Microdroplets

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Coupling Microdroplet Microreactors with Mass Spectrometry: Reading the Contents of Single Droplets Online**

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Emulsions created inside microfluidic devices have generated significant interest in recent years because of their ability to compartmentalize reactions and assays on a microfluidic platform.[1–4] Microdroplets can be generated at rates in excess of thousands per second, with volumes ranging from nanoliters to femtoliters, and can be used to carry out enzymatic assays,[5,6] gene expression,[7] cell-based assays,[8–11] and chemical synthesis.[12,13] Furthermore, multiple operations such as droplet fusion,[14,15] division,[17] and sorting[18–20] can be integrated in lab-on-a-chip devices. The combination of all these advantages provides an extremely powerful platform for high-throughput chemistry and biochemistry.

The utility of this technology however, ultimately depends on the ability to assess the chemical contents of each microdroplet. To date, most approaches have focused on optical techniques to retrieve information from the droplets.[7,10,14,21–23] Fluorescence has been mostly used because of its ease of implementation in microscopic formats, and its widespread use as a sensitive and quantitative spectroscopic tool in biology. The primary disadvantage of a detection scheme based on fluorescence is the requirement that a fluorescent label needs to be involved in the reaction.

Conversely, mass spectrometry (MS) provides a potentially universal label-free method to study chemical reactions. Moreover, electrospray ionization mass spectrometry (ESI-MS) has been successfully integrated with microfluidic formats in an on-line fashion.[24–27] However, its integration with microdroplet microfluidics has remained a challenge.[28] The direct MS analysis of microdroplets is problematic for several reasons. The primary difficulty stems from the presence of the carrier fluid, which is often composed of fluorous or mineral oils as well as significant amounts of surfactant. This continuous phase interferes with the ESI process by both sequestering charge carriers and preventing the formation of a stable Taylor cone.[29]

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Herein, we describe the integration of MS into a detection scheme for microdroplets created within microfluidic channels. We show that we can record mass spectra of compounds encapsulated in microdroplets, identify droplets based on their components, and combine fluorescence screening with MS analysis. Furthermore, we use these proof-of-principle experiments to indicate how similar approaches can be applied to the ambitious goals of on-chip protein evolution and chemical synthesis.

In order to remove the carrier phase from the microdroplet-encapsulated species of interest, we used our previously reported method for on-chip emulsion separation.[30] In this technique, droplets flow parallel to an aqueous stream between a pair of electrodes. Application of a voltage between the electrodes causes coalescence of the droplets with the aqueous stream, which effectively extracts their contents. To perform ESI-MS, we used a silica capillary as an emitter, which was inserted into a stainless steel sheath and placed near the atmospheric sampling inlet of the mass spectrometer. A schematic representation of the microfluidic device used for our studies is shown in Figure 1a. Soft

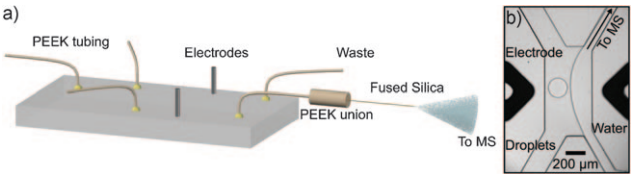

Figure 1. a) Schematic representation of the microfluidic device used for MS analysis of microdroplets. Poly(dimethylsiloxane) channels are molded and sealed by using soft lithography. Fluid connectors are fixed to the chip and connected to the MS emitter and other chip outlets by standard unions. b) Micrograph of the microfluidic device in operation. Aqueous droplets are formed in fluorous oil by using flow focusing. In the absence of an electric field, they flow past a lateral aqueous stream. When an electric field is applied, they coalesce with the lateral stream and their contents are analyzed by MS.

lithography was used to fabricate poly(dimethylsiloxane) (PDMS) microfluidic devices.[31] Poly(etheretherketone) (PEEK) tubes were attached to the devices, and commercial fittings were used to connect them to the syringes that delivered the fluids and to the silica capillary that was used as the MS emitter. A micrograph of a microfluidic device in operation is shown in Figure 1b. The electrodes are shown in black on both sides of the separation chamber. A droplet formed by flow-focusing[32] flows past the lateral stream of water. This stream, which carries the extracted contents of the

droplets, is delivered through the silica capillary to the mass spectrometer for analysis.

