DEP can be used to manipulate the droplet. Activation of electrodes adjacent to a droplet initiates droplet movement. By sequentially turning a series of electrodes on and off, the droplet can be guided toward another droplet until coalescence occurs.[115]

Tan *et al.* used a combination of an expanded channel configuration to bring two surfactant stabilized droplets close

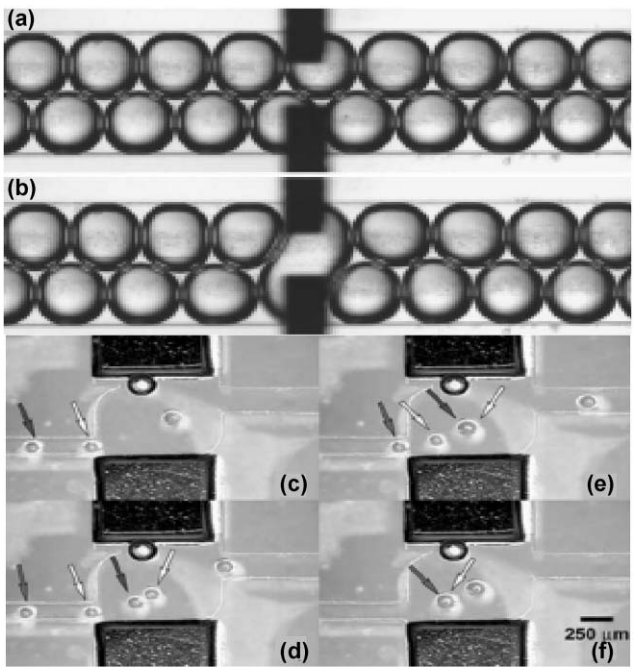

Fig. 6 Active fusion of droplets using electrodes. (a–b) Gold electrodes inducing fusion of NaCl solution droplets (reproduced with permission from ref. 112). (c–f) Fusing of water droplets in n-hexadecane (reproduced with permission from ref. 116).

to one another, and parallel aligned electrodes to fuse the droplets[116] (Fig. 6e–f). Electrofusion has also been applied to more stabilized vesicles such as cells and liposomes.[117] Short DC pulses are applied to the vesicles to form small pores in the membrane. When two porous vesicles are brought in close proximity of one another, their membranes will reorganize and merge together. This application could be used for the creation of hybrid or artificial cells.

Active droplet fusion has also been demonstrated with heating elements as the control mechanism. This method takes advantage of the thermodynamic property of fluid in which the viscosity decreases with increasing temperatures. This principle is applied to the continuous phase to dynamically control the drainage rate of fluid in between droplets. In this design, a fluid resistance bypass was incorporated to allow passage of the lower viscosity continuous phase when the heating element is activated. Drainage of the continuous phase slows movement of the droplet and causes it to merge with the adjacent droplet.[104] Optical tweezers have also been implemented for droplet fusion.[118] This method allows for fusion of specific droplets through direct manipulation with the optical tweezers. However, a disadvantage of this system is its decreased throughput.

Mixing in droplets

Mixing is an important tool required for carrying out and studying the kinetics of biological and chemical reactions. When dealing with fluids in the microscale, one major problem is being able to overcome interfacial forces and promote mixing between two fluid streams. Due to laminar flow conditions, when two fluid streams come into contact with each other, there is no turbulent mixing and the only mixing behavior is diffusive. The same properties that allow adjacent miscible fluids to flow in distinct streams becomes a problem when one needs the fluids to mix. Although the diffusion distance is smaller, the time required to completely mix the two fluids is still long. Even inside droplets, the laminar flow conditions can be preserved and has lead to the development of interesting biphasic particles. Clever channel configurations have been implemented to promote rapid internal mixing within droplets. Electrowetting-based droplet devices have also developed mechanisms to rapidly mix the contents inside droplets.

Passive mixing. When a droplet moves through a straight channel, an equal recirculating flow is generated in each half of the droplet that touches the channel wall.[119] Fluids within each half of the droplet are mixed, but the halves remain unmixed and separated from each other. To enhance internal mixing within droplets, channel geometry is used to create chaotic advection to fold and stretch the droplet contents[120] (Fig. 7a). Chaotic advection can be promoted with the use of bends and turns in the microchannel design. As the droplet traverses through a winding channel, the halves of the droplet experience unequal recirculating flows. One half of the droplet is exposed to the inner arc of the winding channel, a shorter channel section, and thus a smaller recirculating flow is generated compared to the other droplet half which is exposed to a longer channel section (Fig. 7b). The irregular motion along the walls promotes chaos and crossing of fluid streams since the fluid vortexes of each half are asymmetrical. The droplets achieve an alternating asymmetric flow pattern through the serpentine microchannels. The sharp turns also help to reorient the droplet so that it becomes thoroughly mixed as it goes through a series of stretching, reorientation, and folding[121] (Fig. 7c). An advantage of this design is that the degree of mixing is determined by the length of the channel.

Configurations other than winding channels have also been developed to promote mixing. Sarrazin *et al.* investigated the influence of coalescence geometry on mixing of droplets flowing into a straight channel.[122] Instead of fusing the droplets in a symmetrical fashion, the group implemented a shifted coalescence design such that one droplet meets the other perpendicularly. They observed partial mixing in the symmetrical fusion design at 40 ms and complete mixing within 10 ms in the shifted fusion design. This time is comparable to the mixing rate in winding channels.

Liau *et al.* have addressed the issue of mixing difficult biological reagents such as bovine serum albumin which are viscous and tend to adsorb to channel surfaces.[123] The group tested mixing in serpentine microchannels, straight channels, and straight channels lined with small protrusions and found

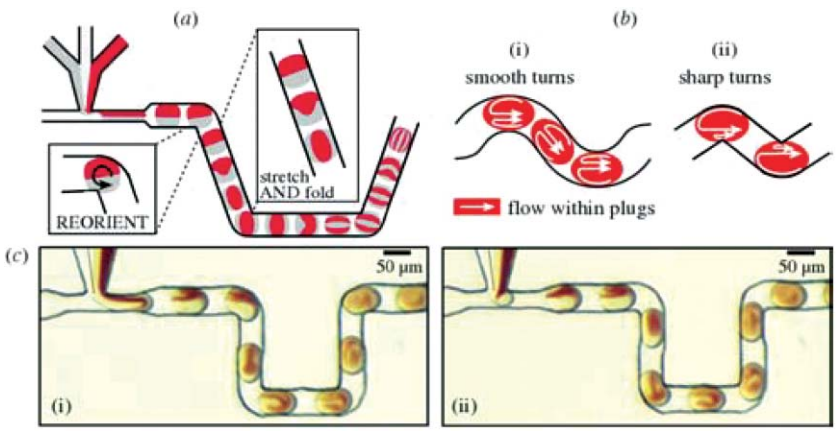

Fig. 7 Passive mixing within droplets. (a–b) Schematic demonstrating mixing patterns within droplets inside winding channels. (c) Experimental results show the rotational pattern (reproduced with permission from ref. 121).

that droplets filled with high concentrations of protein mixed ineffectively in all three cases. However, it was observed that the addition of small protrusions increased circulation within the droplets significantly over straight channels. Thus the group created a mixing design which incorporates protrusions along a winding channel to induce oscillating interfacial shear in the droplets. The protrusions help to improve circulation within the droplets by thinning the oil on one side of the droplet and increasing interfacial stresses. Cabral and Hudson have demonstrated a mixing method which includes larger protrusion in a straight channel.[124] These protrusions work to deform the droplet and promote internal circulation.

Active mixing. The mixing inside of droplets can also be electrically controlled. Electric control can be implemented a number of ways, with either the droplets sandwiched between electrodes, or with the droplets lying on an array of electrodes. The droplets may also be immersed in air or oil, and not in direct contact with the electrodes, but separated by a hydrophobic dielectric layer instead. The manipulation of droplets composed of water, buffers, and biological fluids in both air and oil has been demonstrated.[125–127] Electric control offers advantages such as conservation of space and flexibility from a limited channel design. Electric control allows a droplet to be moved back and forth and mixed in a confined space whereas channel-based mixing requires more elaborate and long winding channels. Each droplet is also individually controlled and its mixing is not dependent on channel design or fluid flow rates.

Droplet movement and manipulation using external electric fields have been demonstrated.[32,128] Paik *et al.* have investigated droplet mixing using EWOD and the influence electrode aspect ratio has on the degree of mixing.[129] Droplets are fused and then moved back and forth on a linear array of electrodes. Mixing is then observed from both the top and side view to ensure accurate measurement of mixing times. The group developed various mixing schemes depending on the aspect ratio of the electrodes. When using electrode aspect ratio below 0.2, mixing was facilitated by incorporating a splitting and fusion technique. Droplets were split, oscillated, and then fused back together over three electrodes. This sequence is repeated multiple times and found to take approximately two seconds for complete mixing. When using higher aspect ratio electrodes, electrode arrays were used to transport and mix droplets. Fused droplets were mixed by transporting them in a circular motion on the electrodes (Fig. 8). Complete mixing was demonstrated on 2 × 4 electrode arrays within three seconds.

