Published in final edited form as:

J Chromatogr A. 2015 February 20; 0: 98–116. doi:10.1016/j.chroma.2014.10.039.

Microfluidics-to-Mass Spectrometry: A review of coupling methods and applications

Xue Wang, Lian Yi, Nikita Mukhitov, Adrian M. Schrell, Raghuram Dhumpa, and Michael G. Roper*

Department of Chemistry and Biochemistry, Florida State University, 95 Chieftain Way, Tallahassee, FL 32306

Abstract

Microfluidic devices offer great advantages in integrating sample processes, minimizing sample and reagent volumes, and increasing analysis speed, while mass spectrometry detection provides high information content, is sensitive, and can be used in quantitative analyses. The coupling of microfluidic devices to mass spectrometers is becoming more common with the strengths of both systems being combined to analyze precious and complex samples. This review summarizes select achievements published between 2010 – July 2014 in novel coupling between microfluidic devices and mass spectrometers. The review is subdivided by the types of ionization sources employed, and the different microfluidic systems used.

Keywords

Proteomics; Metabolomics; Lab-on-a-Chip; Integrated Devices; Bioanalysis

1. Introduction

Microfluidic devices have greatly affected the field of analytical chemistry since their introduction in the early 1990's. These devices, made using a variety of fabrication techniques and materials, offer numerous advantages over comparable bench-top instruments [1, 2]. Miniaturization with the subsequent reduction in sample and reagent volumes is a key advantage. It is now feasible to use and manipulate volumes that are orders of magnitude lower than what was possible a few decades ago. Another advantage specific to microfluidic devices include integration of multiple analytical processes onto a single platform with very little dilution, increasing the overall sensitivity of the assay [3, 4]. The nature of the fabrication process also allows reliable production of parallel analysis domains for automated and high throughput assays, which reduces sample handling and errors associated with manual manipulations [5].

©2014 Elsevier B.V. All rights reserved.

*Address Correspondence to: Dr. Michael G. Roper, Department of Chemistry and Biochemistry, Florida State University, 95 Chieftain Way, Dittmer Building, Tallahassee, FL 32306, Ph 850-644-1846, Fx 850-644-8281, roper@chem.fsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Despite these advantages, there are numerous pitfalls surrounding the use of microfluidic systems. One limitation is the difficulty in coupling these devices to conventional macro-scale systems, such as external pumps or detectors. Because of this difficulty, optical detection, most notably fluorescence detection, is often the detection method of choice due to its ease in obtaining information within the confines of micro-channels [6]. And while this detection method is extremely sensitive, it is non-universal requiring the use of fluorescent reporters. Electrochemistry is also a convenient means of detection in microfluidic systems due to the ability to miniaturize many of the components [7]. However, the same problems with specificity occur with electrochemical detection as encountered with optical detection, in addition to difficulties decoupling detection electronics with the high voltages used to drive fluid flow.

Mass spectrometry (MS) offers a near universal approach for detection than optical or electrochemical methods. However, MS detection from microfluidic systems has not been explored as much as these other methods. One reason for this lack of popularity may be due to the difficulty in coupling the analyses performed on microfluidic devices to the off-chip mass spectrometer. This entails sampling microliter to picoliter volumes from the device after chip-based analysis is performed. And while this coupling can be problematic, the information obtained from MS analysis is often worth many of the difficulties encountered.

The purpose of this review is to highlight several papers published between 2010 – July 2014 that describes novel coupling mechanisms or applications of microfluidic systems to MS detectors. We outlined this review into microfluidic systems coupled to electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), and other ionization methods. Within each of these categories, we subdivided into the main microfluidic systems employed, conventional or analog, droplet, and digital microfluidic systems. We wrote this review assuming the reader understands the fabrication methodologies of microfluidic devices, but point out unique aspects during the discussion of the individual papers. In the conclusion, we note a few intriguing possibilities for future coupling strategies. It should be noted that we did not intend to make this a comprehensive review of the literature, and point the reader to other reviews that provide additional viewpoints or time frames on this subject [8–12].

2. Microfluidics coupled to ESI-MS

Due to its ease in accepting low flow rates, ESI is compatible with microfluidic devices. As a result, it has been a widely exploited ionization method for on-line microfluidic-MS analysis. With the continued development of microfabrication technology, coupling of the main types of microfluidic systems (analog, digital, and droplet microfluidics) to MS via ESI has become more common, and a number of successful examples have been achieved.

2.1 Analog microfluidics ESI-MS

Analog, or conventional, channel-based microfluidics are often utilized due to their wide versatility in a number of analyses, such as sample preparation, preconcentration, micro-reactions, and separation. In this section, we describe the most common means of coupling analog microfluidic systems to ESI-MS followed by select applications using these systems.

2.1.1 Coupling to ESI-MS via integrated emitters—Several approaches have appeared in the literature for coupling emitters from a microfluidic device to interface with ESI-MS. These means of coupling can be classified into two main categories: spraying from integrated emitters and spraying via external emitters.

Integrated emitters are made directly within the device during fabrication producing connections with no, or very little, dead volume. A simple means to produce this type of emitter is to fabricate the channel of interest, such as a separation channel, so that it extends to the edge of the device. Application of the electrospray voltage is achieved through a secondary channel that intersects the separation channel near the edge of the device. In this way, it is also possible to introduce sheath flows containing the appropriate buffers or organic solvents to increase spray efficiency. Although the fabrication of this type of coupling strategy is straightforward, one drawback to this design is wetting of the microfluidic substrate by the separation eluent [13]. The low contact angle that the hydrophilic liquids have on the glass leads to sample spreading on the chip edge and generates a relatively large Taylor cone, which introduces dead volumes at the microchannel exit and may negatively influence the separation. Hydrophobic coatings on the hydrophilic channels have been tested but are not stable over time [14]. Moreover, even on hydrophobic substrates, the Taylor cone tends to form at the site of the most protruding microdefect since it experiences the highest local electric field strength [15]. This inability to accurately control the position of the spray will reduce the spray efficiency. To overcome these limitations, the channel of interest is directed towards a sharpened point on the device to limit the surface area that the eluent comes into contact with as it sprays from the device.

Over the past two decades, Ramsey's group has explored online coupling of microfluidic chips to MS [16]. In recent work, they have developed an integrated capillary liquid chromatography (LC)/capillary electrophoresis (CE)-ESI-MS system to study proteolytic digests [17]. To compensate for flow rate differences between the offline capillary LC system (~500 nL/min) and the microchip CE system (~100 nL/min), a split flow channel was employed after introducing the LC eluent into the chip as shown in Figure 1. With the split channel having a low resistance to flow, the LC eluent delivered to the CE domain was reduced to a level that was compatible with electroosmotic flow (EOF). The glass microchip was coated with 3-(aminopropyl)triethoxysilane (APTES) to produce a positively charged surface, resulting in anodic EOF in acidic buffers, which are also more favorable for generating efficient electrospray. After the CE separation, the channel was directed to the corner of the microchip where electrospray was generated by applying a 3.6 kV voltage to a sheath channel that intersected near the end of the separation channel. By aligning the corner of the chip with the MS orifice, the analytes were successfully injected into the MS. With this multidimensional separation system, the peak capacity was ~1400 within 50 min when analyzing a complex mixture of peptides. The reproducibility for LC retention and CE migration times was 0.32% and 0.75% RSD, respectively. They utilized their LC-CE-MS system to characterize two N-linked glycosylation sites of a monoclonal antibody.

Later, the authors published another work that described the method that was used to modify the microchip surfaces [18]. A commercial chemical vapor deposition (CVD) system was used to coat their glass devices, and two surface modifications were compared, APTES and

(3-aminopropyl)di-isopropylethoxysilane (APDIPES), with both demonstrating stable surface modifications for over 1 week. Highly efficient separations were shown with these modified devices generating theoretical plate counts of ~600,000 for peptides and a peak capacity of 64 within 90 s. An “efficiency coefficient” () was employed for comparing the efficiency of their separations to that of a purely diffusion-limited separation, with $=N_{\text{optimal}}/N_{\text{observed}}$, where N is the plate number. Separations with purely diffusion-limited band broadening would yield a value of 1. With their APDIPES-coated device, they achieved a value of 1.09, indicating a near diffusion-limited separation.

