Microfluidics

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Dilution-Free Analysis from Picoliter Droplets by Nano-Electrospray Ionization Mass Spectrometry**

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The amount of sample required for a chemical analysis is frequently determined not by instrument sensitivity but by the ability to isolate, prepare, and deliver trace analytes to the instrument. For example, in the case of proteomics, about 50 proteins can be identified[1,2] from single-cell-sized (50 pg) tryptic digest samples that are prepared in bulk, diluted, and analyzed by ultrasensitive liquid chromatography/mass spectrometry (LC/MS). However, owing to losses occurring during conventional sample preparation and challenges in working with small volumes on the benchtop, at least four orders of magnitude more starting material are still needed.[3] Microfluidic devices, with their flexible design, precise flow control, and ability to integrate multiple sample handling and analysis steps,[4] offer promise for bridging this gap. A particularly attractive microfluidic approach for sample-limited analyses employs aqueous droplets or plugs encapsulated by an immiscible oil.[5–7] Each droplet serves as a discrete compartment or reaction chamber enabling, for example, high-throughput screening[8,9] and kinetic studies[10–12] of femto- to nanoliter samples, as well as the encapsulation[13–15] and lysis[13] of individual cells with limited dilution of the cellular contents.

A potential challenge for droplet-based platforms, however, is that detection is largely limited to a small number of resolvable species using methods such as fluorescence; chemical separations and more comprehensive detection methods, such as MS, are not readily applied to encapsulated droplets. As such, there is tremendous interest[7,16] in combining the unique characteristics of digital[17] and droplet-based microfluidics with continuous-flow microchannels, which can be accomplished by extracting the droplet contents into an aqueous stream for further processing and analysis. In the case of sample-limited analyses, the entire droplet should ideally be transferred to the aqueous channel with minimal analyte dilution. Transferring droplets from oil to aqueous streams while keeping the carrier liquids separate has proven difficult.[18] Previous efforts have utilized spatially selective surface coatings[18,19] to establish a boundary between the hydrophobic and aqueous phases, or synchronized electrical pulses from embedded electrodes to drive the droplets from the oil to an aqueous stream.[20] The latter approach was recently used to analyze droplet contents by electrospray ionization (ESI) MS.[21] The aqueous channel into which the droplets were extracted operated in excess of 4 $\mu L\,min^{-1}$, and the contents were flowed from the chip through tubing to a conventional, external sheath-flow electrospray source. The large distance to the ESI emitter led to dilution of the droplet contents, and the high flow rates reduced ionization efficiencies,[22] resulting in limited sensitivity (circa 500 μM detection limits for peptides).

We have been developing[23] devices capable of automatically transferring the contents of droplets to an aqueous stream for analysis by nano-ESI MS using integrated electrospray emitters, thus making much higher sensitivity analyses possible. Figure 1 shows an overview of the device. Aqueous 600–800 pL droplets (plugs) are generated on-chip and carried downstream by an immiscible oil (Figure 2a, and Movie S1 in the Supporting Information). The geometry of the droplet generator enables sub-nanoliter droplets to be produced at low frequencies (ca. 0.1 Hz). The oil stream is separated from an adjacent aqueous stream by an array of cylindrical posts, forming 3 μm wide apertures in between. Interfacial tension between the two immiscible liquids prevents bulk transfer for similar pressures in the two channels. However, the aqueous plugs rapidly coalesce with the aqueous stream upon contact through the apertures (Figure 2b, and Movie S2 in the Supporting Information). Although transfer typically occurs through only one or two apertures, the larger number of openings serves to buffer pressure imbalances between the channels, preventing bulk crossover of the oil and aqueous streams. Indeed, we were unable to form a stable oil–aqueous interface with a device having a single 3 μm wide aperture. The rate of droplet transfer to the aqueous stream closely matched the flow rate of the oil channel (ca. 200 $nL\,min^{-1}$), which in this case was more than twice the flow rate of the carrier channel (80 $nL\,min^{-1}$). This rapid transfer resulted in a brief pressure spike in the aqueous carrier channel and minimized dilution owing to mixing with the carrier solution. In fact, as shown by the MS data below, a large portion of each droplet transferred without mixing with the carrier solution at all, enabling essentially dilution-free analysis from the droplets.

The aqueous channel terminated at an integrated nano-ESI emitter described previously,[24] created by making two vertical cuts through the polydimethylsiloxane (PDMS) device, with the channel terminating at the apex. Because

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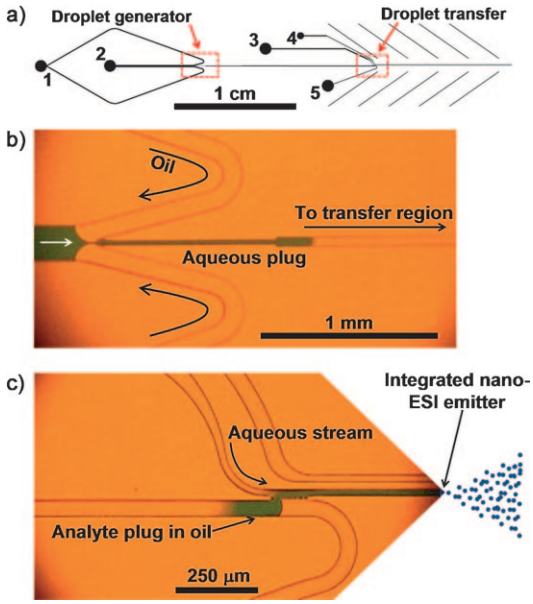