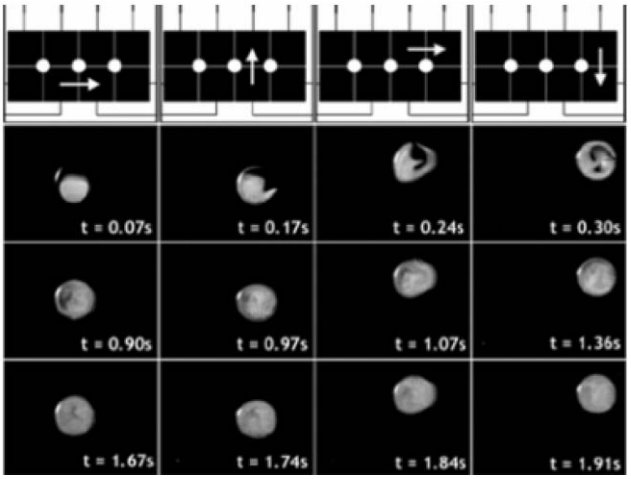

Fig. 8 EWOD-based active mixing achieved by moving droplets across the surface in the pattern described in the top row of images (reproduced with permission from ref. 129).

Droplet sorting

One of the key advantages of droplet microfluidics is the ability to generate unique droplets that can be transported and analyzed individually. Sorting facilitates an array of functions including the isolation of droplets of interest, purification of synthesized samples and the segregation of heterogeneous mix of droplets. Additionally, sorting mechanisms enable individual control of single droplets out of a population.

Sorting can be divided into two types, passive and active. Passive sorting includes systems in which a bias is applied constantly to distinguish the species to be sorted. An active sorting system employs an increased level of complexity, but provides dynamic control over the bias and has more flexibility over the parameters it can sort. It needs to be noted that in passive sorting systems the bias and the sorting parameter is coupled; whereas in active systems the two need not be and thus allows active systems to sort droplets using a variety of characteristics such as particle content or functionality. More precisely, active sorting schemes involve both a mechanism to manipulate the movements of droplets and a method to detect the sorting criteria. Gravity and channel geometry has been employed to sort droplets passively by size and active sorting employs electrical control and has also been used as a mechanism to manipulate the droplets.

Size-based sorting using channel geometry. An example of passive droplet sorting is the system designed by Tan *et al.*[94] In this setup, the microfluidic channels are designed so that the flow stream of the continuous phase carries the smaller satellite droplets into a side channel, whereas the larger primary droplets flows through the main channel. Due to their smaller surface area, the satellite droplets are exposed to the flow projected from only the side branch whereas the larger droplet feels the higher velocity flow stream of the main channel. Since the satellite droplets are byproducts of the droplet generation process, sorting the droplets by size allows the sample to be purified. This concept was also applied to larger droplets (Fig. 9a–e). Size sorting using channel geometry has been demonstrated to differentiate size differences as small as 4 μm.[130] However, it has yet to be shown what limits this method has in terms of its sensitivity to similar sized particles.

Gravity-driven size-based sorting. Another method that sorts droplets by size utilizes gravity. Huh *et al.* developed the μSOHSA—microfluidic sorting device with hydrodynamic separation amplification—which combines gravity-based sorting and channel design to purify perfluorocarbon droplets from a polydispersed emulsion (Fig. 9 f–g).[131] The basis of this

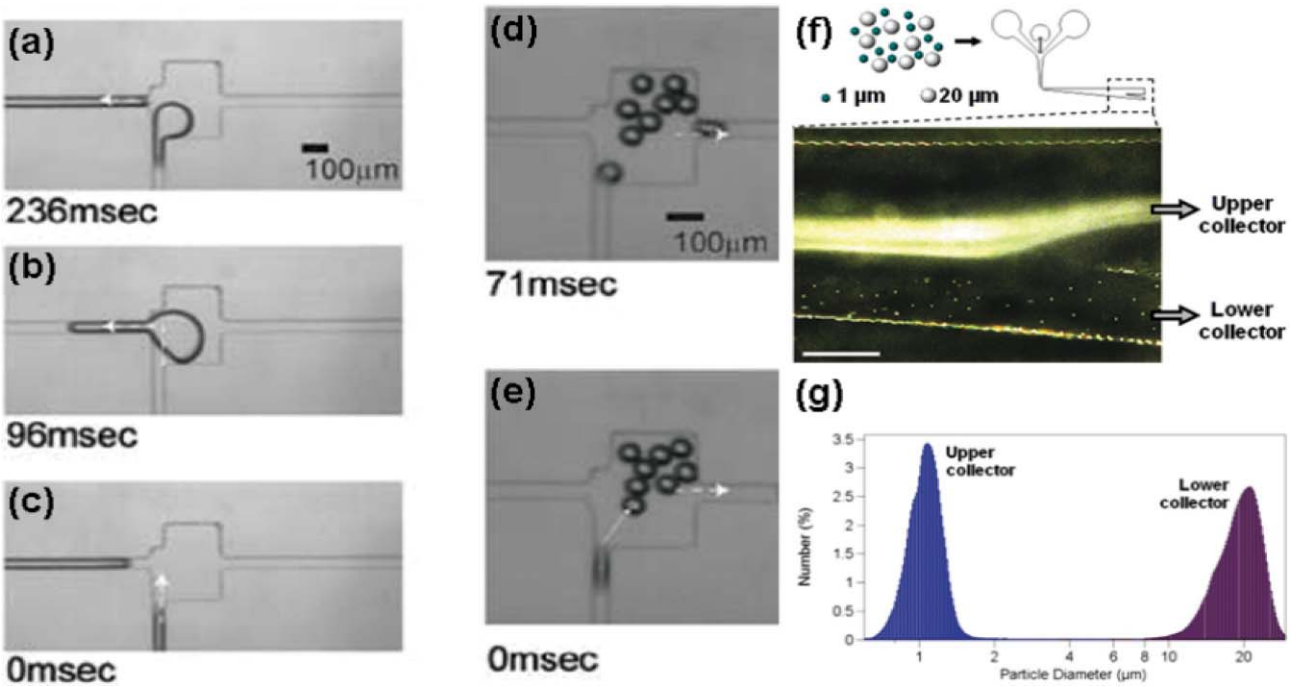

Fig. 9 Passive size-based sorting using channel geometry. (a–c) Larger droplets move to the left channel (white arrowhead). (d–e) Smaller droplets move to the right channel (white arrowhead). Size-based sorting utilizing sedimentation effect (reproduced with permission from ref. 130). (f) Top—schematic of particles loaded into the device. Bottom—micrograph of widening separation region shows distinct separation. (g) Collected particles indicate high efficiency in sorting (reproduced with permission from ref. 131).

sorting scheme is that the sedimentation velocity of larger particles is greater than that of smaller particles at a given density. This difference results in the separation of larger droplets away from the smaller droplets. This effect is greatly enhanced by the asymmetrical widening design of the separation channel which has a different downward velocity depending on the position along the vertical axis. The combination of greater sedimentation velocity and higher downward velocity moves larger particles towards the bottom of the channel effectively. The μSOHSA was shown to be able to sort out virtually all small (<6 μm diameter) perfluorocarbon droplets out of a polydispersed sample.

DEP-based sorting. An example of active sorting, DEP-based schemes allow manipulation of individual droplets, particles, or cells within microfluidic channels.[132,133] DEP has recently been demonstrated by Wang *et al.*, showing the ability to controllably direct particles into five distinct channels, opening up possibilities for complex sorting mechanisms (Fig. 10a–d).[134] In the design created by Ahn *et al.*, indium tin oxide (ITO) electrodes are placed beneath PDMS microfluidic channels to manipulate water droplets in hexadecane.[135] The electric field created between the electrodes provides the force to steer the droplets into designated downstream channels. The location and shape of the electrodes are designed to maximize the field gradient and effectiveness in deflecting the droplets. In addition to demonstrating the ability to control an entire stream of droplets, the device could also sort out a single droplet out of the stream at a generation rate of 1.6 kHz. The ability to manipulate and sort out single droplets combined with a suitable detection scheme will enable a platform capable of high throughput screening for various applications.

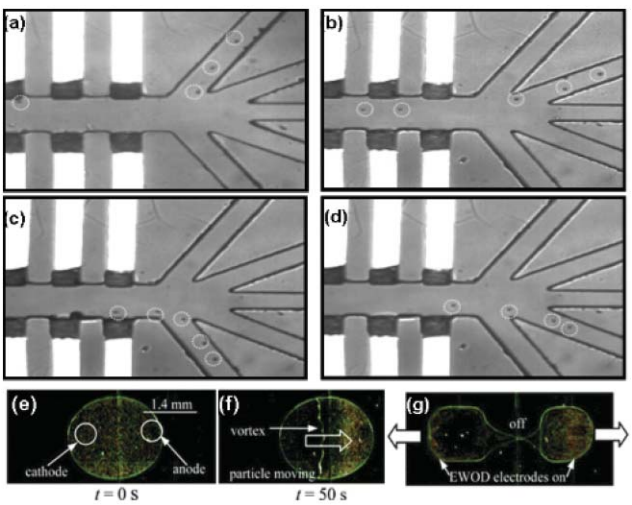

Fig. 10 Active sorting mechanisms. (a–d) DEP-based selective sorting of beads into five channels (reproduced with permission from ref. 134). (e–g) EWOD-based sorting of particles inside the droplet followed by compartmentalization of particles into separate droplets (reproduced with permission from ref. 136).