Belder’s group incorporated an organocatalytic reaction and an aqueous dilution feature before analysis by microfluidic electrophoresis-MS detection using an integrated nanospray emitter [19]. The enantioselective vinylogous Mannich reaction, which is catalyzed by chiral phosphoric acid, was used as a model system. The non-aqueous reactants were mixed together in the device followed by dilution with the aqueous separation buffer. This mixture was then injected into the separation channel by a pinched injection. After separation, electrospray was generated from the end of the separation channel with the help of a makeup flow. To achieve separation of the chiral products, a longer separation channel was used (increased from 7.5 cm to 23 cm) with a chiral selector, hydroxylpropyl- β -cyclodextrin, added to the separation buffer. With this device, they successfully separated the enantiomeric products on-chip with integrated MS detection. The total time for one analysis took less than 5 min, which the authors state may allow for fast monitoring of reaction intermediates or short-lived species.

In another report [20], a microfluidic device produced in SU-8 used a cross-T separation channel design with a sheath channel intersecting near the end, similar to that described above. The authors have previously used this material for device fabrication and have stated that SU-8 is advantageous as the direction of the EOF can be reversed from cathodic to anodic by adjusting the pH below 3 [21]. A sheath liquid channel was used for aiding in the electrospray and was also used to deliver a peptide fragment for use as an internal mass calibrant in the mass spectrometer. The authors stated that with their integrated system, electrophoretic peaks corresponding to peptide fragments had peak widths as narrow as 0.6 s. With this device, human muscle cell lysates were analyzed and seven proteins were identified with sequence coverages as high as 93% recorded.

Later, the same device was incorporated with a means to perform on-chip liquid phase micro-extraction (LPME) for measuring drug metabolites from urine samples [22]. A polypropylene membrane was placed on top of the sample inlet reservoir, which had been filled with an acidic acceptor solution. A drop of an alkaline urine sample was placed on top of the membrane and on line micro-extraction was conducted. The extracted drug metabolites of tramadol and paracetamol were separated and analyzed. A comparison was made of the metabolites detected using off-line solid phase extraction (SPE) and the LPME method, both analyzed by the microfluidic CE-ESI-MS system. The authors demonstrated that the LPME method offered advantages over off-line SPE, such as integrated analysis, small sample requirements, and fast analysis speed.

A hybrid glass/poly(dimethyl siloxane) (PDMS) microfluidic device was used for enantiomeric separation of 3,4-dihydroxyphenylalanine and amino acids via a partial filling technique [23]. A syringe pump was used to partially fill the separation channel with a plug of chiral selector, sulfated β -cyclodextrins (labeled “Sulfated CD” in Figure 2). When voltage was applied, the sulfated CD migrated to the anode, while analytes and bulk EOF moved to the cathode, enabling high-resolution chiral separations. A microfabricated monolithic nanoelectrospray emitter using a make-up fluid from an arm channel was employed to couple to ESI-MS, which allowed the detection of native amino acids without derivatization. The authors stated that this microfluidic ESI-MS method had detection limits one order of magnitude lower than conventional CE-MS analyses (LODs: 0.04 μ M vs. 0.50 μ M). Additional integration of on-chip cell culture to this platform using a 0.22 μ m membrane demonstrated the ability to study stereochemical preference in living cells. The authors suggested the combined advantages of specific MS detection and microfluidic electrophoretic separations could enable separation and quantification of enantiomers in a successive manner with a temporal resolution of < 25 s.

Another approach has been used to integrate the electrospray electrode into the spraying channel to minimize dilution of the sample [24]. A pyramid shaped tip (15 μ m $\times$ 50 μ m) was formed with the channel in the center of the tip (Figure 3). A V-shaped electrode channel was fabricated so that the point of the “V” just intersected the sample channel forming what the authors called a “micropore”. The V-shaped channel was filled with a low melting point alloy (LMA) that solidified and was used to apply the electrospray voltage. The electrode was in direct contact with the sample solution via the micropore. During filling of the LMA channel, a stable phase interface was required so that the LMA did not leak into the separation channel. This stable interface was dependent on the surface tension of the LMA and the size of the micropore, which was dictated both by the angle of the “V” channel and the distance between the “V” channel and sample channel prior to etching. The total ion current (TIC) of the electrospray showed a relative standard deviation (RSD) of 4.4% over a 6 h continuous experiment.

PDMS has been widely used as a substrate for microfluidic devices, but can be problematic to couple to ESI-MS since it has a large chemical background due to the presence of uncrosslinked oligomers and other contaminants from the substrate. To overcome this, the Smith group used a series of solvents to extract the contaminants and showed that the background signal was reduced while the sample peak intensity was increased $\sim$ 1.8 fold [25]. The authors also stated that the flow rates could be reduced as low as 10 nL/min for stable operation by optimizing the emitter geometry.

Later, this PDMS based emitter was further improved to facilitate better control over the spray [26]. Instead of fabricating the channel in a thick PDMS slab, they used rapid prototyping methods to produce channels in a thin PDMS slab. A second membrane was used to enclose the channel, producing a total device thickness of $\sim$ 100 μ m, comparable to the outer diameter of conventional emitters. Two PDMS slabs were used to sandwich the thin pieces for support, with the inner pieces extending out of the outer pieces by $\sim$ 5 mm (Figure 4A–D). The emitter tip was made into a triangular shape, and was aligned with the mass spectrometer inlet. The reduced surface area at the emitter tip (Figure 4E–F) enhanced

the electric field and reduced wetting of the surface by the electrospray solvent. Moreover, it allowed stable sprays to be achieved at lower potentials and lower flow rates. At 10 nL/min, the RSD of the TIC from a 1 μ M leucine enkephalin solution was ~3.5%. Due to the smaller surface area that the spray encountered on this membrane-based emitter compared to the device using only the larger PDMS slabs, a higher organic solvent component (e.g. 90% methanol) could be utilized. Although the chip design is straightforward, the authors noted that this PDMS based chip has great potential to integrate complex multilayer microfluidic devices because its fabrication procedure is similar to that used to produce pneumatic valves in PDMS.

To improve spray efficiency in glass devices, 3-dimensional (3D) electrospray tips were fabricated using a series of fabrication steps [27, Figure 5A–B]. The authors fabricated the 3D glass emitters relying only on standard micro-fabrication techniques and all manual machining steps were omitted. The fabrication of the 3D emitters utilized a variety of methods such as CVD of polycrystalline silicon on the glass surface, photolithography, reactive ion etching (RIE), hydrofluoric acid wet etching, and thermal bonding. But, the benefit was that these sharp, 3-dimensional emitters required very low ESI voltage (1.4 – 1.75 kV) to produce a stable spray and enabled the use of low percentage organic solvent such as 10% methanol without losing spray stability. When integrating the emitter with CE separation (Figure 5C), a plate number of $\sim 1.7 \times 10^5$ was achieved in separating a mixture of ammonium halides.

Lu *et al.* developed a polyimide device with dual channels, one for an aqueous sample and the other for a methanol/acetic acid buffer, ending at a single emitter which allowed mixing in the Taylor cone [28]. The authors stated that since good ionization efficiency requires the addition of organic solvents and volatile acids that can be detrimental to biological macromolecules, this device eliminated the requirement of mixing the sample with organic solvent. The device was fabricated by using a laser to ablate two channels on a polyimide film. The aqueous, sample channel was ablated on the bottom side of the polyimide with the outlet of the channel patterned towards the V-shaped emitter tip (Figure 6A). The channel for the organic solvent was also ablated on the bottom side of the polyimide, parallel to the sample channel, but stopped approximately halfway down the device (Figure 6A). A hole was punched into the polyimide film allowing the organic solvent to flow to the top of the polyimide film where it then traveled in another ablated channel that ended at the same location as the end of the aqueous sample channel. In this way, the methanol/acetic acid mixture met the aqueous samples on the opposite side of the device tip, eliminating the on-chip mixing step. The depths of the two channels dictated the distance between the two channels as shown in Figure 6B. The optimized inter-channel distance was found to be 9 μ m in terms of both the ion intensity and the RSD of the TIC when characterized with A β 16, a peptide involved in Alzheimer's disease.

To achieve high mass accuracy for CE-ESI-MS, Ramsey's group developed a dual emitter microfluidic chip to sequentially introduce sample and a reference compound sequentially to the MS, with the reference compound used to calibrate the mass spectrometer [29]. The two emitter tips were 3.6 mm away from each other as shown in Figure 7. By applying electric potentials to selected reservoirs, sample and reference compounds were sequentially infused

into the separation channel and the emitter tip, and analyzed by MS. The fastest switching time between the two electrospray signals was ~70 ms. This dual emitter device allowed more versatility in choosing the reference compound and eliminated the requirement of mixing the sample with a reference from a make up flow that usually introduces extra band broadening at low flow rates. The mass accuracy was less than 3 ppm after the calibration, when evaluating the performance with leucine enkephalin. The authors also stated that this device has the potential of studying gas-phase ion/ion reactions and monitoring multiple microfluidic separations with a single mass spectrometer.