Figure 1. Device design. a) Representation of the device. Oil and the analyte-containing aqueous solution are supplied through ports 1 and 2, respectively. The aqueous carrier solution into which droplets are transferred is infused through port 3. High voltage (ca. +3 kV) to drive the electrospray is supplied at the stainless-steel needle of the syringe providing carrier solution to port 3. Port 4 supplies electrically conductive solution through a channel for an in-Taylor-cone liquid junction[24] to enable electrophoretic separations, and was not used for these experiments. Port 5 is the waste reservoir for the oil. The angled lines that are not connected to the fluidic circuitry served as guides for accurate cutting of the PDMS devices to form nano-ESI emitters, enabling variable distances from the droplet transfer region to the emitter to be easily obtained. b) Droplet generator. c) Droplet transfer region. The interface between the aqueous and oil channels is comprised of 6 cylindrical columns, each 15 μm in diameter, leaving 3 μm wide apertures in between. Channel widths are 50 μm for the droplet/oil channel, and 20 μm for the aqueous stream leading to the ESI emitter. The channel above the aqueous stream in (c) was connected to port 4 and was not used for this work.

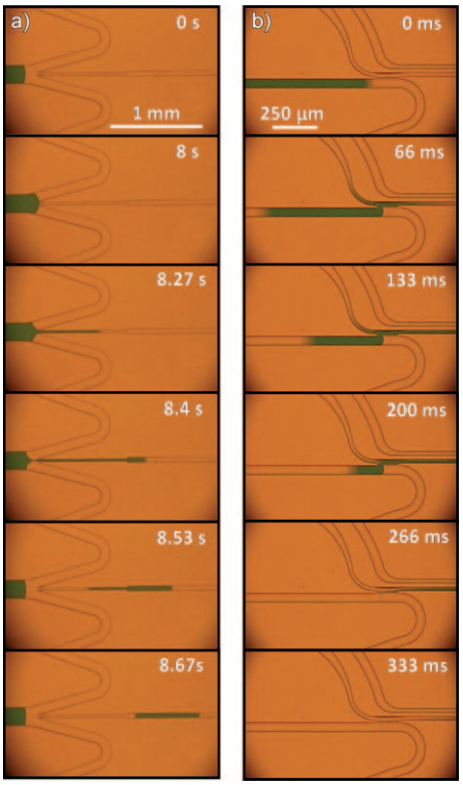

Figure 2. Micrograph sequences depicting device operation. a) Droplet generation. Flow rates were 10 nL min^{-1} for the aqueous solution (green) and 200 nL min^{-1} for the oil (clear), and matched those used to collect experimental data reported herein. b) Droplet transfer. The flow rate in the aqueous channel was 80 nL min^{-1} and it also matched experimental conditions. The blurred appearance of the aqueous plug prior to transfer is due to the long exposure time of the imaging device.

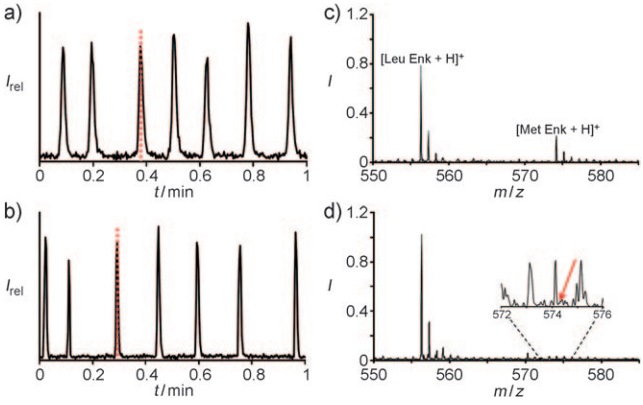

Figure 3. MS Data. a,b) Extracted ion traces for leu enkephalin (m/z 556–557) obtained for $d = 6$ mm (a) and 1 mm (b). c,d) Mass spectra obtained at the apexes of the peaks indicated by the dashed red lines in (a) and (b), respectively. The red arrow in the expanded window in (d), indicating m/z 574.2, highlights the absence of met enkephalin from the carrier solution stream.

the nano-ESI source was integrated with the device and operated stably at 80 nL min^{-1}, it was possible to achieve much higher MS sensitivity for droplets than previously reported.[21] In the present design, the distance d from the droplet transfer region to the ESI emitter can be as short as 1 mm or as long as 3 cm, depending on where the cuts defining the emitter are made; the longer distances are intended for capillary electrophoresis (CE) separations. Herein, we evaluated the effect of d on ESI-MS performance for direct infusion in terms of droplet peak widths and MS signal intensities as d was varied from 1 mm to 6 mm. Extracted ion traces for leu enkephalin are shown in Figure 3 a and b for $d =$ 6 mm and 1 mm, respectively. A plot of peak width versus d (Figure S3, Supporting Information), which also provides approximate post-transfer residence times for different distances, shows the considerable peak broadening that can occur over a distance of just 5 mm due to Taylor dispersion and diffusion.