EWOD-based sorting. Another example of active sorting mechanisms is EWOD-based droplet manipulation. As mentioned in the droplet generation section, EWOD uses electrodes to change the interfacial energy between the droplet and the surface to cause droplet movement. This phenomenon can

also be used to move droplets along a path. Cho *et al.* demonstrated sorting of droplets and its contents using this method by first separating two types of particles into opposite regions of a single droplet using electrophoresis, then splitting the droplet in half using EWOD (Fig. 10e–g).[136] The two daughter droplets then can be directed to move along different paths and thus separating and sorting two droplets with different contents. The advantage of this system is its ability to perform separation inside the droplets prior to sorting. However, it is limited in its throughput as current EWOD technology has yet to match the generation and operation speed of flow-focusing based droplet devices.

Other manipulation/sorting technologies. In addition to the methods mentioned above, many techniques that have been designed to manipulate objects such as particles or cells could be applied to droplets. For example, optical manipulation inside microfluidic devices demonstrated by Ozkan *et al.* using vertical cavity surface emitting laser (VCSEL) could potentially be adapted to droplet manipulation and sorting.[137] The group showed sorting of objects such as cells and polystyrene beads. In addition, localized heating caused by a laser was used to control generation and sorting of droplets.[138] Since most droplets generated in a microfuidic device are on the same size scale as beads and cells, an optical sorting mechanism is another possibility. An example was demonstrated by Jeffries *et al.*[139] The group used an optical vortex trap to expand or shrink single droplets, which enable alterations to the chemical concentration in each aqueous droplet.

Wang *et al.* fabricated vertical electrodes inside SU-8 channels to create a device that is capable of switching using magnetohydrodynamic (MHD) force.[140] The vertical electrodes serve as MHD pumps that drive the fluid flow in the channels. Three sets of MHD electrodes are used in conjunction to create a sorting junction that is capable of directing cell flow into two separate channels. Since this scheme controls flow based on the continuous phase, it can be used for a variety of sorting parameters when operating on droplets. This sorting scheme can also be scaled up to enable sorting into multiple channels.

Electroosmotic flow has also been used as the basis of sorting. Dittrich and Schwille have been able to achieve high specificity sorting of fluorescent particles and cells utilizing electroosmotic flow to direct the flow of the continuous phase.[141] The particles to be sorted are focused at the center of the channel and are directed into two downstream channels. The switching of the fluid stream is actuated by the activation of perpendicular flow driven by electroosmotic forces. This perpendicular flow forces the stream of particles into either the left or right channel depending on the fluorescence signal. This is another method that could be incorporated into droplet-based microfluidic systems.

Phase change in droplet

Droplet-based microfluidics provides a robust platform for the manipulation of a variety of fluids and is capable of performing an array of operations and reactions. However, many biomedical applications require materials that are not liquid but in the form of solids or gels.[142] Solid particles made from polymeric and biological materials are used in drug delivery[143–146] and hydrogels[147] are being studied for encapsulation of cells for implantation and drug studies. Many droplet-based systems have been designed to create solid particles as well as hydrogel beads through different means.[148–150]

There are two main methods that have been used to change the phase of the droplets from liquid to either solid or gel in microfluidic systems. First, ultra-violet (UV) light has been used to initiate polymerization of soluble polymers into solid particles. The UV light activates photo-initiators in the droplet which causes monomers to link to each other and solidifies the droplets. UV-initiated polymerization has been used to create particles with interesting properties. Chemical agents have also been employed to induce polymerization. Unlike UV light, fluidic channel design becomes an important factor in introducing chemical agents to droplets.

In addition to forming particles by polymerization, solvent extraction/evaporation methods had been used in combination with droplet microfluidics to create monodisperse polymer particles.[151] Lorenceau *et al.* and Hayward *et al.* created polymersomes by first forming water/oil/water emulsions with diblock polymers dissolved in the oil phase, and then removing the solvents in the oil phase through evaporation.[152,153] This creates a thin polymer membrane separating the two water phases. Kobayashi *et al.* created tripalmitin particles by evaporating hexane after collecting the droplets in a sample bottle.[154] PLGA particles of various sizes have also been synthesized by Hung and Lee using a combination of droplet microfluidics and miniaturized solvent extraction/evaporation processes.[155]

Photo-initiated polymerization. Photo-initiated polymerization uses light, usually UV to activate photo-initiators. The photo-initiators could then become a reactive radical. Radical polymerization then links the monomers and solidifies the droplet. Due to the use of optically clear polymer and glass, many microfluidic platforms are capable of integrating light sources into the set up to allow photo-initiated polymerization. Groups have demonstrated particle synthesis using this method with a variety of materials. More interestingly, novel particle shapes have been created using microfluidic platforms that cannot easily be made using traditional methods.

Due to the simplicity of photopolymerization, a number of different designs have been established utilizing this method. De Geest *et al.* uses a flow focusing design combined with UV illumination to create dex-HEMA gel beads.[156] A solution of dex-HEMA and photoinitiator is sheared by a continuous phase consisting of mineral oil, and the droplets are collected along with the oil in a vial. The vial is then immediately irradiated and the droplets solidified by UV. The group also tested the functionality of the beads by incorporating FITC-BSA in the microgels to study the degradation of and release of protein from the beads. Ikkai *et al.* designed an array of microchannels that was used to create UV-polymerized *N*-isopropyl acrylamide gel beads.[157] In this design, the droplets are generated in a modified T-junction and are collected in a vial similar to the method used by De Geest *et al.*

Droplets can also be polymerized *in situ* by designing channels that allow droplets to be irradiated by UV while remaining on chip. The primary challenge of on-chip UV polymerization is the need to provide sufficient exposure without prematurely polymerizing the reagents. Different strategies have been developed to overcome this issue. Jeong *et al.* fabricated a glass capillary-PDMS hybrid device that was used to synthesize polymeric particles with embedded enzymes.[158] Droplets are generated by using mineral oil as the continuous phase in a sheath flow type design. The group protects reagents against unwanted UV exposure by using aluminum foil to cover up the entire device except for a small window left open to polymerize the droplets. Horseradish peroxidase was incorporated into the microparticles and was demonstrated to have retained its activity even after UV irradiation. Barnes *et al.* utilized Raman spectroscopy for the analysis of monomer composition and degree of conversion of droplets in microfluidic channels.[159] Microfluidic devices were fabricated using borosilicate glass slides in order to minimize the fluorescence effects in the Raman data.

Zourob *et al.* uses a different strategy to supply the required UV for particle polymerization.[160] The group created molecularly imprinted polymer beads using a spiral channel design fabricated in polycarbonate. As opposed to creating a small irradiation window, the device has long spiral channels that have a total length of almost 2 m. By controlling the flow velocity, the particles experience an exposure time of 100 s.

It should also be noted that a novel technique termed continuous-flow lithography has been developed to generate a variety of irregularly-shaped particles by photopolymerizing shapes defined by photomasks.[161] With coflowing hydrophobic and hydrophilic streams, followed by photopolymerization to define their shape; nonspherical amphiphilic polymer particles can be generated.[162] These particles will then self assembled into larger more complex structures.

Catalyst-initiated polymerization. Unlike photo-initiated processes, this mechanism utilizes chemical species that trigger polymerization. Since the droplets are carried in the continuous phase, introducing the chemical trigger is not trivial. Two primary techniques have been developed to achieve this goal. First, the crosslinking agent, such as ions in the case of ionic crosslinking, could be contained in the continuous phase. After the generation of droplets, the crosslinker diffuses into the droplet and causes the droplet to be solidified or gelled.[163,164] Zhang *et al.* used this method to create capsules using a variety of materials including alginate, kappa-carrageenan, and carboxymethylcellulose.[165] The group was able to control the residence time in the chip and concentration of crosslinking agent in the continuous phase to create different types of particles. The process was terminated by putting the particles into a large volume of crosslinking agent-free solution. Unlike traditional methods in which droplets are dropped into a polymerization solution, particles are synthesized *in situ* thus allowing continuous, high throughput processing.