2.1.2 External emitters—Despite the robustness of integrated emitters, fabrication can be a challenge, and those new to microfluidics may prefer to utilize conventional emitters. As a result, there are multiple applications and approaches described in the literature that utilize external emitters placed within the microfluidic substrate.

In one example where LC was performed on a microfluidic device and coupled to MS, a micro-structured fiber (MSF) was used as the emitter [28, 29]. The MSF is a bundle of hollow fibers, or channels, designed to confine and guide light; however, the Oleschuk group have used these devices for spraying from microfluidic devices. In one report [30], a capillary was used to deliver sample to a cyclic olefin copolymer (COC) plate containing 3.5 μm C8 particles while the MSF was used as the outlet to spray sample. Fabrication of the microfluidic system consisted of several bonding steps that were optimized to fully seal the device without deforming the channels. The MSF was composed of 54 parallel channels, each with a diameter of 3.8 μm (Figure 8). The diameter of the region containing the hollow fibers was ~75 μm , while the outer diameter of the MSF was ~230 μm which allowed facile coupling to external fluidic system components. Due to the small inner diameter of the individual fibers, the MSF also served as a retaining frit for the LC packing material. The particles were observed to pack tightly against the MSF and no packing material was lost during chip operation, indicating that the MSF worked well in this capacity. As an electrospray emitter, the MSF showed a good solvent tolerance even at extreme solvent compositions (all water or all acetonitrile), which makes it an excellent choice when performing gradient elution on LC. The authors stated that the benefit of moving to this type of emitter was that the rate of clogging of this multi-emitting device was much lower than the rate of clogging by a single emitter. In the first report of the MSF as an emitter [31], the authors compared spray performance of the MSF to a commercial, tapered emitter. Leucine enkephalin in a 50/50 (v/v) acetonitrile/H₂O solution containing a mixture of nonvolatile salts used for cell culturing was used as the test solution. After introducing the concentrated salt solution, the TIC from the conventional emitter sharply declined and eventually dropped to 0, indicating a clogged tip. In contrast, the TIC from a 30-channel MSF emitter continued to produce spectra even after the current dropped to 0, which, the authors stated, indicated that ions were still being sprayed. This microchip [30] was used to effectively separate small drug molecules by isocratic elution and larger peptides using gradient elution.

There have been several applications of external emitters used in microfluidic ESI-MS platforms towards proteomics. In one example reported by the Wilson group, a protein digestion microreactor in a microfluidic device was coupled prior to ESI-MS. As shown in Figure 9A, this system utilized a wide (1.5 cm) and shallow (30 μm) reactor filled with 20

μm pepsin-agarose beads. Due to the low flow resistance in this channel, low pressures could be utilized while maintaining high flow rates. Using myoglobin, ubiquitin, and bovine serum albumin as model proteins, sequence coverage of 64–99% were observed with a residence time < 4 s in the digestion reactor. Later, this same group developed a means to integrate hydrogen/deuterium exchange (HDX) labeling upstream of this system to study protein structure and dynamics [33] as well as allosteric effects in enzyme inhibition [34]. For the most recent application [34], a variable position micromixer was used to adjust the reaction time of the beta lactamase enzyme, TEM-1, with a covalent inhibitor clavulanate. As shown in Figure 9B, TEM-1, was delivered via a capillary into a microfluidic channel while the inhibitor was delivered in a coaxial arrangement. By varying the position of the capillary, the reaction time between TEM-1 and clavulanate could be varied. After allowing the reaction to proceed, a fixed volume of deuterated water (D₂O) was introduced followed by HDX quencher. The quenched reaction was followed by pepsin digestion and transfer to ESI was performed via an inserted capillary. Digestion and transfer were achieved in ~6 s, which resulted in undetectable back exchange of backbone protons. This system was successfully applied to characterize structural changes and dynamics that occur upon acylation of TEM-1 by clavulanate. Both rapid structural changes at the active site and subtle changes in active-remote regions were identified. The authors predicted this approach would be a facile and powerful tool to study allosteric effects in protein function processes.

In a study by the Harrison group [35], a multifunctional microfluidic device in which integrated trypsin digestion, SPE, electrophoretic separation, and ESI-MS was demonstrated (Figure 10). Two packed beds filled with 40–60 μm diameter beads, loaded with either immobilized trypsin, reversed-phase packing, or both, were used as the digestion and SPE column, respectively. An example of on-chip digestion of cytochrome c showed a residence time of 3 min in the trypsin immobilized bed was enough for complete digestion, in contrast to the 2 h with conventional solution phase digestion. The digested peptides were then concentrated 13-fold by the SPE bed and a double T injector was used to introduce the eluent into the CE separation channel. Electrospray was initiated from an inserted gold-coated nano-electrospray capillary. On chip digestion with SPE concentration improved the sensitivity for MS detection and showed higher sequence coverage (~80%) compared to the same sample without SPE (~40%). This integrated system demonstrated the possibility of performing automated protein processing on a single microfluidic device.

Harrison's group further improved this system by incorporating a polymer monolithic micro-SPE bed and a separation channel coated with a polycationic coating [36]. The micro-SPE bed was formed *in situ* using a UV-initiated process to form a cationic polymer. The separation channel was coated with polyE-323, a polymeric amine coating, to reduce protein and peptide adsorption. The authors demonstrated that these modifications to the SPE bed and separation channel could be used to generate anodic EOF suitable for separation and electrospray. Digestion was carried out by incubating solution phase trypsin with captured proteins on the monolithic polymer bed for 30 min under stop flow conditions. The monolithic SPE column showed reproducible and linear calibration curves at constant preconcentration factors. Analysis of cytochrome c and BSA digests indicated minimal trypsin autodigestion products and high sequence coverages of 88% and 56%, respectively.

The authors stated that this system had the potential to be multiplexed for high throughput sample preparation.

Micro-SPE is not only a powerful preconcentration technique, but it also plays a critical role in sample cleanup. Over the last 5 years, Lin's group has developed several microfluidic devices where SPE, as well as other functional domains, are integrated with ESI-MS for the study of drug evaluation and cell signaling. In one example shown in Figure 11A, a microfluidic system was used to investigate the uptake of methotrexate, an anticancer drug, on HepG2 and Caco-2 cells [37]. An on-chip gradient generator was used to produce a range of drug concentrations to parallel cell culture chambers. Downstream micro-SPE columns were used prior to online ESI-MS and the authors found that intracellular drug concentrations correlated in a dose-dependent manner with increased cell cytotoxicity.

Lin's group developed similar systems with multiple cell chambers to study drug-to-cell as well as cell-to-cell interactions. For example, a multiple cell co-culture system integrated with micro-SPE and ESI-MS was employed to study the inhibition of growth hormone secretion from GH3 cells by dopamine released from PC12 cells [38]. ESI-MS was used to detect the secretion dynamics of growth hormone during the cell-to-cell communication under various conditions.

In a recent study from this group [39], a surface tension plug (STP) was integrated on a microfluidic device to inhibit cell-to-cell communication (Figure 11B). The STP was a channel containing a gas-liquid interface, which could stop flow between two cell culture chambers. Epinephrine released from 293 cells (mimicking a human kidney) and glucose from L-02 cells (mimicking a human liver) were concentrated via SPE and detected by ESI-MS.

Drug permeability was also characterized using a microfluidic platform with a semipermeable polycarbonate membrane sandwiched between a cell culture channel and a channel containing a drug (Figures 11C–D) [40]. Caco-2 cells were attached on a Matrigel-coated membrane and curcumin could be perfused from either side of the membrane. The authors demonstrated that curcumin transported through the monolayer could be preconcentrated online by SPE and quantified by ESI-MS. The authors stated that their automated systems offered potential for high throughput drug discovery and development.

