Microfluidics

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From Microdroplets to Microfluidics: Selective Emulsion Separation in Microfluidic Devices**

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Microdroplets show great promise as a new high-throughput technology in chemistry, biochemistry, and molecular biology.[1–3] Microdroplets can be generated at rates in excess of several thousands per second and accurately formulated using minute amounts of small molecules, DNA, proteins, or cells.[4–6] Furthermore, integrated active elements can be used to control individual droplets. With the technology for creating,[7–9] dividing,[10,11] fusing,[12–14] interrogating,[12,15] and even sorting[11,16] microdroplets already developed, one of the main problems to be resolved is how to access their contents.

Droplets are naturally self-contained microreactors that prevent sample loss, diffusion, and cross-contamination, general issues that afflict traditional microfluidics. However, the isolated nature of droplets prevents physical access of their contents on-chip. Even though this does not represent a problem for many of the applications that have already been demonstrated, it limits the integration of microdroplets with other platforms. Analytical techniques such as mass spectrometry, capillary electrophoresis, and liquid chromatography have been successfully integrated with continuous-flow microfluidic devices,[17] but their integration with microdroplets remains challenging. If the contents of microdroplets could be readily extracted on demand, the carrier fluid discarded, and the microdroplets converted into a continuous stream, microfluidic functionality could be combined with the advantages of microdroplets.

In this paper we present a technology that bridges the fields of microdroplets and continuous-flow microfluidics by extracting on-chip the contents of microdroplets and incorporating them into a continuous stream. The extraction is achieved through electrocoalescence: droplets are forced to coalesce with an aqueous stream by application of an electric field across the channel. The extraction is controlled through the voltage applied at microfabricated electrodes on each side of the channel and can be performed in a continuous or discrete fashion. The discrete collection of droplets can be controlled by an external electrical signal related to the contents of the droplets. As a proof of principle, we have implemented a fluorescence intensity based detection system to control the collection of the droplets, resulting in a device capable of selectively incorporating the contents of droplets of interest to a continuous microfluidic stream.

We used flow-focusing to generate microdroplets.[7] An aqueous stream was focused between two oil streams as they pass through a junction. Shear forces make the aqueous thread break up into monodisperse droplets. Droplet size and frequency were controlled by a combination of channel dimensions and flow rates. We used a mixture of fluorous oil (FC-77) and 1*H*,1*H*,2*H*,2*H*-perfluorooctanol (70:30 by weight) as the carrier phase. The oil and aqueous flows at the flow-focusing device were adjusted to generate the desired droplet frequency, typically ranging from 10–250 Hz. The flow of the lateral aqueous phase was adjusted so an interface was held in the region between the electrodes without overflow in either direction.

Figure 1a shows a scheme of a typical device where droplets flow parallel to a stream of water between two electrodes. In the absence of an electric field, the droplets are not perturbed by the presence of the aqueous stream and follow the geometrically determined flow lines. Figure 1b and c show micrographs of such a device in operation. Droplets of a dye generated at the flow-focusing device flow past the

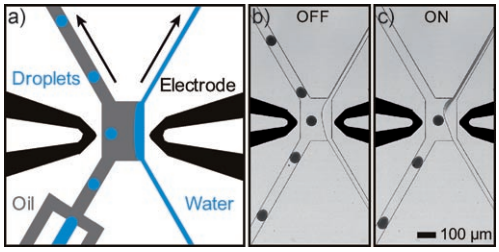

Figure 1. a) Scheme of a typical device. Droplets generated on-chip flow parallel to an aqueous stream between two electrodes. b) Micrograph of a typical device in operation in the absence of an electric field. Droplets of a dye flow past the electrode region without interacting with the aqueous stream. c) In the presence of an electric field, droplets coalesce with the lateral aqueous stream as they enter the electrode region.

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electrode region in the absence of a field (Figure 1b), whereas they coalesce with the lateral stream when a field is applied (Figure 1c). As a result the dye contained in the droplets is transferred from its discrete carriers into a continuous stream. Figure S1 in the Supporting Information shows mass spectra of a peptide solution encapsulated in droplets and of the same solution after emulsion separation. After emulsion separation the spectrum shows the signals corresponding to the peptide, whereas the signal from nonseparated droplets is dominated by the carrier phase.

In general, coalescence occurs when two or more interfaces approach below a critical distance for a sufficient length of time.[18] Electrocoalescence follows the same general mechanism but is modified as a result of electric forces appearing at the interfaces.[19] When a voltage difference is applied to the electrodes, an electric field in the direction perpendicular to the flow is created. This field alters the trajectory of the droplets and polarizes the interfaces. Above a threshold voltage, it induces coalescence between the droplets and the aqueous stream. In our experiments, typical voltages required to induce 100% coalescence range from 1.5 to 3 kV. These values of applied voltage generate an electric field of $\approx 10^7\ V\,m^{-1}$, which is significantly larger than those reported for both bulk[19,20] and microfluidics,[21,22] typically in the range of 10^4–$10^5\ V\,m^{-1}$. This larger electric field might be explained in part by a larger distance between the interfaces and a shorter time of contact, since coalescing droplets are usually not moving with respect to each other.