Calcium alginate gels are commonly used for the encapsulation of cells for a variety of purposes. Their synthesis have been studied by a number of groups using droplet-based microfluidic systems. Although calcium alginate beads can be made using the method mentioned above, researchers have developed other strategies for the generation of alginate gel beads. Sugiura *et al.* uses a design they termed micro-nozzle array to generate an array of alginate solution droplets.[166] The micro-nozzles are holes created by reactive ion etching through a 500 μm thick silicon plate. The design allows multiple droplets to be generated at once; greatly improving the throughput of the process. Downstream of the alginate droplet generation region, droplets containing $CaCl_2$ are generated through an identical array. The two types of droplets are allowed to fuse and the reaction between alginate and the Ca^{2+} ion causes the droplet to become a gel bead. Although a large number of droplets are generated, synthesis of gel beads relies on random fusion events between the two different types of droplets. In addition, there is no way to ensure droplets of the same type do not fuse and create droplets of various sizes.

Liu *et al.* tackles this problem by creating a device that generates calcium alginate beads through controlled droplet fusion.[167] Alginate and $CaCl_2$ droplets are each generated at a shear-focusing generation junction. The droplets are then directed to a synthesizing channel that has a fusing chamber. The fusing chamber is simply a segment of channel that has larger diameter than the other regions. As mentioned earlier in the review, this drains the continuous phase in between the droplets and promotes coalescence. As in the previous method, fusing of droplets allows the alginate to interact with the calcium ion to form a gel bead. The frequency of each droplet generation is tuned to match so that pairs of droplets will fuse to create the calcium alginate gel bead. The authors not only demonstrated the controllable synthesis of alginate beads; but provide a tunable platform to perform reactions using droplet fusion.

Irregular particles

The mechanisms used in the processes mentioned above all have equivalents in batch fabrication processes. However, synthesis techniques have been developed using droplet-based microfluidic systems to create particles that are difficult if not impossible to create macroscopically.[168] These novel techniques take advantage of the unique properties of microfluidic platforms such as laminar flow and local control of flow conditions. These particular properties allow the research groups to create objects such as non-spherical particles, Janus droplets, and double emulsions.

Non-spherical yet monodispersed particles are very difficult to create macroscopically due to the fact that surface forces will force droplets into spherical conformations in their spending medium. However in microfluidic systems, the shape of droplets can be controlled by creating microchannels that physically confine and dictate their shapes.[169,170] Both Dendukuri *et al.* and Seo *et al.* created disc and rod-shaped particles in addition to spherical particles using droplet-based systems.[149,161] First, droplets are formed using either T-junction or flow-focusing methods. The droplets are then allowed to flow into the shape-defining region. Rod-shaped particles can be created by designing channels that have width and height smaller than the diameter of the droplet. When the

droplets reach such channels, it will be force to elongate and adopt a rod-like morphology. UV light is then applied to polymerize the particle and allow it to retain its shape. To create disc-like particles, the height of the channel is made to be smaller than the diameter of the droplets while the width is made to be larger. When the droplet enters such a channel it will be "flattened" into a discoid shape. In addition to photopolymerizable materials, Liu *et al.* generated discoids and long - few hundred micrometers - threads using calcium alginate gels.[167] Because gelling of the particles is a time-dependent process, the size and shape of the particles were controlled by the flow rates of the continuous and dispersed phases. Self-assembly is another approach that can be employed to create irregular particle shape[171] or jammed colloidal shells on droplets.[172]

The term Janus particle is used to describe a particle that has two distinct surface regions.[173] One example is the surface-modified particle created by Paunov and Cayre.[174] The group used PDMS to physically block one side of polystyrene latex beads while sputtering gold on the open side and creating two distinct surfaces on one particle. However, this method suffers from the need of multiple fabrication steps and low throughput processing. Recently, droplet-based microfluidic platforms have been used to create Janus particles that not only have distinct surface properties, but also two different internal compositions. Nisisako *et al.* first demonstrated the generation of Janus particles using a microfluidic platform by mixing either carbon black or titanium oxide with isobornyl acrylate to create black and white monomer solutions respectively.[42] The two types of monomers are injected into a flow focusing type device to generate the Janus droplets (Fig. 11a). In order to create symmetrically divided droplets, the group reported that matching the viscosities of the two monomer streams is important. The created droplets are collected and thermally polymerized at approximately 90 °C. Due to the different electrical properties of carbon black and titanium oxide, the particles can be rotated *via* electric field and was shown to be potentially useful in display technologies.

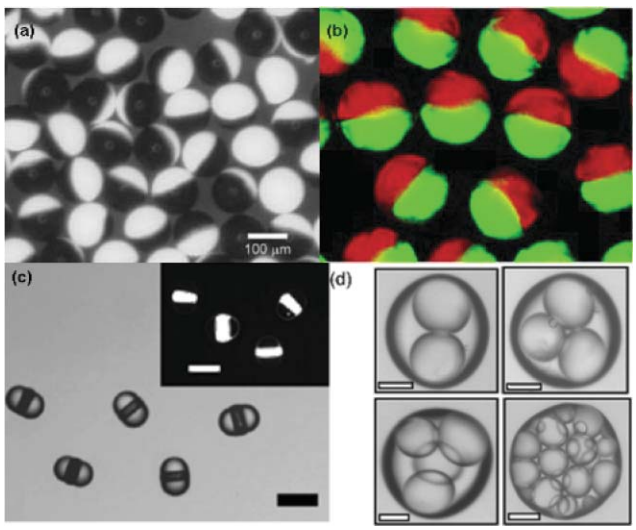

Fig. 11 (a) Electrically distinct Janus particles (reproduced with permission from ref. 42). (b) Dual fluorescence colloid-filled hydrogel granules (reproduced with permission from ref. 175). (c) Ternary particles formed by break up of fluid stream containing three distinct phases (reproduced with permission from ref. 176). (d) Double emulsions with 2, 3, 4 and multiple inner emulsion droplets (reproduced with permission from ref. 61).

Shepherd *et al.* generated dual-fluorescence labeled Janus particles using core–shell fluorescent particles and acrylamide gel.[175] Silica microspheres, 500 nm in diameter, containing either RITC (red) or FITC (green) fluorescent dyes are each suspended in a mixture with acrylamide, crosslinkers, and photoinitiators. The two streams are injected into a flow-focusing device with mineral oil acting as the continuous phase. The Janus droplets are photo-crosslinked through UV illumination immediately after their generation. Fluorescence imaging shows that the particles are hemispherically distinct in both spherical and discoid configurations. (Fig. 11b) These particles are unique in that fluorescent dyes are not just encapsulated in a particle, but crosslinked with the nanometer sized colloids to form a larger organized structure.

In addition to Janus particles, ternary particles can also be synthesized using microfluidic devices. In their report, Nie *et al.* generated ternary particles with two distinct components in an A–B–A configuration (Fig. 11c).[176] Three streams of monomers flowed through a flow-focusing set up with one material sheathing the other. The streams are then sheared by an aqueous stream with 2% SDS acting as the continuous phase. The droplets are then exposed to UV light to solidify them. The group also demonstrated selective functionalization of the Janus particles they created. FITC-conjugated bovine serum albumin (BSA) was covalently linked to the surface of the hydrophilic portion of the Janus particles. This method offers the ability to control the coverage of surface modification on the particle through changes in the volume ratio of the materials used to create the Janus droplets.

The uniform double emulsion is another type of droplet system that is unique to microfluidic systems. Although batch methods exist to produce double emulsions,[177,178] polydispersity is a major hurdle in producing useful double emulsions. Various groups have successfully produced double emulsions using droplet-based microfluidic systems.[179,180] For instance, Nie *et al.* used silicon oil, ethylene glycol dimethacrylate (EGDMA) and water to create core–shell particle structures by UV-polymerizing the EGDMA after forming double emulsions using a flow-focusing device (Fig. 11d).[61] Nisisako *et al.* used a combination of three T junctions to create double emulsions in which two distinct inner aqueous droplets are encapsulated by an oil droplet suspend in an outer aqueous phase.[21] Chu *et al.* recently demonstated the ability to create monodisper multiple emulsions using glass capillaries.[181] The group was able to achieve precise control over the size and contents of each level of the emulsions. The ability to produce not only monodispersed but complex multilayer emulsions suggests that droplet-based microfluidic platforms have the potential to create novel delivery vehicles for therapeutic agents.

Droplet applications

In nature, chemical and biological operations are carried out in micron-sized spaces such as in cells and their organelles. Droplet microfluidics offers the capability to form femto- to

picoliter sized droplets and to compartmentalize and mimic reactions and molecular processes within individual droplets. With the development of tools for the transport and manipulation of droplets and particles, a number of possibilities exist for combining these fluidic elements to carry out synthesis and functionalization of particles for biomedical applications. For this reason, droplet-based microfluidic platforms, with the ability to transport, mix, split, and sort droplets, are being applied to particle synthesis for therapeutic delivery, biomedical imaging, drug discovery, biomolecule synthesis, and diagnostics.