The mass spectrum of the nonapeptide bradykinin (RPPGFSPFR), which was recorded online while peptide-containing droplets were extracted into the lateral stream in a continuous fashion, is shown in Figure 2a. The spectrum

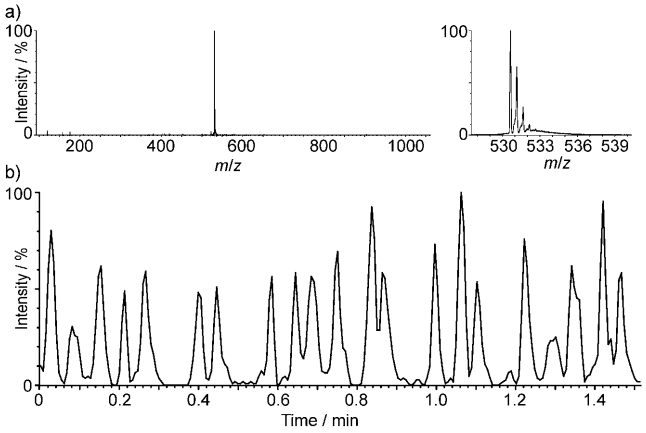

Figure 2. a) MS analysis of bradykinin encapsulated in microdroplets and extracted in a continuous fashion. Several low-intensity features with a lower m/z value were identified as chemical-noise components, including a signal at m/z 365 that corresponds to 1H,1H,2H,2H-perfluorooctan-1-ol). b) Ion current for the mass range associated with bradykinin, which shows peaks corresponding to the extraction of single droplets. Electric pulses were used to extract and analyze individual droplets while the process was monitored by using high-speed video (see the Supporting Information).

shows a clear signal, which was assigned to doubly charged bradykinin (m/z 530.79 $[M+2H]^{2+}$, monoisotopic) following tandem MS. These results show that by using this technology, it is possible to separate the contents of the droplets from the oil and analyze them online by using MS.

Individual droplets were extracted on-chip for MS detection. The ion current for the mass range corresponding to the $[M+H]^+$ ion of bradykinin is shown in Figure 2b. We generated short pulses at a fixed frequency of 0.3 Hz, which extracted approximately one out of 30 droplets. There was no synchronization between extraction pulses and droplet generation, therefore the resulting extraction efficiency of this experiment was not optimal and a single pulse occasionally extracted two droplets. These results show that it is possible however to extract and analyze droplets individually, which allowed studies where different compartmentalized reaction products are analyzed by using MS. The variability observed in the peak height for each droplet arises from our current emitter geometry, as well as limitations in the scan rate of the instrument and residual oil present after emulsion separation (see the Supporting Information for a detailed discussion). These issues could be greatly improved by microfabrication of an integrated emitter of adequate geometry and conductivity.[24]

To demonstrate label-free identification of microdroplets, we generated droplets that contained different compounds, extracted the droplets individually, and analyzed them online by using MS. In our experiment, we formed droplets of angiotensin (NRVYIHPFHL, m/z 648.354 $[M+2H]^{2+}$, monoisotopic) and bradykinin by using separate flow-focusing devices, and then combined the streams of droplets before they entered the emulsion separation chamber. We then generated pulses at a fixed frequency of 0.1 Hz and extracted droplets individually.