We frequently observed a decay in the percentage of coalescence after establishing an electric field. One of the possible solutions to this problem is the use of pulsed fields, which have increased coalescence efficiency in bulk.[19] A very interesting feature of using pulsed fields in a microfluidic environment is the ability to address individual droplets, as this provides a tool to access the contents of a single droplet on demand.

Figure 2 shows a sequence of micrographs in which an individual droplet is selected from a stream. Droplets flow past the electrodes at an applied potential insufficient for coalescence. After an additional square pulse is applied, an individual droplet is fused, the applied voltage is returned to its previous value and droplets flow past again. After the extraction, the contents of the droplet (KSCN) are incorporated into the aqueous lateral stream ($Fe(NO_3)_3$) and react with it, forming a colored compound. To select an individual droplet it is critically important to ensure that only one droplet enters the region between the electrodes during the length of the pulse. Therefore the pulse width and starting point must be carefully adjusted.

The pulses used to induce coalescence can be controlled by an external electrical signal. To demonstrate the potential of selective emulsion separation we chose to combine it with fluorescence intensity detection, which is one of the most useful and commonly used techniques in biochemistry and has been successfully implemented in microfluidics[17] and microdroplet systems.[12,15] Figure S2 in the Suppporting Information shows the setup used to induce electrocoalescence based on fluorescence intensity detection. The detector (photomultiplier tube, PMT) reads out a signal which is proportional to the fluorescence of the excited droplet. When the signal exceeds the threshold of the pulse generator trigger, a high-voltage pulse is applied across the electrodes. The gain of the PMT can be adjusted to allow the triggering to take place at any level of fluorescence, with the signal-to-noise ratio determining the reliability.

Figure 3a–d shows a sequence of micrographs in which a fluorescent droplet is detected and fused while a nonfluorescent droplet flows past the electrodes undisturbed. Using a device comprising two separate flow-focusing devices, we generated a stream of alternating fluorescent and nonfluorescent droplets. When the fluorescent droplets flow past the laser, the emitted light is gathered by the detector whose

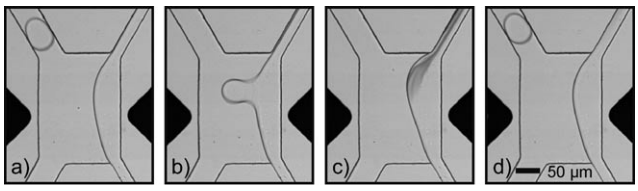

Figure 2. Sequence of micrographs showing the extraction of the contents of an individual droplet. a) Below the threshold voltage, the electric field is insufficient to induce coalescence. b) When an additional square pulse is applied, an individual droplet is selected and c) its contents are incorporated to the lateral aqueous stream. d) The applied voltage returns to its original value before the next droplet enters the electrode region and therefore flows past without coalescing. The droplet contain KSCN which reacts with the $Fe(NO_3)_3$ in the lateral stream forming a colored complex.

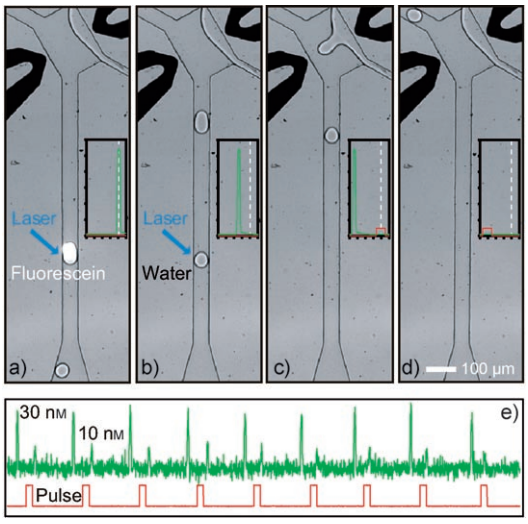

Figure 3. a–d) Micrographs showing the selection and extraction of a fluorescent droplet. Insets show 10 ms of signal from the detector (green) and pulse generator (red) with the current frame position marked in white. a) A droplet containing 12 mM fluorescein flows through the laser spot and emits fluorescent light which is detected by the PMT. b) A water droplet passes through the laser spot without fluorescing. c) An electric pulse fuses the droplet containing fluorescein with the lateral stream. d) The field is removed before the water droplet passes between the electrodes so it flows past. e) Trace from a separate experiment showing the signal from the PMT (green) and pulse generator (red) for a stream of droplets containing 30 nM and 10 nM fluorescein droplets. Only the droplets containing 30 nM fluorescein trigger the pulse generator.

signal triggers a pulse that induces coalescence. Nonfluorescent droplets do not trigger a pulse and therefore are not incorporated into the lateral stream. The laser detection point is arbitrarily chosen; a time delay between the detection and the pulse is introduced to account for the distance the droplets have to cover between the laser and the electrodes.