Chemical reactions

Several of the applications discussed in this review rely on the chemical reactions that occur within droplets. For applications ranging from protein expression to organic compound synthesis, performing reactions in the microscale conserves expensive and precious reagents, reduces exposure to hazardous chemicals, and allows multiple reactions to be carried out in highly parallelized experiments.[182] In batch processes, there is high risk involved when performing exothermic reactions where large excess amounts of heat can be released. However, by scaling down the reaction in microreactors, parallel reactions can be performed with minimized risk. Reactions can also be done much quicker due to shorter diffusion and heat and mass transfer distances. Mixing inside microdroplets also benefits from the internal vortex circulation directed by channel geometry.

Chemical reactions performed in microreactors are mostly done with single phase flow,[183] however both research and industrial interest has increased in the use of multiphase segments to peform isolated reactions.[184–186] Droplet-based microfluidics offers greater control over droplet reactions and has been used for titration of formic acid[187] and anticoagulants,[188] precipitation reactions,[189,190] crystal growth,[191] and particle synthesis of cobalt[192] and titania.[193] Comparison between hydrolysis reactions occurring due to mass transfer between two phases and ones taking place in single phase was reported by Ahmed *et al.*[194] In the first condition, aqueous droplets composed of sodium hydroxide solution droplets were formed in a continuous organic phase consisting of p-nitrophenyl acetate dissolved in toluene. The reaction rate depends on a number of factors including the droplet size, flow rate, and external temperature. A comparison with the same reaction done in a conventional flask showed a dramatic increase in yield with the microreactor method (Fig. 12b). Further comparisons done with Heck reactions provided more evidence that segmented flow enhances chemical reaction rates and efficiency.

Burns and Ramshaw measured the diffusion time between multiple phases with two simple acid–base reactions in droplets.[195] A pH indicator was used to measure the progress of the reaction of acetic acid with potassium hydroxide and acetic acid with sodium hydroxide. Kerosene was mixed with acetic acid and formed the organic continuous phase for the aqueous basic droplets. When the pH indicator changes color to specify a transformation from acidic conditions to neutral pH, it also signifies that an equal number of molecules of acetic

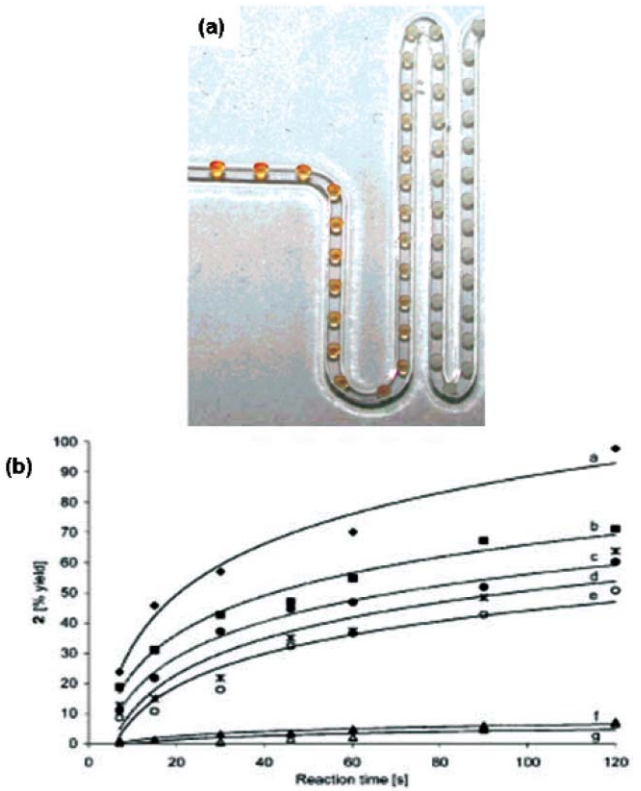

Fig. 12 (a) Bromination reaction performed in droplet microreactors. Change in color indicates completion of reaction. methods (reproduced with permission from ref. 47). (b) Comparison of hydrolysis reaction yields of *p*-nitrophenyl acetate under various conditions. Segmented flow (curves a–e) shows significant improvement in yield over bulk methods (curves f–g) (reproduced with permission from ref. 194).

acid has diffused into the droplet as there are molecules of KOH or NaOH in the droplet. These reaction rates were found to depend on both the concentration ratios of the acid and bases, and the length of the droplet segment. Smaller droplets are expected to have a higher reaction rate due to their greater surface area to volume ratio, which will enhance the diffusion-dependent reaction process.

Droplets have also been used in organic-phase reactions. However, use of harsh organic solvents can cause deformation of channels that can lead to cross contamination between adjacent channels or chemical leakage. Thiolene has been used because of its greater solvent resiliency than PDMS, which is known to swell and deform in the presence of organic solvents. Thiolene has been demonstrated to be surface treatable for use in forming water-in-oil and oil-in-water emulsions, and has been shown to be compatible with cell culture on chip.[196] Cygan *et al.* uses thiolene microfluidic devices to carry out the bromination of alkenes inside benzene droplets (Fig. 12a).[47] Hexane or toluene droplets were formed in both T-junctions and flow-focusing based designs with sodium dodecyl sulfate (SDS) in deionized water as the continuous phase. Flow-focusing was found to more stably control benzene droplet formation by minimizing droplet wetting to the channel walls. For the bromination experiment, an orange colored stream of

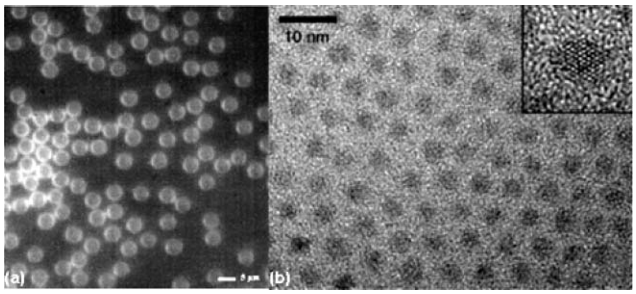

Fig. 13 (a) Microbubble encapsulating perfluorocarbon (PFC) gas with lipid shell (reproduced with permission from ref. 86). (b) Transmission electron micrograph of CdSe nanocrystals synthesized inside microfluidically generated droplets (reproduced with permission from ref. 198).

bromine in benzene was combined with a styrene stream to form droplets in a flow-focusing junction. Flow rates were used to control the concentrations of each reagent in the droplet and used to determine the conditions needed for optimum reaction rate. Long winding channels were used to promote mixing within the droplets and for visualization purposes. The transition of the droplet from orange to clear to indicate reaction completion could be visualized clearly by eye. These examples show the potential of droplet-based microfludics for streamlining organic reactions.

Semiconductor CdSe nanoparticles have been generated in microfluidic gas-liquid segmented flow[197] and liquid slugs[198] under high-temperature synthesis (Fig. 13b). Glass chips with local temperature control can define reaction conditions and enable size tuning within a continuous process. CdS nanoparticle synthesis was presented in a droplet fusion channel where droplets of two reagents were alternatively generated and merged to allow chemical reaction and synthesis inside each droplet.[108] CdS/CdSe core–shell nanoparticles were generated by droplet generation, mixing, and merging in a multi-step synthesis process.[199]

Droplet-based microfluidics can offer better compound reproducibility and the ability to perform reactions under safer conditions, however future work to prevent clogging during precipitating reactions is still required. The increased surface area between the immiscible fluids present technical challenges due to molecular adsorption that interfere with the reactions within the droplets. Other challenges include the interconnects between macro fluid volumes and the micrometer scale inlet ports, as well as methods to collect the products from the discretized droplets without contamination from the continuous phase.

Therapeutic agent delivery

Due to the wide range of materials and methods available, the combination of polymers and other colloid particles can be used to alter drug release profiles, affect drug absorption rates, improve site specific targeting, and a number of other drug distribution characteristics.[200–202] Current batch methods result in polydisperse particles, thus microfluidic generation of microparticles and microcapsules with reproducible size and femtoliter to nanoliter volume is a useful tool for therapeutic agent delivery. Control over particle size and minimization of the size distribution is important for the use of particles in the route of administration and controlled release of encapsulated materials such as drugs, dyes, enzymes, *etc*.[203] The droplets can be filled with various hydrophilic or hydrophobic compounds and the capsule shell thickness can be altered to control compound release rates. Unlike diffusion limited continuous-flow microreactors, droplets with well-defined three dimensional boundaries allow rapid mixing and transport of reagents.[204] Microdroplets have demonstrated the ability to encapsulate polymer beads, metal particles, and dyes for delivery and analysis,[165,203,205,206] and microbeads transferred between liposome vesicles were utilized by an electrofusion technique with the potential fusion-based delivery of artificial microstructures into cells.[207]

Biocompatible or biodegradable materials are popular models for therapeutic agent encapsulation and delivery. Microparticles prepared in microfluidic channels as carriers to encapsulate cells have attracted the most interest for use in cell therapy. Hydrogels such as alginate and agarose are the most commonly used polymers because of their relatively simple preparation.[208,209] Upon contact with polycations, alginate transforms into gel immediately, thus the droplet fusion technique has been applied to simply merge alginate and calcium ion droplets.[166,210] Another contact approach is to direct droplets into a bulk solution containing calcium ions to form the alginate gel beads.[205,211,212] (Fig. 12a) Alginate and calcium ions can also be separated by a buffer stream inside the same droplet to prevent premature mixing.[213] Mixing can then be initiated with winding channels. Cell encapsulation was also demonstrated in aqueous and lipid droplets.[213–215] Cell viability was monitored over weeks and the survival of the cells was attributed to the fact that the droplets contained culture medium and that the droplet shell isolates the cells from harsh environment.