Figure 3a–c shows micrographs of droplets in the emulsion separation chamber before they were extracted (top) and

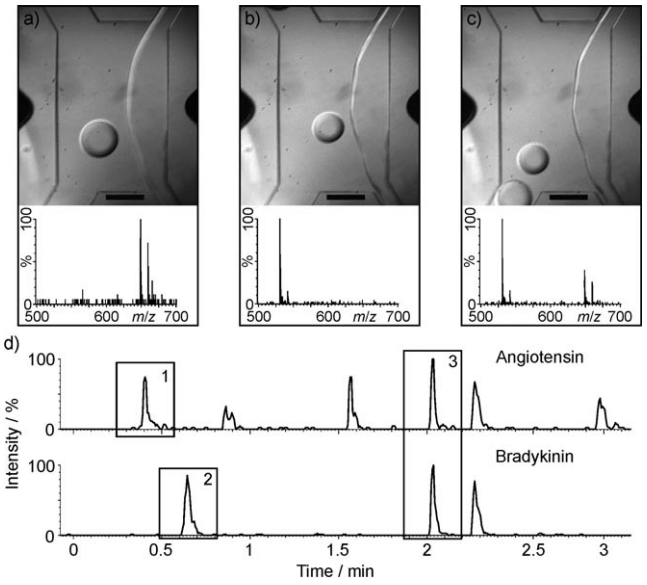

Figure 3. MS identification of single droplets. Upper panels: Micrographs showing droplets of a) angiotensin, b) bradykinin, and c) both before extraction (see the Supporting Information for a video). Scale bars: 200 μm. Lower panels: MS data corresponding to each extraction event showing the signals associated with a) angiotensin (m/z 648.354 $[M+2H]^{2+}$), b) bradykinin (m/z 530.79 $[M+2H]^{2+}$) and c) both peptides. d) Ion currents for the signals corresponding to doubly charged angiotensin (upper spectrum) and bradykinin (lower spectrum).

MS data corresponding to each extraction (bottom). When droplets of angiotensin (Figure 3a) or bradykinin (Figure 3b) were extracted individually, a clear peak, which corresponds to the peptide that was encapsulated in each droplet, is observed in the mass spectrum. When two consecutive droplets entered the separation chamber and were extracted together, the spectrum shows peaks for both compounds (Figure 3c).

Ion currents for selected regions in the mass spectra of angiotensin and bradykinin are shown in Figure 3d. Peaks that correspond to the extraction of an individual droplet (labeled 1 and 2) are observed in each trace. We also observe concurrent signals, which correspond to the simultaneous extraction of both types of droplets (labeled 3), in both traces. These results show the potential for applications in combinatorial chemistry and drug discovery, since compounds or combinations of them could be encapsulated or synthesized in droplets, assayed, and then identified by using MS.

The integration of fluorescence detection and sorting of droplets with MS analysis will allow advanced experiments in which droplets are screened for biological activity prior to determining the chemical identity of their content. To demonstrate this proof-of-principle, we separately generated droplets of a peptide (angiotensin) and its fluorescent analogue (angiotensin–FAM), and then extracted them individually either in an uncorrelated fashion or selectively by using fluorescence to control the extraction. To realize the selective extraction of fluorescent droplets, we developed a portable laser-induced fluorescence (PLIF) device. This device featured additional channels perpendicular to the fluidic channel, within which we inserted an optical fiber. We then used this fiber to deliver the excitation beam from the laser to the device and to carry the emitted light from the device back to the detector.

The experimental setup used to combine fluorescence screening and MS analysis is shown in Figure 4a. We focused a laser into an optical fiber that was inserted into the microfluidic device. The fiber delivered the excitation light and collected the emission light, which was measured by a photomultiplier tube, from the droplets. The signal from the photomultiplier tube was used to trigger the pulse generator, which allowed the selective extraction and analysis of fluorescent droplets. The process was monitored by using high-speed video.