In recent studies by the Peterson group [41, 42], liquid-liquid extraction on a microfluidic device was used to extract and concentrate drugs or metabolites from a reaction solution and coupled online to ESI-MS. As shown in Figure 12, a supported liquid membrane (SLM) was sandwiched by two channels in a polymethyl methacrylate (PMMA) device. In the top sample channel, rat liver microsomes were delivered, while in the bottom channel, an acceptor solution was flowed in the counter direction and coupled to ESI-MS. The SLM was composed of a water immiscible organic solvent, 2-nitrophenyl octyl ether, immobilized in a porous membrane. Metabolites could be selectively extracted into the acceptor channel by application of a DC voltage across the SLM. This system was employed for the online analysis of metabolism of amitriptyline *in vitro*. The MS signal for the more polar hydroxylated metabolites was improved by a factor of ~5 when the extraction voltage was

15 V. The extraction step also effectively prevented reaction material and buffer salts from interfering with the ESI-MS detection. Because changes in the reaction chamber could be monitored 9 s later by MS, the authors suggested their methodology allowed drug metabolism studies with high temporal resolution while consuming very small amounts of reagents.

2.2 Droplet microfluidics ESI-MS

Droplet microfluidics has emerged as a powerful technique in which highly monodispersed droplets in the picoliter to nanoliter volume range can be generated and manipulated with high frequency. Droplets are formed in a multiphase environment, and due to their independent nature, each droplet can be considered as a microreactor with no cross-contamination between droplets [43]. Mass spectrometry analysis of droplet composition has attracted considerable attention due to the possibility of high throughput detection in a label-free and online manner. For analysis by MS, droplets can either be extracted from the multiphase flow, or sent to the MS directly without extraction. Both of these coupling methods, as well as their applications in recent years, will be addressed in the followed discussion.

In droplet microfluidic systems, oil is typically used as the carrier fluid. One of the limitations of integrating two-phase droplet systems with MS is interference from the oil in formation and detection of sample ions [44]. Therefore, it may be necessary to extract droplets into a continuous aqueous phase to be delivered to an emitter for electrospray. In a recent study by Zhu and Fang [45], a novel droplet extraction interface based on a hydrophilic tongue structure was developed and applied for the study of reaction kinetics. As shown in Figure 13, this glass microfluidic device integrated a droplet generator, a droplet extraction interface, and a monolithic emitter. The segmented flow channel was deeper than the ESI channel and was made hydrophobic by modification with octadecyltrichlorosilane (OTS). The ESI carrier flow channel and the hydrophilic tongue, where the segmented flow channel and ESI carrier channel met, were not modified and remained hydrophilic. The tongue interface functioned to extract droplets as they passed by, drawing the droplet into the ESI carrier flow channel. By changing the waste reservoir heights, the backpressure in the segmented flow channel was optimized to achieve extraction without leakage of oil into the ESI carrier flow channel. Using optimum conditions, droplets containing angiotensin II were extracted and delivered to the mass spectrometer. The peak heights from the extracted ion currents showed reproducibility of 5.2% RSD and indicated no carryover between droplets. The authors suggested that this system was a promising tool in the study of reaction kinetics.

Kennedy's group has found that droplets separated by air or oil can be pumped directly into a fused silica nanospray emitter tip for MS analysis without extraction of the droplets [44, 46 – 48]. The authors found that high viscosity oils, like FC-40 and perfluorodecalin, did not generate signal at low spray voltages, but that the electrospray current and signal rapidly stabilized as each new aqueous droplet entered the emitter so a series of droplets segmented by selected oil plugs could be analyzed without an extraction step. This simple coupling method was applied in the screening of enzyme inhibitors [47]. Solutions of 25 test compounds were introduced as an array of droplets separated by perfluorodecalin in a tube.

Enzyme, substrate, and quenchant were introduced into the droplets through a series of PDMS tees. Reaction mixtures were injected through an electrospray emitter tip into MS detector. A good reproducibility with RSD < 3% in droplet peak height for each test compound was observed. The half maximal inhibitory concentration of three known inhibitors tested with this approach agreed with reported values. A carryover of < 1% was observed for the emitter and addition of more PDMS tees caused higher carryover. This system demonstrated feasibility in screening of enzyme inhibitors with high throughput and 1000-fold lower sample volume requirements than conventional methods. The authors suggested modification of the tees or channel dimensions was necessary to reduce carry over. They predicted this method may be broadly applied in screening reactions and parallel channels can be incorporated for higher throughput applications. This coupling method was also used for *in vivo* monitoring of acetylcholine in the brain of live rats [48]. Although these examples were not performed in planar microfluidic devices, the simple methodology offers the potential to couple many droplet microfluidic systems directly to ESI emitters.

In a study by Ji *et al.*, a similar methodology was employed for coupling a droplet based proteolysis microfluidic device as an interface between LC protein separation and MS detection [49]. As shown in Figure 14, proteins separated by LC were mixed with trypsin online and the mixture was encapsulated into droplets. After fast digestion in droplets, buffer was introduced to modify the pH and matrix of sample for compatibility with ESI-MS. Perfluorodecalin was used as the oil phase and electrospray voltage was optimized for highest signal from the sample droplets and minimum signal from the oil segments. A mixture of myoglobin, lysozyme, and cytochrome c was separated into three fractions by LC and after encapsulated with trypsin in droplets, the digests were successfully identified with MS on this system with sequence coverages of 67, 61, and 53%, respectively.

2.3 Digital microfluidics ESI-MS

A different way to manipulate droplets is through the use of digital microfluidics (DMF) [50]. This technique is based on the manipulation of discrete droplets using an array of patterned electrodes. By applying electrical potentials to selected electrodes, the droplets can be moved to appropriate positions for various operations, such as mixing, dispensing, extraction, and derivatization. The benefits of DMF are similar to droplet microfluidics, discrete droplets enable minimal crosstalk between analyses, while an additional benefit is a particular microfluidic channel layout does not need to be designed prior to experiments because the electrode array enables any number of user-designed analyses to be performed on a single device. To couple DMF devices to ESI-MS, two main problems need to be addressed: transferring droplets from DMF to an ESI emitter and dissociating the voltages used to perform droplet manipulations and the ESI spray voltage.

Wheeler's group has pioneered the coupling of DMF to ESI-MS. In one of their earlier designs, a hybrid DMF-microchannel device was developed [51]. A microchannel was fabricated underneath the DMF plate and was used to transfer the droplet from the DMF layer to the emitter. During fabrication (Figure 15A), an access hole was drilled in the DMF layer before patterning the electrodes and was aligned with the bottom channel prior to bonding. A droplet was delivered to the access hole, where application of a high voltage to

the DMF top-plate electrode produced electrospray from the edge of the microchannel at the corner of the chip. This device was used to analyze amino acids from dried blood spots (DBS) on a filter paper by performing extraction and derivatization on DMF and detection on ESI-MS (Figure 15B). The authors compared their method to more conventional methods developed for similar applications, and showed that their method allowed a reduction in reagent use (20 μ L vs. 170–450 μ L) and a decrease in analysis time (1 h vs ~3.5 h).

Our group used an educator for online DMF-ESI-MS [52, Figure 16]. A fused silica capillary was placed on the DMF device and coupled to a standard ESI needle. The outlet of the ESI needle was inserted and aligned into a tapered gas nozzle and when N₂ was delivered through the gas nozzle, a pressure differential was produced across the fused silica capillary. Due to the pressure difference, droplets were pulled from the surface of the DMF chip to the ESI needle and finally injected into the N₂ flow to the MS inlet. This interface not only successfully delivered the droplet from the DMF device to MS, but also allowed decoupling of the spray voltage from the DMF voltages allowing droplets to be continuously actuated while electrospray was occurring.

For nanospray ESI, Wheeler's group inserted one end of a fused silica capillary between the top and bottom DMF plates of the DMF chip while the other end was tapered and used as the electrospray emitter [53]. Transfer of the droplet to the MS was initiated by capillary action after actuation of the droplet to the inlet of the capillary (Figure 17A). The DMF voltages were then turned off and a DC voltage was applied to the top plate to generate the nanospray as shown in Figure 17B. On this device, they continued their study on DBS samples for screening of metabolites, including succinylacetone, from newborns. Their results from the DMF-MS device were comparable to that of the standard techniques used at Newborn Screening Ontario.

To extend the applicability of DMF devices coupled with ESI-MS, Wheeler's group developed a DMF device with an integrated emitter [54]. The device contained a two- to one-plate DMF interface and a folded nano-ESI emitter, all of which were formed from a single flexible polyimide substrate (Figure 18). After fabricating the bottom plates and the top plate separately using conventional methods, the device was assembled so that the bottom plate extended past the top plate forming the one-plate region. The one plate region was then formed into a cone with a 50 μ m orifice. Droplets were transferred from the two-plate region to the one-plate region, actuated through the cone, and ESI was generated by application of a voltage from an electrode within the folded cone region. This device was used to study the Morita–Baylis–Hillman reaction. Mixing of the reactants was performed in the two-plate region while dilution and introduction of the makeup fluid were conducted in the one-plate region. They observed that as reaction times increased, the peak intensities of the product increased as well. The authors stated that this highly integrated DMF-ESI-MS device would be useful for real-time monitoring of microscale synthetic processes.