Before and after encapsulation, laser optical trapping and photolysis of single cells were applied to manipulate cells.[216] Fluorescenty-labeled proteins conjugated with polymer matrix during droplet generation were also reported.[217] (Fig. 14b–c) Experimental results indicate some ion exchange is allowed between the droplets and the environment. Ampicillin encapsulated by monodispersed chitosan microparticles was reported.[218] The group found that the drug release rate could be controlled by droplet size and due to the control over the small size distribution, these particles offer the potential for controlled-release drug delivery. Continuous fabrication of biocatalyst-immobilized microparticles in microfluidic systems initiating oxidase-catalyzed reaction have also been demonstrated.[158] The fast generation and photopolymerization process reduced the risk of chemical or optical damage to the entrapped enzymes and biological substrates.

PLGA nanoparticles synthesized by Hung *et al.* can be used as effective drug delivery vehicles due to their small size and monodispersity.[219,220] Additionally, multifunctional particles have been created using droplet-based microfluidic systems. Microbubbles with therapeutic-containing shells have been demonstrated by Hettiarachchi *et al.*[221] The particles combine the ability of microbubbles to act as ultrasound contrast agents with a cargo-carrying shell structure to create a system that

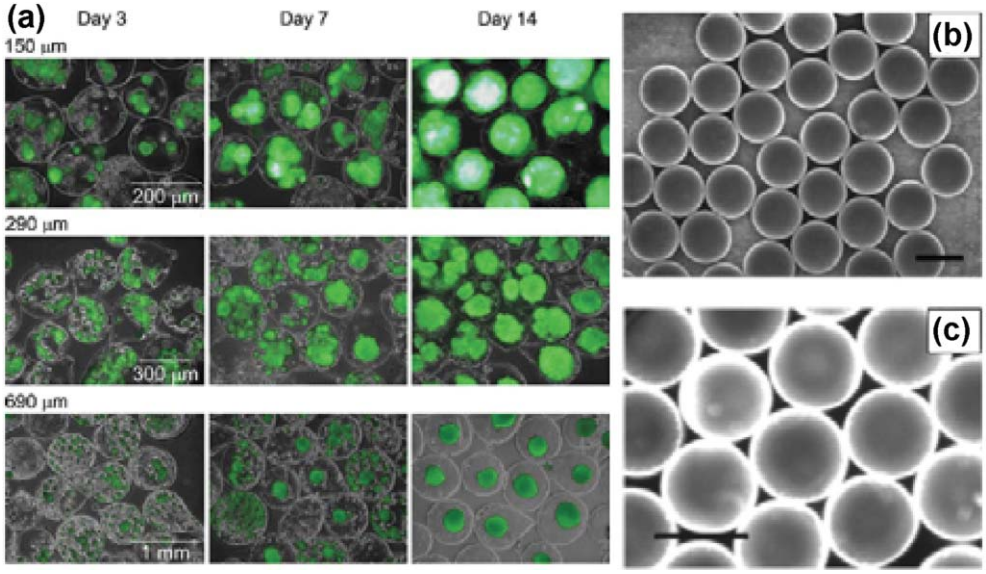

Fig. 14 (a) Alginate-PLL-alginate particles of various sizes encapsulating CHO/NK4-GFP cell (reproduced with permission from ref. 211). (b) Triproplylene glycol diacrylate (TPGDA) and acrylic acid (AA) copolymer particles generated in a droplet-based microfluidic device. (c) Fluorescent image of TPGDA/AA particles conjugated with FITC-BSA (scale bar represents 100 μm) (reproduced with permission from ref. 217).

simultaneously provides imaging functionality and function as a drug delivery vehicle.

To realize the tremendous promise of microfluidic droplet based therapeutic agents, continued improvement is needed to chemically stabilize the droplets when the desired particle sizes are reached. Also, the ability to collect the therapeutic particles while avoiding contamination from the continuous phase will be critical in controlling the final quality of the particles and their therapeutic efficacy.

Imaging

Microfluidic devices have been used for continuous synthesis, control, and characterization of high-quality particles and bubbles, in which the synthesis process can deliver monodispersed size distribution without subsequent size selection processing.[222] Compared to conventional particle synthesis, microfluidic reactors enable the enhanced control of heat and mass transfer. Microreactors with single phase continuous flow or laminar flow designs bring reagent streams into contact but lack the mixing efficiency. Droplets overcome such limitations by enhancing mixing and chemical reactions with internal recirculation. In addition, the use of small volumes require smaller diffusion distances than in bulk solutions.

Microbubbles have been used as ultrasound contrast agents in ultrasound imaging to improve the ability to image damaged tissues and serve as a tool for early detection of diseases. Improved sensitivity and specificity of 2-D and 3-D ultrasound imaging can be achieved by increasing the reflection of the sound waves. The microbubbles increase sensitivity by enhancing the contrast in the obtained image. The high echogenicity of the microbubbles allows them to reflect the soundwaves differently compared to surrounding tissues, creating a high-contrast sonogram.

The sizes of ultrasound contrast agents affect not only their ability to cross the pulmonary circulation but also the degree of ultrasound reflectivity. Optimum reflectivity occurs with microbubbles 2–5 μm in diameter, a size range also suitable for passing through the capillaries in the lungs. Traditional methods of generating microbubbles rely on bulk mechanical agitation which result in a polydisperse size distribution. Hettiarachchi *et al.* reported an optimized microfluidic approach for monodisperse microbubble generation in the optimal diameter range required for the use of ultrasound contrast agents.[86,223,224] Gas composition and membrane components determined the stability of the lipid microbubble contrast agents and how long they could be sustained in the body. Microbubble contrast agents composed of higher molecular weight gases and more rigid lipid-based shell showed longer survival times.

Semiconductor nanoparticles made of CdS and CdSe exhibit unique size- and shape-dependent physical, optical, and electronic properties. Also known as quantum dots, their size-dependable fluorescence spectrums have found applications in optical devices and for use in biological sensing. CdSe nanoparticle synthesis in microfluidic systems is reported in the Chemical Reactions section above, and has been demonstrated to show improved imaging properties compared to conventional and continuous flow synthesis.[108] Homogeneous polymer microcapsules generated in microfluidic devices were also used as carriers to encapsulate quantum dots in each polymer matrix providing amplified and reproducible fluoresence-based analysis.[152,225,226]

Biomolecule synthesis

Biologists have long been on a quest to build artificial cells[227,228] to understand the kinetics and biology behind life's most fundamental reactions. An artificial cell holds the advantage of having well-defined components that will allow scientists to study biological activities otherwise impossible. Micron-sized aqueous compartments that are capable of

performing biological reactions are a first step to creating an artificial cell. Droplet microfluidics is not limited to the synthesis of particles and capsules, but can also be used for the synthesis of biological molecules such as protein and DNA. Droplet microfluidics with its ability to rapidly create highly uniform aqueous droplets with controlled contents, could serve as an important component for the creation of artificial cells. Since droplets can be made micron-sized or smaller, encapsulation of a single template copy of DNA can be realized. The integration of heating elements and the ability to precisely control droplet movement allow these vesicles to serve as microreactors for *in vitro* protein expression, DNA amplification, and other biochemical reactions. Although biomolecule synthesis is done well in living cells, synthesis in droplets is advantageous due to its ability to isolate and control specific reactions, increase effective concentrations of reagents, parallelize experiments, synthesize proteins lethal to cells, and for its potential in high throughput molecular engineering.

Many cell-free biological reactions have been carried out in droplets, such as ATP synthesis using microbubbles,[229] and protein expression in emulsions,[230] and membrane transport has been studied in micelles and liposomes.[231] Emulsions of aqueous droplets in oil are a popular choice for containment since the majority of biological reactions occur in aqueous solutions. Fig. 15a shows a schematic of a potential molecular evolution experiment conducted in a droplet-based system. Advantages droplet-based systems have over continuous flow platforms is the ability to isolate molecular processes and reagents, thus preventing unwanted adsorption onto channel surfaces, cross contamination, and evaporation of solvents.