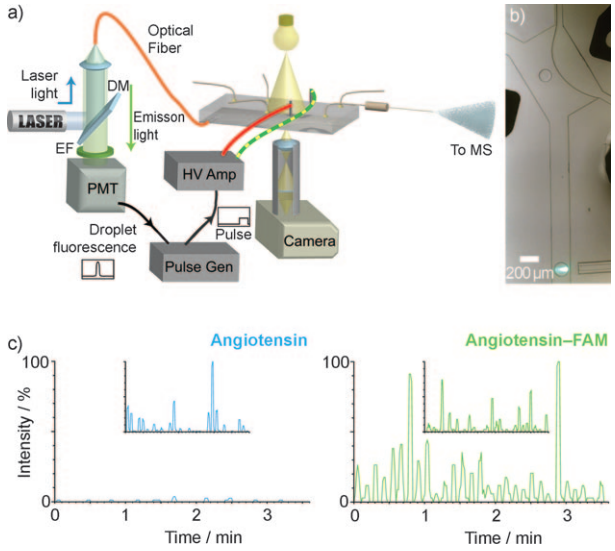

Figure 4. Integration of fluorescence screening and MS analysis of microdroplets. a) Schematic diagram of the experimental setup for fluorescence intensity measurements. Light for fluorescence measurements is carried to the chip using an optical fiber. The fluorescence signal is used as a trigger for the extraction. High-speed imaging is used to determine the extraction parameters and monitor the process. b) Micrograph of the microfluidic device in operation, which shows the fluorescence emission of fluorescein within a droplet as it passes the optical fiber carrying the laser light. c) Ion currents for the signals corresponding to angiotensin (left) and fluorescein-labeled angiotensin (right). When electric pulses were generated periodically, peaks for both compounds are observed (shown as insets). When the electric pulses were triggered by the fluorescence signal no peaks for angiotensin and regularly spaced peaks for its fluorescent analogue were observed.

A micrograph of such a device in operation is shown in Figure 4b. The optical fiber within its channel is shown at the bottom right of the image and the path of the laser is indicated by green fluorescence emission across a fluorescein-containing droplet. The detection limit obtained for fluorescence data acquired in this configuration is comparable to those reported previously,[19,30] thus providing an excellent signal-to-noise ratio for the selection experiments carried out.

The ion current for the m/z range that corresponds to angiotensin- (left spectrum) and fluorescein-labeled angiotensin (right spectrum) is shown in Figure 4c. When the extraction pulses were uncorrelated with the fluorescence signal of the droplets, peaks that corresponded to either peptide were observed (see insets in Figure 4c). When the extraction pulses were correlated with the fluorescence signal, only peaks that corresponded to the fluorescent compound were observed. In addition, the peaks are regularly spaced and the extraction efficiency increases because of the more precise timing afforded by the fluorescent trigger pulse. In these experiments, a 100% selection efficiency at 0.1 Hz was achieved. The selection throughput was limited by analyte dispersion after separation and instrument scan rate, since droplets can be sorted at kHz rates.[18–20] These results show that it is possible to combine one of the most useful approaches for screening biological compounds within microdroplets, fluorescence, with MS analysis, paving the way for ambitious studies in the areas of directed evolution, drug discovery, and combinatorial chemistry.

In summary, we have developed a technology that enables online MS analysis of individual microdroplets. We have also developed a portable fluorescence screening technology that is compatible with MS and used it to combine microdroplet sorting and online MS analysis. Further developments will be required to overcome our current limits of detection for single droplets (ca. 500 µM bradykinin). However, we envision that the experimental apparatus described here will significantly influence the general utility of microdroplet microreactors, especially in the area of directed evolution, screening for protein–protein interactions, and single cell assays.

Experimental Section

Soft lithographic techniques were used to fabricate PDMS microfluidic channels and oxygen plasma was used to seal the channels with PDMS-coated glass slides.[31,33] Solder electrodes were fabricated using microsolidics.[34] PEEK tubes were glued onto oxygen-plasma activated devices using NOA (Norland optical adhesive) and connected through PEEK unions to the tubing and the MS emitter. The silica capillary that was used as emitter was 375 µm in OD and 75 µm in ID. A typical device presents 200 µm wide channels for droplet formation and an 80 µm wide channel for the lateral stream. The channel width in the electrode area varies between 480 µm and 680 µm. Channels are 75 µm deep. Positive mode electrospray MS data was recorded using a Synapt HDMS instrument (Waters, Milford

MA, USA). Further experimental details are available in the Supporting Information.

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