3. Microfluidics coupled to MALDI-MS

MALDI is another popular technique for coupling microfluidics to mass spectrometry. The use of microfluidics with MALDI has revolved primarily around integration of sample

preparation steps, such as fraction collection from separations or protein digestion steps. Additionally, automation of MALDI spotting can minimize user variability, which is a desirable feature given that matrix mixing and spot application can strongly influence sample analysis [55].

3.1 Interfacing external MALDI plates to microfluidics

Several examples have been developed of automated systems that utilize microfluidics for spotting analyte to external MALDI plates. In one example [56], a COC microfluidic device was developed for contact spotting the outlets of 8 parallel channels to a MALDI plate. Spotting was performed by moving the outlet of these channels within 200 μm of the MALDI plate. The outlets of the channels were coated with Teflon AF to increase hydrophobicity and improve droplet transfer to the plate. After a particular time, the device was elevated 2 mm and moved to the next location for the next series of spots. Flow rates of 12 $\mu\text{L}/\text{min}$ resulted in droplet volumes ranging from 104 – 190 nL. The authors also demonstrated that this automated spotting system would be useful for fraction collection by coupling it to a microfluidic LC device. Twenty fractions, each 2 min in duration, from the four-peptide separation were spotted onto a custom nanofilament silicon plate and analyzed by laser-desorption ionization (LDI). Each of the peptides was observed in specific fractions with co-elution of two of the later eluting peptides. The authors stated that this method is amenable to faster and higher separation resolution by adjusting the spotting time interval.

In another report [57], Murray's group used a bioreactor on a PMMA microfluidic chip to perform trypsin digestion coupled via tubing to a MALDI plate placed on a motorized xyz stage (Figure 19). The 340 nL bioreactor was 4 cm $\times$ 200 μm $\times$ 50 μm (length $\times$ width $\times$ depth) containing an array of 50 μm diameter cylindrical micropost support structures with a 50 μm inter-post spacing producing a 16 mm² surface area. Trypsin was immobilized onto the surface of the bioreactor using surface modification protocols developed in the authors' laboratory [58]. A solution of cytochrome c, myoglobin, β -casein, and BSA were passed through the bioreactor and digestion ensued. Following digestion, the eluent was deposited onto a MALDI plate in either a continuous (Figure 19A) or a spot deposition mode (Figure 19B). The MALDI plate was pre-coated with α -cyano-4-hydroxycinnamic acid (CHCA) matrix, or alternatively, the matrix could be mixed with the sample on chip prior to spotting. The system was optimized and continuous deposition at low flow rates through the bioreactor was found to be optimal for highest sequence coverage.

There is much interest in hormone secretions from islets of Langerhans due to defective secretion being hallmarks of type I and II diabetes. While temporally resolved secretion profiles from islets have been measured using a variety of methods on microfluidic chips [59–62], there have been few reports demonstrating the ability to measure multiple hormones simultaneously [62]. To address this, Bllaci *et al.* utilized surface acoustic waves (SAW) to sample hormone release from islets of Langerhans [63]. The SAW atomizer consisted of a piezoelectric substrate, single crystal lithium niobate, with two sputtered chromium-aluminum single-phase unidirectional transducer (SPUDT) electrodes (Figure 20). Application of a voltage waveform to the SPUDT electrodes produced a traveling SAW that made aerosol droplets from the liquid sample, which were then masked by a pinhole and

collected on a MALDI plate. The methodology was applied to analyze secretions from islets placed directly, or via a membrane spacer, on the piezoelectric substrate. The limit of detection (LOD) of insulin was 100 amol, and the authors stated that the signal-to-noise (S/N) could be improved by an order of magnitude with the addition of transferrin to the matrix. A number of peptides secreted from islets were observed including insulin, C-peptide, glucagon, amylin, somatostatin, and glucagon-like peptide 1.

3.2 MALDI-MS from analog microfluidic devices

A microfluidic LC device (Figure 21) was developed that used EOF pumps to generate flow through 10–20 mm long LC channels packed with C18 resin [64]. Field strengths between 1000–2000 V/cm in the EOF pumping channels were utilized to produce flow rates from 30–100 nL/min in the LC channel. The EOF pumps were used to perform elution of tryptic peptides in the LC column using a step gradient. Separation was deemed complete when the first eluted compound reached the end of the LC column, leaving a distribution of sample components along the separation column. The voltage to the EOF pumps was then removed, turning off the flow rate in the LC column. Hundreds of small diameter channels running perpendicular to the LC channel were then used to elute the components from the LC column into analysis reservoirs with each reservoir sampling 2 mm of the LC separation channel. Finally, the reservoirs were dried and 0.5 μ L of CHCA matrix was added for MALDI analysis. The authors demonstrated this system using a sample containing a mixture of peptides digested from 13 proteins and while most peptides were spread through only 2–3 collection reservoirs, the data did allow identification of all 13 proteins and the authors stated that this method showed promise for proteomic applications. In a sample of a cell extract from MCF-10A breast epithelial cells, analysis of a 1 μ L sample, at 2 μ g/ μ L, enabled identification of 205 proteins. The authors stated that while the LC separation was not ideal, the chip could be used as a prefractionation device.

In another report, solution phase isoelectric focusing (sIEF) was integrated with MALDI analysis [65]. Protein or microdialysate samples were separated on a glass “SlipChip” by sIEF resulting in enhancement of sample concentrations by more than 50-fold. Once the separation and concentration enhancement were finished, the two plates were slipped, cutting the focused bands into reservoirs on the device. The spots were then frozen, the device disassembled, matrix applied to the spots, and interrogated by MALDI. The integration of sIEF with MS allowed the authors to determine the pI of the protein, with the smallest difference in pI of 0.07 pH units, as well as their identities.

The Sweedler group demonstrated a method for quantitation of analyte concentration using MALDI to image adsorption of the analyte along a length of a PDMS microfluidic channel [66]. They demonstrated through simulations and experiments that the length of the channel containing analyte was linearly proportional to the concentration of analyte (Figure 22A). This methodology was used to analyze the secretion of acidic peptide (AP) and α -bag cell peptide (α BCP) secretions from *Aplysia californica* bag cell neurons. To minimize sample loss in the cell culture chamber while maximizing sample adsorption in the analysis channel, the sample reservoir was coated with 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (OEG) and the analysis channel was coated with OTS. These coatings were essential for

enhanced quantitation of the peptide secretions with minimal adsorption of the analytes in the culture chamber. The *Aplysia* samples were placed in the OEG reservoir and solution withdrawn through the OTS-coated channel by a syringe pump. Flow rates of 0.1 $\mu\text{L}/\text{min}$ were utilized for a channel dimension of 200 μm wide $\times$ 50 μm deep. Once the sampling was finished, the complementary glass slide was peeled off, the PDMS channels dried, and the matrix, 2,5-dihydroxybenzoic acid (DHB), was sprayed on. Finally, MALDI imaging was used to determine the length of coverage and to relate it to analyte concentration (Figures 22B and 22C). Results demonstrated an LOD of 600 and 400 fmol for AP and αBCP , respectively.

3.3 MALDI-MS from droplet or digital microfluidic devices

Recently, a method was published which demonstrated the coupling of droplet-based microfluidics with MALDI [67]. Aqueous reagents were mixed and 3 nL droplets were formed in volatile perfluorinated oil. A capillary was used to direct droplets from a microfluidic T-junction onto a MALDI plate mounted on a motorized x-y stage (Figure 23A). The plate had been previously coated with Teflon and hydrophilic spots produced via laser ablation to capture the aqueous droplets. Droplet placement onto the hydrophilic spots was aided by optical detection of the droplets just before exiting the capillary with reliable droplet deposition rates up to 7 Hz achieved. The aqueous droplets remained on the hydrophilic regions and both the oil and aqueous solvent was allowed to evaporate. The MALDI matrix, DHB, was then applied to the dried droplets. The use of this methodology allowed the enzymatic conversion of angiotensin II to angiotensin I to be monitored over time with each droplet/MALDI spot corresponding to a different time point in the enzymatic reaction over the course of 3 hours (Figure 23B). Similar to the Kennedy group's work coupling droplet microfluidics to ESI-MS, this methodology was not performed on a conventional microfluidic system, but again, it presents a simple and promising method for coupling microfluidic systems to MALDI. In a different manuscript, the authors applied this methodology to collect fractions from nano-LC prior to MALDI-MS detection [68].