Water-in-oil emulsions have been utilized in a variety of novel biological applications.[232,233] *In vitro* compartmentalization (IVC) using microdroplets has been demonstrated to be an effective tool in improving the throughput of molecular evolution experiments.[234–236] Nakano *et al.* has demonstrated single molecule reverse transcription polymerase chain reaction (RT-PCR) in emulsion droplets as a highly sensitive method to detect biological agents.[237] However, these studies used emulsions formed in batch by stirring or agitation which results in droplets with broad size distributions. One method to narrow the distribution is to increase the emulsion process speed. However, a correlation with loss of activity of the proteins to increased emulsion processing has been discovered.[238] In addition, the droplets could not be individually addressed, sorted or analyzed. A few groups have taken advantage of microfluidic-based systems to achieve protein expression inside droplets. Dittrich *et al.* have demonstrated protein expression within monodisperse droplets generated by a microfluidic platform (Fig. 15b).[239] The necessary components for transcription and translation, and the protein-encoding gene for green fluorescent protein (GFP) were encapsulated in each droplet with a Y-channel configuration, and after incubation at 37 °C, fluorescence measurements were taken to verify *in vitro* protein expression completion.

Huebner *et al.* performed protein expression by encapsulating single *E. coli* cells in droplets to express a yellow fluorescent protein.[240] Droplets were formed also by a Y-channel configuration that used oil to shear streams of LB medium with cells and LB medium only. The two flow rates of the two aqueous streams were used to control cell occupancy in each droplet, and thus the degree of fluorescent protein production. In this manner, proteins can be expressed *in vitro* in cell-sized vesicles and probed for function in a single device. The application of droplet microfluidics for protein expression offers the potential for high throughput directed evolution of proteins.

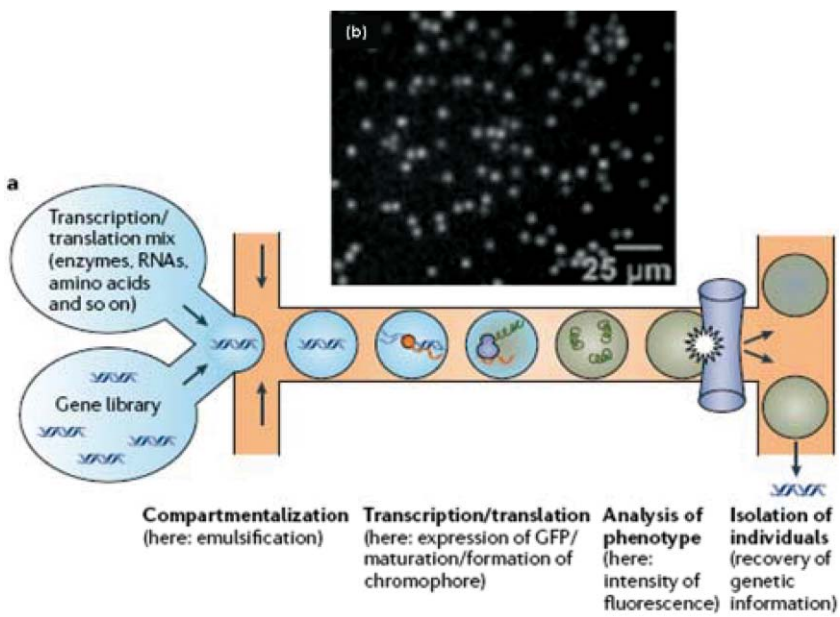

Fig. 15 (a) Conceptual schematic of *in vitro* protein evolution process performed utilizing a droplet-based microfluidic platform (reproduced with permission from ref. 204). (b) Fluorescence microscopy image of cell-free expression of green fluorescent protein (GFP) in water-in-oil droplets produced with a microfluidic device (reproduced with permission from ref. 239).

Microfluidics has also proven useful for the amplification of DNA. A thorough review of PCR microfluidics is given by Zhang *et al.*[241] The majority of PCR microfluidics has been demonstrated in continuous flow however droplet-based microfluidics has gained recent interest as a platform to perform PCR. Zhang *et al.* investigated PCR using continuous flow or droplet-based microfluidics and proved theoretically that the droplet-based method not only performed better, but did so with a simpler design.[242] Wang *et al.* designed a droplet-based chip for DNA amplification.[243] The chip integrates all the components necessary for the denaturation, annealing, and extension processes of PCR such as heating elements and paths to direct droplet transport. To verify amplification, human papilloma virus-DNA was used in the chip and the amplification products were analyzed by gel electrophoresis. The group also compared their PCR system with PCR performed in a traditional PCR instrument, the Rotor-Gene™. The conventional method required over 2 hours of processing, whereas the droplet-based method took less than 15 minutes. Beer *et al.* recently demonstrated real-time single-copy PCR utilizing a droplet-based system. The group also showed cycle number reduction that suggests the potential of improve sensitivity as well as reaction times.[244]

The large surface area of droplets present opportunities for amphiphilic biomolecular assays. However, the large interfacial area also presents difficult challenges in unwanted biomolecular adsorption. The attempt to truly mimic biological syntheses and reactions will require the generation and manipulation of much smaller droplets (sub femtoliters) which currently is not feasible. Although much progress is still needed in this area, droplet microfluidics has shown its potential as a method of carrying out high throughput directed evolution of proteins, PCR, and for forming artificial cells.

Diagonostic chips

The main motivation behind the field of microfluidics is to create Lab-on-a-Chip devices based on the concept of the micro total analysis system (μTAS) first introduced by Andreas Manz in the early 1990s.[2] The prospect of reducing processing time and consumption of reagents has prompted the development of many novel technologies aimed to replace traditional laboratory equipment. A large number of microfluidic devices have been designed to process biological reagents including cells, proteins and DNA.[245–250] Droplet-based platforms have the benefits of working on the microscale by having decreased diffusion distance, faster mixing, and laminar flow; but also the added advantage over continuous systems in that they can produce large numbers of microreactors to allow parallel processing while keeping each reactor independent and isolated. These unique properties have enabled a wide array of biochemical diagnostic assay to be performed using droplet-based microfluidic systems.

Optical methods have been integrated into droplet-based microfluidic platforms for bioassays and detection. Srisa-Art *et al.* developed a confocal fluorescence spectroscopy detection scheme for a fluorescence resonance energy transfer (FRET)-based DNA assay.[251] This detection method was also used to demonstrate a biotin/streptavidin assay based on FRET. One example of on-chip biological experimentation is the electrochemical detection and electroporation device demonstrated by Luo *et al.*[252] The device integrates gold microelectrodes with droplet generation for the analysis of aqueous droplets produced in soybean oil. The size of a droplet can be estimated by measuring the change in impedance between the electrodes and correlating the residence time on top of the electrodes with the flow rate. The electrodes can also measure the ionic concentration of the aqueous droplets as they pass by. Additionally, the device is capable of performing electroporation of encapsulated yeast cells. The group demonstrated successful electroporation through the incorporation of fluorescein into the yeast cells after passing over the electrodes. This study shows the potential of a multifunctional droplet-based platform for analysis of cell content and high throughput cellular electroporation.

In addition to optical and electrochemical detection of the contents of droplets, processing of biological fluids using droplet microfluidics has also been demonstrated. Srinivasan *et al.* designed an EWOD-based droplet platform that is capable of measuring glucose concentration from a variety of biological fluids including saliva, serum, plasma, and urine.[253,254] Droplets containing the sample and reaction mixture are fused and mixed using electrodes as described in previous sections of this review. All the fluids can be actuated using the EWOD mechanism at 20 Hz with a voltage below 65 V. The glucose level is monitored using a colormetric assay based on Trinder's reaction in which the absorbance of quinoneimine is measured from a series of reactions involving the production of peroxidase from glucose and glucose oxidase. The concentration of glucose can be determined by the rate of absorbance change of quinoeimine over time. The absorbance is measured using a LED as the illumination source and a photodiode. The ability to accommodate a variety of samples as well as performing detection on-chip is a clear indication of the promise droplet -based systems hold in creating lab-on-a-chip devices.

The handling of complex biological fluids has been extended to the processing of blood and its components by Song *et al.*[188] In their device, the group evaluated the blood clotting response by adapting the activated partial thromboplastin time (APTT) test to a droplet-based microfluidic system. The time-dependent clotting behavior of the blood droplets were monitored using long winding channels. Not only was the group able to test a variety of plasma/blood samples, but they also showed that the values obtained from the microfluidic assay were comparable to that of conventional methods.