These experiments are in concept similar to a fluorescence-activated cell sorter (FACS) but with a number of added features: time of reaction can be accurately controlled, droplet formulation is carried out using microfluidic techniques, and the contents of the target droplets are extracted on-chip allowing further processing. For high-throughput screening, it is essential to be able to select droplets containing low concentrations of fluorophores from an array of concentrations very similar to the target. To demonstrate the real potential of this system for such studies we selected droplets containing 30 nM fluorescein from a stream that contained droplets of 30 and 10 nM concentrations. Figure 3e shows the trace of successful pulse triggering for droplets containing 30 nM fluorescein, whereas droplets 10 nM in concentration do not trigger pulses. Analysis of a larger sequence of the trace (not shown) shows that all 30 nM and less than 1% of the 10 nM droplets were selected.

Solid-supported chemistry and biochemistry play a key role in biotechnology, drug discovery, and combinatorial chemistry, areas that have driven the search for higher-throughput technologies. The possibility of combining solid-supported chemistry and microdroplets offers exciting avenues for future research, especially if combined with the large number of methods and technologies available for the manipulation of microspheres in a microfluidic environment.[23,24] We used selective emulsion separation to detect and extract fluorescent beads encapsulated in microdroplets and incorporate them into a continuous microfluidic stream. These results could be extended to assays performed on fluorescence-reporting beads as well as cell-based assays.[5,25]

To study the selection of bead-containing droplets, we generated droplets of a solution containing 2-μm diameter fluorescent beads (0.005 vol%) in phosphate buffer. This concentration results in approximately 10^4 beads per liter. With an approximate droplet volume of 50 pL, the number of beads per droplet is roughly 0.6. This resulted in most of the droplets containing either one or no beads. The fluorescence intensity emitted by the beads was used, as previously, as the signal to trigger a high-voltage pulse. Figure 4 shows a sequence of micrographs in which a droplet containing a fluorescent bead is selected and merged with the lateral stream. The bead can be seen both inside the droplet before fusion (Figure 4a,b) and within the stream after fusion (Figure 4c).

The beads used contained approximately 3×10^7 molecules of dye which if homogeneously distributed within a droplet would correspond to a concentration of $\approx 100\ \mu M$. Such a concentration results in a large signal if the bead and the laser spot overlap. In the experiment shown in Figure 4 the overlap between the laser and the droplets resulted in 89% of the droplets containing beads being selected successfully. This could be improved by further expanding the beam or by increasing the amplification of the PMT signal.

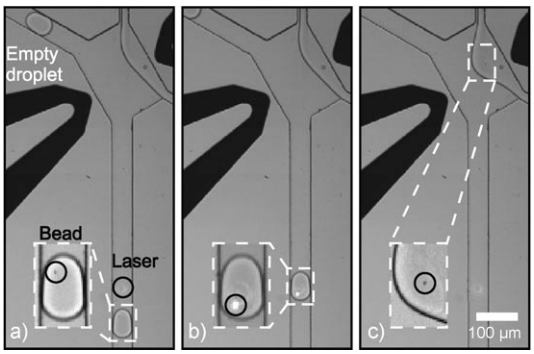

Figure 4. Micrographs showing the selection of a droplet containing a fluorescent bead. a) A droplet containing a fluorescent bead approaches the fluorescence detection point. b) As the droplet flows through the laser, the bead emits detectable fluorescence. c) The fluorescence signal triggers an electric pulse which fuses the bead-containing droplet with the lateral stream. The bead can be seen within the lateral stream after the droplet is merged.

In summary, we have demonstrated a new technology capable of extracting the contents of microdroplets on-chip and incorporating them into a continuous microfluidic stream. We are able to select individual droplets based on their contents. As a proof of principle, we have implemented a fluorescence detection system and used it to collect droplets containing low levels of a fluorescent dye as well as single fluorescent beads. This technology opens the door for applications in the fields of directed evolution, enzyme inhibition studies, high-throughput drug screening, etc. This device has the potential to combine all of the available microfluidic techniques with microdroplet-based screening. Moreover, further control can be provided by adjusting the composition of the receptor stream. This stream can be used to quench reactions so that their endpoints are accurately determined, ensure that cells do not encounter any adverse environments or, on the other hand, lyse cells to study their contents on-chip after a reaction carried out in droplets. We believe that this technology will have a great impact on microdroplets and their integration with microfluidics.

Experimental Section

We used soft lithographic techniques to fabricate poly(dimethylsiloxane) (PDMS) microfluidic channels and oxygen plasma to seal the channels with PDMS-coated glass slides.[26,27] Solder electrodes were fabricated using microsolidics.[28,29] Extra channels for the electrodes were included in the mold used to fabricate the fluidic channels. After the plasma treatment, the devices were placed on a hot plate at 130°C (solder melting point 60°C). When the device temperature had equilibrated, we introduced solder rods in previously punched holes, filling the cavity completely with solder by capillarity. Before removing the device from the hotplate, while the solder was still liquid, we introduced copper wires in the solder channels to serve as electrical contacts. Our typical device (Figure 1a) presents 50-μm-wide channels for droplet formation and a 20-μm-wide channel for the lateral stream. The channel in the electrode area is 170 μm wide, with the electrodes 10 μm from the walls. Channels are 25 μm deep.

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