Digital microfluidic methodologies have had great success in coupling to MALDI due to ease with which the sample can be analyzed, typically utilizing the DMF device as the MALDI plate. In one report, a DMF device was developed for collecting fractions from a CE separation for analysis by MALDI [69]. Eluent from the CE was collected into droplets of background electrolyte or MALDI matrix at the end of the capillary. The droplets were then collected onto a DMF device, which consisted of a fluorinated food wrap placed over the DMF board. The food wrap was removed at the end of fraction collection and transferred to a MALDI plate with the dried samples intact for analysis. This method allowed the authors to successfully separate, fraction collect, and identify 4 standard peptides (angiotensin, adrenocorticotrophic hormone (ACTH), bradykinin, and insulin).

DMF can also be used for automating complex sample preprocessing steps. In a paper by Chatterjee *et al.* [70], a DMF system was used to automate various proteomic sampling steps such as disulfide reduction, alkylation, enzymatic digestion, and addition of MALDI matrix. These steps were followed by MS analysis directly from the DMF device. Carbonic anhydrase, ubiquitin, and cytochrome c were digested on chip utilizing a trypsin solution,

co-crystallized with DHB, and yielded a MALDI-MS spectrum sufficient for protein identification. Disulfide reduction and alkylation was demonstrated with bovine insulin, where the A- and B-chains were readily detected. The authors also addressed a problem with using high concentration protein solutions on DMF devices, which can precipitate and inhibit droplet movement. 5 μ M BSA was difficult to move, but an off-chip 100-fold dilution to 0.05 μ M allowed easy droplet movement at the cost of S/N in the mass spectrum. The authors also investigated the use of different surfactants to aid movement of protein solutions. Pluronic F-127 and n-octyl β -D-glucopyranoside (OBG) were used and while both aided in droplet movement, only OBG resulted in a movable droplets and peptide fragment detection in the MS spectrum. In addition, different coatings on the top and bottom DMF plates were tested for highest MS sensitivity. The authors found that it was ideal for the top plate to be coated in Teflon to minimize protein and analyte adsorption when the top plate was removed. However, it was more ideal for the bottom plate, which contained the DMF electrodes and is normally coated in Teflon, to have the Teflon removed leaving a layer of the dielectric, Parylene-C, on the electrode where the crystallization was performed.

Later the same year, Nelson *et al.* [71] demonstrated protein processing on a DMF device with integrated temperature control to yield more efficient peptide digestion and accelerated droplet evaporation. To allow temperature control within the device, one of the square actuation electrodes was replaced with a patterned multifunctional electrode site that could be used for droplet movement, a heater, or as a thermistor temperature sensor. Importantly, the authors also integrated hydrophilic regions around the heater, which helped to maintain the position of droplets during heating steps. Digestion and disulfide reduction were tested at different temperatures on cytochrome c and insulin, respectively. Insulin reduction was performed by merging a droplet of insulin and a reducing reagent followed by heating at 22, 30, 40, 50, 60, and 70 $^{\circ}$ C on the multifunctional electrode. After allowing the reduction to occur, the reaction was quenched and mixed with DHB. Increased reduction efficiency was observed in the MALDI spectrum at the higher temperatures, with 70 $^{\circ}$ C having the most optimal signal. Proteolytic digestion was performed by mixing a trypsin droplet with cytochrome c and comparing digestion between room temperature and 40 $^{\circ}$ C. The room temperature reaction showed 15% sequence coverage, while the 40 $^{\circ}$ C showed 74% sequence coverage.

Expanding further on the idea of utilizing DMF devices for sample processing for MALDI-MS analysis, Luk *et al.* demonstrated the first successful use of hydrogel microreactors for enzymatic digestion on a DMF device [72]. A hydrogel disc was created and trypsin or pepsin was covalently attached to the discs. The discs were then placed on the microfluidic device and solutions of BSA or lysozyme were delivered to the gels and allowed to incubate. The digested peptides were drawn off of the DMF device using a ZipTip and eluted onto a MALDI plate using 100% acetonitrile. The efficacy of the digestion on the hydrogel disc digestion was compared to both high and low concentrations of trypsin in solution, 40 mg/ml and 0.2 mg/ml, respectively. The protein solution incubated in the low trypsin solution showed incomplete digestion, while the high concentration trypsin solution resulted in autolysis of the enzyme. The proteins digested in gel on the microfluidic device showed higher sequence coverage as well as a smaller standard deviation of that coverage between

runs (Figure 24). Although this was not a direct coupling of DMF to MALDI, the ability for protein processing on chip leaves the strong potential for the coupling of these processes in the future.

Due to matrix interference in MALDI, it can be difficult to perform low molecular weight analyses. To overcome this, matrix-free LDI can be performed. In one report of this method coupled with DMF [73], a conventional DMF electrode array was used as the base plate. The cover plate used a highly doped silicon substrate that was partially etched with an HF/AgNO₃ solution to produce a ~1 μm thick coating of silicon nanowires. These nanowires were then coated with OTS to produce a superhydrophobic surface. The OTS was removed in small regions leaving patches of superhydrophilic silicon nanowires. As aqueous droplets passed by these patches, a volume of ~100 pL was left behind. The device was then opened and the silicon nanowire interface acted as the inorganic target for LDI. Using the setup, the authors were able to detect [Des-Arg1]-bradykinin ($m/z = 904.5$ Da) at concentrations from 10 – 50 fmol μL⁻¹.

4. Microfluidics coupled to other ionization methods

There have been several reports of microfluidic devices coupled to ionization schemes other than ESI or MALDI. One of the attractive methods for coupling microfluidics to MS is using paper as a sample delivery substrate [74]. The use of paper gives advantages such as low cost, portability and disposable sample cartridges. Recently, SAW was used to transfer analytes from paper into a mass spectrometer [75]. A polyester-cellulose paper was used as a wick to draw sample from a reservoir. The liquid moving across the paper then encountered the high order SAW vibrations produced by a pair of gold-chromium SPUDT electrodes (Figure 25A). The acoustic energy produced micrometer-sized charged aerosol droplets from the sample, which then evaporated and contained gas phase ions. These ions were directed to the MS inlet under ambient condition. This paper-based SAW device was used to detect the presence of caffeine and fluorouracil in whole blood (Figure 25B) and plasma. The device was also used for detection of heavy metals in a water sample.

Another work demonstrated offline coupling of droplet-based microfluidics to paper-based ionization MS [76]. In this work, a 3-layer microfluidic device was used to examine the kinetics of acetylcholine hydrolysis using potassium hydroxide (KOH). The top glass layer had a circular groove patterned around the inlet access hole as well as flow guide channels. The bottom layer was a piece of unpatterned glass, and the middle layer was a Teflon membrane that was used as spacer (Figure 26A). To generate droplets, the device was held vertically and buffer was flown into the inlet access hole. The buffer was confined within the circular groove due to the surface tension at the air-liquid interface until it reached a critical volume and broke off forming ~0.5 μL droplets. The droplets moved downwards inside the guide channels by gravity, which avoided the use of any carrier liquid such as oil. The hydrolysis reaction was initiated by merging acetylcholine and KOH droplets with the length of the guide lanes controlling the reaction time. The reaction was stopped by merging a droplet containing a quencher, and the final droplet was collected by chromatography paper at the end of the guide channel (Figure 26B). A 3 kV voltage was applied to the paper to ionize the contents into the MS inlet (Figure 26C). Using KOH at various concentrations

and measuring the amount of acetylcholine to choline hydrolysis, the second-order rate constant was extracted from the mass spectra data and shown to be $14.71 \text{ M}^{-1} \text{ s}^{-1}$, which the authors stated was larger than the hydrolysis rate constant in water.

The Girault group utilized their electrostatic-spray ionization technique to selectively ionize aqueous droplets in an oil carrier phase [77]. A polyimide microfluidic device was used and channels fabricated by laser ablation. A $50 \mu\text{m}$ diameter “spyhole” was drilled through the top of the device and an ion transfer capillary to the MS was aligned above the spyhole. Aqueous droplets were generated at a rate of 0.1 Hz and transferred into the device via a capillary. When the droplets passed by the spyhole, an electrode under the device applied a 10 Hz, 8 kV square waves, which ionized the droplets (Figure 27). The voltage caused an electrostatic charge on the droplet thereby ionizing the low viscosity solution (water) while the oil continued to flow to the microchip outlet. This approach eliminated the need for a dilution step or an oil removal step and was used to study tryptic digestion of cytochrome c and ACTH, as well as the reaction between β -lactoglobulin and α -tocopheryl acetate.