Lastly, molecular imprinted polymer (MIP) particles can be prepared using a droplet-based microfluidic platform. Kubo *et al.* created atrazine-imprinted particles using polymerized methacrylic acid (MMA).[255] Droplets containing the pesticide atrazine, MMA and photoinitiator are generated using a Y-shaped variation of a T-junction design with an aqueous continuous phase. The droplets were collected after generation on the device and cured using UV irradiation. After removal of atrazine, the particles were shown to have preferential binding to atrazine suggesting the imprinting fabrication was successful. This selective binding to a pesticide could potentially be very useful in areas of chemical detection

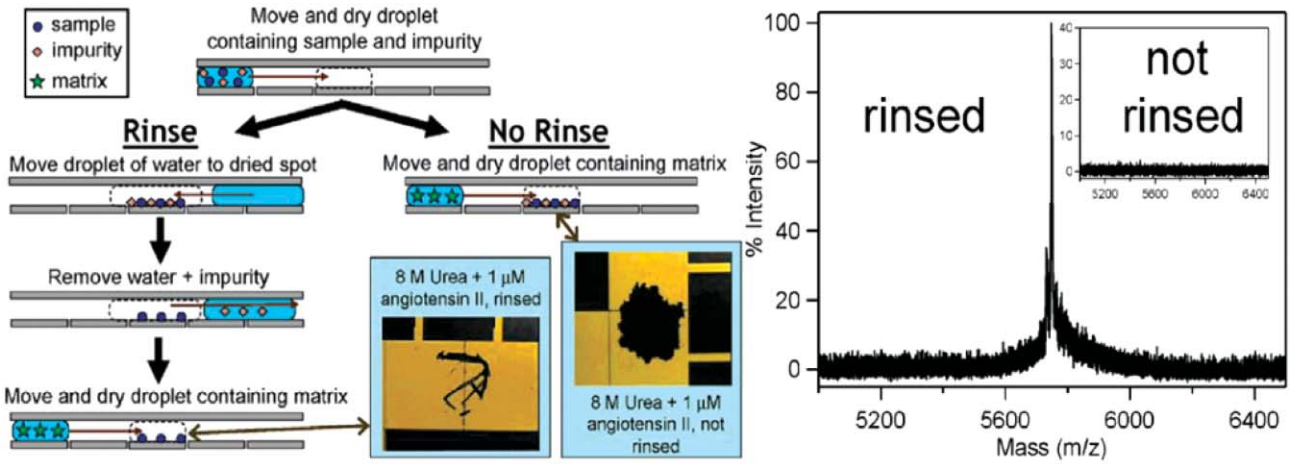

Fig. 16 (a) Schematic operation of EWOD-based on-chip purification for MALDI analysis. Inset shows comparison of rinsed *versus* un-rinsed matrix. (b) Results showing significant increase in signal for the rinsed sample (reproduced with permission from ref. 253).

and food safety monitoring. This shows that the platform could be used to synthesize particles useful in high sensitivity diagnostic tests.

Drug discovery

The applications described previously have all dealt with the production or synthesis of materials in droplet-based microfluidic platforms. However with the addition of optical and amperometric detection components such as mass spectroscopy, gel electrophoresis, and X-ray crystallography, droplet microfluidics has evolved into a platform with the potential for use in more sophisticated applications. Large diverse compound libraries can be quickly generated and screened in a single microfluidic device. This makes droplet-based microfluidics an ideal method for the discovery and study of new drug compounds.

An important tool used for protein analysis is matrix-assisted laser desorption/ionization (MALDI). Work has been done to integrate MALDI mass spectroscopy with droplet-based microfluidic devices as a high throughput method of achieving protein and chemical compound analysis.[256] Wheeler *et al.* developed an in-line sample purification device that uses EWOD to remove impurities from the sample (Fig. 16).[257] EWOD is also used to transport the sample to a spot where it is analyzed by MALDI-TOF mass spectrometry.[258] This allows for automated sample handling and increased throughput for use in peptide and small molecules drug library screening.

Further characterization of protein drug candidates is done though X-ray diffraction of protein crystals. Due to the low consumption of reagents and ability to perform parallel combinatorial chemical experiments, droplet microfluidics has been applied successfully for screening optimal conditions for protein crystallization. Lau *et al.* tested forty different conditions within a single device to crystallize catalase, glucose isomerase, thaumatin, and ferritin (Fig. 17).[259] Extensive work has also been done to simultaneously screen and optimize crystallization experiments.[260–262] Li *et al.* demonstrated the quick optimization of model membrane protein crystallization.[263] Nearly 1300 crystallization trials were initiated by one person in less than twenty minutes.

Cells play an important role in pharmaceutical screening, from its utilization in the production of drug candidates to the study of a eukaryotic cell's reaction to the presence of a drug. This traditionally requires large automated machinery and multiple microwells to complete a single experiment. These microwells contain tens to hundreds of microliters of solutions, which are orders of magnitude larger than volumes consumed in microfluidic operations. Due to the large well size, large number of cells must be cultivated for each experiment. Recent work has been done on the culturing of cells in microfluidic devices using segmented flow.[35,264,265] At certain cell concentrations, single monoclonal cell cultures could be achieved in each droplet. In addition, by separating the cultures into small discrete compartments, large batches of cells are no longer necessary, and the length of time needed for the cultivation of slow growing cells is reduced. It is advantageous to use microdroplets to cultivate rare or slow growing cells, since in large-scale batch cultures, these cells risk being overgrown by faster growing cell types. Sgro *et al.* have demonstrated on-chip freezing of cells encapsulated in aqueous droplets using a thermoelectric cooler (TEC).[266] The mouse lymphocytes maintained relatively good viability even after the

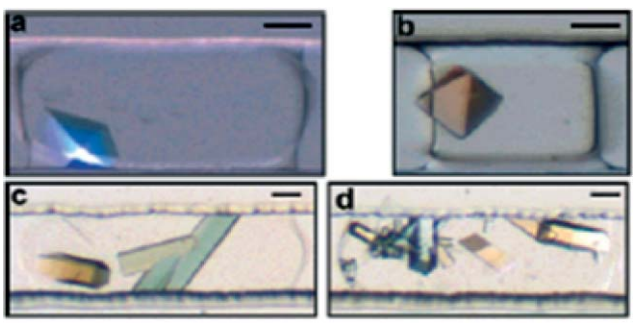

Fig. 17 Micrographs of protein crystals formed inside droplets: (a, b) thaumatin, (c) bovine liver catalase, and (d) glucose isomerase (reproduced with permission from ref. 256).

freeze-thawing process. Reversal of the voltage of the TEC allows the microfluidic chip to be heated instead of cooled, thus showing potential of the device for thermal manipulation of droplets for applicatins ranging from performing crystallization reactions to archiving frozen cells.

Kinetic studies are another important way of characterizing a drug compound. These measurements can be used to better understand enzymatic and chemical activity. Hsieh *et al.* have demonstrated millisecond resolution binding kinetics using molecular beacons.[267] Their scheme achieves highly increased sensitivity by using properties unique to droplet-based microfluidic platforms. The signal is amplified many folds through the accumulated fluorescence of thousands of droplet microreactors containing molecular beacons and the oligonucleotide of interest. Gong *et al.* studied the nucleation kinetics of thermoresponsive microgel particles and measured the growth rate over different temperatures.[268] Song and Ismagilov achieved kinetic measurements of ribonuclease A (RNase A) with millisecond temporal resolution.[269] The enzyme activity is measured by the fluorescence which results from cleavage of a fluorogenic substrate by RNase A. The intensity of fluorescence is measured to determine the extent of reaction completion.

Another critical aspect of therapeutic formulation is the solubility of the compounds being used. Droplet-based microfluidics has been used for solubility screening of chemical compounds. Laval *et al.* created two-dimensional arrays of adipic acid droplets at different concentrations and temperatures.[270] Solubility measurements were taken over an hour, demonstrating better temperature control, and faster screening than traditional methods.

In order to have wide spread adaptation of microfluidic droplets for drug discovery, automation, high throughput processing, and compatibility with current drug discovery tools and platforms are critical. This will involve the development of standardized inlet and outlet fluid ports, microfabrication foundries that allow quick turn around of new designs, automated imaging systems, and turnkey user interfaces. The microfluidic droplet devices, for the most part, should not fundamentally change the way drug discovery is performed currently but provide added functionality and performance to existing technology platforms.

Conclusions

This paper provides a comprehensive review of the field of ''droplet'' or ''digital'' microfluidics and its applications. This nascent field has attracted a diverse group of researchers to study the fundamentals of two phase dynamics in microchannels and also to develop novel solutions for biological and chemical applications that are superior to conventional techniques. Many examples are highlighted in this paper. Researchers in microelectromechanical systems (MEMS) have been developing novel microfabrication and surface treatment techniques to optimize droplet generation and manipulations in microdevice platforms. Microfluidic researchers have been focused on the control of droplet generation, droplet fission/fusion, mixing, and sorting to enable the high throughput analysis and synthesis conditions of large number of femto- to nanoliter volume droplets. Chemists are intrigued at being able to control reactions with precise concentrations and kinetic conditions and at the same time study them in large numbers. Biologists are seeing a rare opportunity to study biomolecular and cellular events in cell-like environments and the notion of building ''artificial cells'' is much more realistic with the control that droplet microfluidics provides. Biomedical engineers are keen to developing ''microsystems'' with better robustness and reproducibility in order to enable new applications and industries at the interface of biomedicine and engineered devices/instruments. There are already companies that are developing droplet-based microfluidic products for the biomedical and biopharmaceutical industries. It is the belief of the authors that the rapid development of this field in just a few years and the vast amount of literature that is being generated points to a revolution in the field of Lab on a Chip that will continue for the next 5–10 years. This review paper is an attempt to capture a snapshot of the field at this critical stage.

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Additions and corrections

Ref. 30 was incorrectly cited and should read as follows: A. Gunther and K. F. Jensen, Lab Chip, 2006, 6(12), 1487–1503.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.