The extent to which the droplet contents were diluted by the aqueous carrier solvent is examined in Figure 3 c,d and Figure 4. At a post-transfer distance of 6 mm (Figure 3 c), met enkephalin, spiked into the carrier solvent, is prominently observed at a peak apex in the leu enkephalin extracted ion

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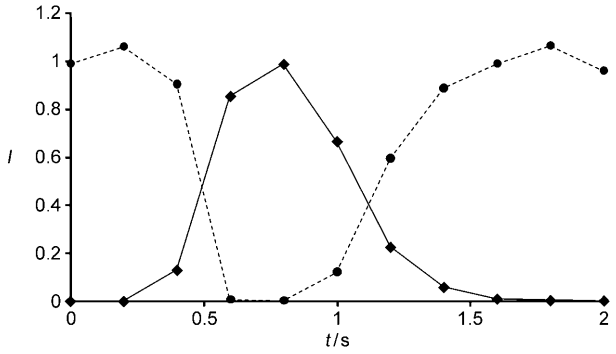

Figure 4. Plot of MS intensities of leu enkephalin (originating from the droplet; ◆, ——) and met enkephalin (originating from the carrier solution; ●, •••••) along the profile of a transferred droplet peak. The distance from the droplet transfer region to the emitter was 1 mm.

trace resulting from a transfer event, indicating the extent of dilution. Furthermore, the normalized peak intensity of leu enkephalin, obtained by comparing the ratio of its absolute intensity to that of met enkephalin in between transfer events with their in-solution intensity ratio, further verifies the dilution. Figure 3d shows a similar spectrum for $d = 1$ mm. In this case, the normalized intensity is unity, and the met enkephalin signal intensity decreases by 99.7% to chemical background levels, such that the maximum extent of dilution by the carrier solvent is about 0.3%. The leu enkephalin peak (Figure S4, Supporting Information) disappears completely between transfer events, indicating that cross-contamination of droplet contents is not an issue. As Figure 3c,d shows MS data only at the apex of the transferred peaks, Figure 4 provides a profile for both leu enkephalin and met enkephalin defining a complete transfer event for $d = 1$ mm, and shows that a droplet can provide multiple spectra unaffected by dilution.

The device described herein combines droplet-based microfluidics with continuous flow microsystems in a manner that enables high-sensitivity nano-ESI MS detection. A gain in sensitivity of about three orders of magnitude is achieved over previous work.[21] The simple method described for droplet transfer and the efficient coupling of droplet technology with MS detection have numerous potential applications. For example, the rapid transfer times (ca. 200 ms) should enable a new method for monitoring diffusion coefficients by MS,[25] and are sufficiently fast to serve as injection events for rapid, high resolution microchip CE separations. Such an injection mechanism for microchip CE should provide a significant advantage for sample-limited analyses over conventional cross injectors,[26] which send the majority of the sample to waste. Droplet-mediated multi-dimensional separations are then feasible with this injection mechanism as well, provided that the transferred droplets comprise the compartmentalized eluent[27] from an orthogonal separation (for example, LC). Finally, by using the droplets to encapsulate and prepare individual cells in a lossless fashion, and coupling the platform with new technologies designed to maximize ionization and transmission efficiencies such as low-pressure nano-ESI MS[28] for optimum sensitivity, this platform could open the door to effective single-cell, MS-based proteomics measurements.

Experimental Section

Leucine enkephalin (YGGFL) and methionine enkephalin (YGGFM) were purchased as solids from Sigma–Aldrich (St. Louis, MO, USA), diluted to 100 μM in water, and further diluted to their final concentrations of 1 μM each in the carrier solvent; the ESI-compatible carrier solvent consisted of 9:1 water/methanol containing 0.1% acetic acid. The oil used was perfluorodecalin (Sigma). Devices were fabricated in PDMS using conventional soft lithography from a single photomask, followed by irreversible bonding to a PDMS cover plate by treatment with a corona source as described previously;[24] channel depths were about 20 μm. Following bonding, devices were heated to 120°C overnight to recover the hydrophobicity of the surface.[18] Liquids were infused from 50 μL syringes (Hamilton, Reno, NV, USA) controlled by Harvard PHD 2000 syringe pumps (Holliston, MA, USA). Through-holes and the fluidic connections between the fused silica capillaries and the microdevices were made as described previously.[24] MS measurements were made using an ion funnel-modified[29] orthogonal time-of-flight instrument (Agilent Technologies, Santa Clara, CA, USA) set to record spectra at 5 Hz. The in-solution intensity ratio of the two peptides was determined by direct infusion ESI MS of an equimolar mixture using a 20 μm inside-diameter chemically etched[30] fused silica emitter operating at 100 nL min^{-1}.

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