In a different approach to ionize samples, a microfluidic device capable of generating a micro-plasma was developed for real-time chemical reaction analysis by MS [78]. Two pieces of glass with patterned electrodes were used to sandwich UV-curable glue. UV exposure through a photomask cured the glue into the desired channel pattern while also sealing the device (Figure 28). The electrodes were not damaged because there were no high temperature steps required during the fabrication process. Plasma was generated by applying an AC signal of 600V at 25 kHz across the electrodes in the presence of helium gas. The authors demonstrated successful ionization and detection of caffeine, as well as measuring an online Fisher esterification of ethanol.

5. Conclusions

It has been approximately two decades since microfluidic chips were first coupled with mass spectrometers. This review discussed recent developments in online coupling based on various ionization sources including ESI, MALDI, SAW nebulization, and paper spray. ESI, due to its ease in interfacing, continues to be the most widely developed and applied technique among the ionization techniques. For a new user to the field, the choice of which of these methods to choose from should be guided by the scientific question at hand. As is often the case in analytical chemistry, each method has its own advantages and disadvantages where the user must balance factors such as cost, resolution, sensitivity, and ease of use.

Many reports in the literature demonstrate how microfluidics can be used to automate sample processing of complex samples prior to information-rich analysis by MS. Novel coupling strategies are still being developed and many more are still possible. For example, methods that can be easily integrated into the microfluidic architecture are ideal candidates for continued development. One model methodology is SAW, which can be used to manipulate particles and fluids as well as nebulize samples, indicating great potential for continued development [79, 80]. Lastly, the use of miniaturized mass analyzers [81] with microfluidics would greatly increase the possibility of on-site analysis.

Acknowledgments

This work was supported by a grant from the National Institutes of Health (R01 DK080714).

References

1. Quake SR, Scherer A. From micro- to nanofabrication with soft materials. *Science*. 2000; 290(5496):1536–1540. [PubMed: 11090344]
2. Haeberle S, Zengerle R. Microfluidic platforms for lab-on-a-chip applications. *Lab Chip*. 2007; 7(9):1094–1110. [PubMed: 17713606]
3. Khandurina J, Guttman A. Bioanalysis in microfluidic devices. *J Chromatogr A*. 2002; 943(2):159–183. [PubMed: 11833638]
4. Roman GT, Kennedy RT. Fully integrated microfluidic separations systems for biochemical analysis. *J Chromatogr A*. 2007; 1168:1–2. 170–188.
5. Hong J, Edel JB, deMello AJ. Micro- and nanofluidic systems for high-throughput biological screening. *Drug Discov Today*. 2009; 14:3–4. 134–146.
6. Johnson ME, Landers JP. Fundamentals and practice for ultrasensitive laser-induced fluorescence detection in microanalytical systems. *Electrophoresis*. 2004; 25(21–22):3513–3527. [PubMed: 15565706]
7. Wang J. Electrochemical detection for microscale analytical systems: a review. *Talanta*. 2002; 56(2):223–231. [PubMed: 18968498]
8. Gao D, Liu H, Jiang Y, Lin J-M. Recent advances in microfluidics combined with mass spectrometry: Technologies and applications. *Lab Chip*. 2013; 13(17):3309–3322. [PubMed: 23824006]
9. Kirby AE, Wheeler AR. Digital microfluidics: An emerging sample preparation platform for mass spectrometry. *Anal Chem*. 2013; 85(13):6178–6184. [PubMed: 23777536]
10. Lee J, Soper SA, Murray KK. Microfluidics with MALDI analysis for proteomics – A review. *Anal Chim Acta*. 2009; 649(2):180–190. [PubMed: 19699392]
11. Sikanen T, Franssila S, Kauppila TJ, Kostiaainen R, Kotiaho T, Ketola RA. Microchip technology in mass spectrometry. *Mass Spectrom Rev*. 2010; 29(3):351–391. [PubMed: 19514079]
12. Ohla S, Belder D. Chip-based separation devices coupled to mass spectrometry. *Curr Opin Chem Biol*. 2012; 16:3–4. 453–459. [PubMed: 22387134]
13. Mellors JS, Gorbounov V, Ramsey RS, Ramsey JM. Fully integrated glass microfluidic device for performing high-efficiency capillary electrophoresis and electrospray ionization mass spectrometry. *Anal Chem*. 2008; 80(18):6881–6887. [PubMed: 18698800]
14. Xue Q, Foret F, Dunayevskiy YM, Zavracky PM, McGruer NE, Karger BL. Multichannel microchip electrospray mass spectrometry. *Anal Chem*. 1997; 69(3):426–430. [PubMed: 9030055]
15. Rohner TC, Rossier JS, Girault HH. Polymer microspray with an integrated thick-film microelectrode. *Anal Chem*. 2001; 73(22):5353–5357. [PubMed: 11816559]
16. Ramsey RS, Ramsey JM. Generating electrospray from microchip devices using electroosmotic pumping. *Anal Chem*. 1997; 69(6):1174–1178.
17. Mellors JS, Black WA, Chambers AG, Starkey JA, Lacher NA, Ramsey JM. Hybrid capillary/microfluidic system for comprehensive online liquid chromatography-capillary electrophoresis electrospray ionization-mass spectrometry. *Anal Chem*. 2013; 85(8):4100–4106. [PubMed: 23477683]
18. Batz NG, Mellors JS, Alarie JP, Ramsey JM. Chemical vapor deposition of aminopropyl silanes in microfluidic channels for highly efficient microchip capillary electrophoresis-electrospray ionization-mass spectrometry. *Anal Chem*. 2014; 86(7):3493–3500. [PubMed: 24655020]
19. Fritzsche S, Ohla S, Glaser P, Giera DS, Sickert M, Schneider C, Belder D. Asymmetric organocatalysis and analysis on a single microfluidic nanospray chip. *Angew Chem Int Ed Engl*. 2011; 50(40):9467–9470. [PubMed: 21948447]
20. Nordman N, Sikanen T, Aura S, Tuomikoski S, Vuorensola K, Kotiaho T, Franssila S, Kostiaainen R. Feasibility of SU-8-based capillary electrophoresis-electrospray ionization mass spectrometry

- microfluidic chips for the analysis of human cell lysates. *Electrophoresis*. 2010; 31(22):3745–3753. [PubMed: 21077242]
21. Sikanen T, Tuomikoski S, Ketola RA, Kostiaainen R, Franssila S, Kotiaho T. Fully microfabricated and integrated SU-8-based capillary electrophoresis-electrospray ionization microchips for mass spectrometry. *Anal Chem*. 2007; 79(23):9135–9144. [PubMed: 17973354]
 22. Nordman N, Sikanen T, Moilanen ME, Aura S, Kotiaho T, Franssila S, Kostiaainen R. Rapid and sensitive drug metabolism studies by SU-8 microchip capillary electrophoresis-electrospray ionization mass spectrometry. *J Chromatogr A*. 2011; 1218(5):739–745. [PubMed: 21185563]
 23. Li X, Xiao D, Ou XM, McCullm C, Liu YM. A microchip electrophoresis-mass spectrometric platform for fast separation and identification of enantiomers employing the partial filling technique. *J Chromatogr A*. 2013; 1318:251–256. [PubMed: 24354006]
 24. Zhu Y, Pan JZ, Su Y, He QH, Fang Q. Fabrication of low-melting-point alloy microelectrode and monolithic spray tip for integration of glass chip with electrospray ionization mass spectrometry. *Talanta*. 2010; 81(3):1069–1075. [PubMed: 20298895]
 25. Sun X, Kelly RT, Tang K, Smith RD. Ultrasensitive nanoelectrospray ionization-mass spectrometry using poly(dimethylsiloxane) microchips with monolithically integrated emitters. *Analyst*. 2010; 135(9):2296–2302. [PubMed: 20617264]
 26. Sun X, Kelly RT, Tang K, Smith RD. Membrane-based emitter for coupling microfluidics with ultrasensitive nanoelectrospray ionization-mass spectrometry. *Anal Chem*. 2011; 83(14):5797–5803. [PubMed: 21657269]
 27. Sainiemi L, Sikanen T, Kostiaainen R. Integration of fully microfabricated, three-dimensionally sharp electrospray ionization tips with microfluidic glass chips. *Anal Chem*. 2012; 84(21):8973–8979. [PubMed: 23045954]
 28. Lu Y, Liu F, Lion N, Girault HH. Dual-channel electrospray microchip. *J Am Soc Mass Spectrom*. 2013; 24(3):454–457. [PubMed: 23385957]
 29. Chambers AG, Ramsey JM. Microfluidic dual emitter electrospray ionization source for accurate mass measurements. *Anal Chem*. 2012; 84(3):1446–1451. [PubMed: 22220742]
 30. Mats L, Gibson GT, Oleschuk RD. Plastic LC/MS microchip with an embedded microstructured fibre having the dual role of a frit and a nanoelectrospray emitter. *Microfluid Nanofluid*. 2014; 16:1–2. 73–81.
 31. Su S, Gibson GT, Mugo SM, Marecak DM, Oleschuk RD. Microstructured photonic fibers as multichannel electrospray emitters. *Anal Chem*. 2009; 81(17):7281–7287. [PubMed: 19715359]
 32. Liuni P, Rob T, Wilson DJ. A microfluidic reactor for rapid, low-pressure proteolysis with on-chip electrospray ionization. *Rapid Commun Mass Spectrom*. 2010; 24(3):315–320. [PubMed: 20049884]
 33. Rob T, Liuni P, Gill PK, Zhu S, Balachandran N, Berti PJ, Wilson DJ. Measuring dynamics in weakly structured regions of proteins using microfluidics-enabled subsecond H/D exchange mass spectrometry. *Anal Chem*. 2012; 84(8):3771–3779. [PubMed: 22458633]
 34. Rob T, Gill PK, Golemi-Kotra D, Wilson DJ. An electrospray ms-coupled microfluidic device for sub-second hydrogen/deuterium exchange pulse-labelling reveals allosteric effects in enzyme inhibition. *Lab Chip*. 2013; 13(13):2528–2532. [PubMed: 23426018]
 35. Wang C, Jemere AB, Harrison DJ. Multifunctional protein processing chip with integrated digestion, solid-phase extraction, separation and electrospray. *Electrophoresis*. 2010; 31(22):3703–3710. [PubMed: 20967777]
 36. Hua Y, Jemere AB, Harrison DJ. On-chip solid phase extraction and enzyme digestion using cationic PolyE-323 coatings and porous polymer monoliths coupled to electrospray mass spectrometry. *J Chromatogr A*. 2011; 1218(26):4039–4044. [PubMed: 21616495]
 37. Gao D, Li H, Wang N, Lin JM. Evaluation of the absorption of methotrexate on cells and its cytotoxicity assay by using an integrated microfluidic device coupled to a mass spectrometer. *Anal Chem*. 2012; 84(21):9230–9237. [PubMed: 23039268]
 38. Wei H, Li H, Mao S, Lin JM. Cell signaling analysis by mass spectrometry under coculture conditions on an integrated microfluidic device. *Anal Chem*. 2011; 83(24):9306–9313. [PubMed: 22022860]

39. Mao S, Zhang J, Li H, Lin JM. Strategy for signaling molecule detection by using an integrated microfluidic device coupled with mass spectrometry to study cell-to-cell communication. *Anal Chem*. 2013; 85(2):868–876. [PubMed: 23240962]
40. Gao D, Liu H, Lin J-M, Wang Y, Jiang Y. Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device. *Lab Chip*. 2013; 13(5):978–985. [PubMed: 23340920]
41. Petersen NJ, Foss ST, Jensen H, Hansen SH, Skonberg C, Snakenborg D, Kutter JP, Pedersen-Bjergaard S. On-chip electro membrane extraction with online ultraviolet and mass spectrometric detection. *Anal Chem*. 2011; 83(1):44–51. [PubMed: 21142026]
42. Petersen NJ, Pedersen JS, Poulsen NN, Jensen H, Skonberg C, Hansen SH, Pedersen-Bjergaard S. On-chip electromembrane extraction for monitoring drug metabolism in real time by electrospray ionization mass spectrometry. *Analyst*. 2012; 137(14):3321–3327. [PubMed: 22679624]
43. Zhu Y, Fang Q. Analytical detection techniques for droplet microfluidics-a review. *Anal Chim Acta*. 2013; 787(17):24–35. [PubMed: 23830418]
44. Li Q, Pei J, Song P, Kennedy RT. Fraction collection from capillary liquid chromatography and off-line electrospray ionization mass spectrometry using oil segmented flow. *Anal Chem*. 2010; 82(12):5260–5267. [PubMed: 20491430]
45. Zhu Y, Fang Q. Integrated droplet analysis system with electrospray ionization-mass spectrometry using a hydrophilic tongue-based droplet extraction interface. *Anal Chem*. 2010; 82(19):8361–8366. [PubMed: 20806885]
46. Pei J, Li Q, Lee MS, Valaskovic GA, Kennedy RT. Analysis of samples stored as individual plugs in a capillary by electrospray ionization mass spectrometry. *Anal Chem*. 2009; 81(15):6558–6561. [PubMed: 19555052]
47. Sun S, Slaney TR, Kennedy RT. Label free screening of enzyme inhibitors at femtomole scale using segmented flow electrospray ionization mass spectrometry. *Anal Chem*. 2012; 84(13):5794–5800. [PubMed: 22656268]
48. Song P, Hershey ND, Mabrouk OS, Slaney TR, Kennedy RT. Mass spectrometry “sensor”. for in vivo acetylcholine monitoring. *Anal Chem*. 2012; 84(11):4659–4664. [PubMed: 22616788]
49. Ji J, Nie L, Qiao L, Li Y, Guo L, Liu B, Yang P, Girault HH. Proteolysis in microfluidic droplets: an approach to interface protein separation and peptide mass spectrometry. *Lab Chip*. 2012; 12(15):2625–2629. [PubMed: 22695710]
50. Wheeler AR. Chemistry: Putting electrowetting to work. *Science*. 2008; 322(5901):539–540. [PubMed: 18948529]
51. Jebail MJ, Yang H, Mudrik JM, Lafreniere NM, McRoberts C, Al-Dirbashi OY, Fisher L, Chakraborty P, Wheeler AR. A digital microfluidic method for dried blood spot analysis. *Lab Chip*. 2011; 11(19):3218–3224. [PubMed: 21869989]
52. Baker CA, Roper MG. Online coupling of digital microfluidic devices with mass spectrometry detection using an eductor with electrospray ionization. *Anal Chem*. 2012; 84(6):2955–2960. [PubMed: 22384846]
53. Shih SC, Yang H, Jebail MJ, Fobel R, McIntosh N, Al-Dirbashi OY, Chakraborty P, Wheeler AR. Dried blood spot analysis by digital microfluidics coupled to nanoelectrospray ionization mass spectrometry. *Anal Chem*. 2012; 84(8):3731–3738. [PubMed: 22413743]
54. Kirby AE, Wheeler AR. Microfluidic origami: A new device format for in-line reaction monitoring by nanoelectrospray ionization mass spectrometry. *Lab Chip*. 2013; 13(13):2533–2540. [PubMed: 23412052]
55. Garden RW, Sweedler JV. Heterogeneity within MALDI samples as revealed by mass spectrometric imaging. *Anal Chem*. 2000; 72(1):30–36. [PubMed: 10655631]
56. Tsao CW, Tao S, Chen CF, Liu JK, DeVoe DL. Interfacing microfluidics to LDI-MS by automatic robotic spotting. *Microfluid Nanofluid*. 2010; 8(6):777–787.
57. Lee J, Soper SA, Murray KK. A solid-phase bioreactor with continuous sample deposition for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spec*. 2011; 25(6):693–699.

58. McCarley RL, Vaidya B, Wei S, Smith AF, Patel AB, Fend J, Murphy MC, Soper SA. Resist-free patterning of surface architectures in polymer-based microanalytical devices. *J Am Chem Soc.* 2005; 127(3):842–843. [PubMed: 15656615]
59. Godwin LA, Pilkerton ME, Deal KS, Wanders D, Judd RL, Easley CJ. A passively operated microfluidic device for stimulation and secretion sampling of single pancreatic islets. *Anal Chem.* 2011; 83:7166–7172. [PubMed: 21806019]
60. Dishinger JF, Reid KR, Kennedy RT. Quantitative monitoring of insulin secretion from single islets of Langerhans in parallel on a microfluidic chip. *Anal Chem.* 2009; 81(8):3119–3127. [PubMed: 19364142]
61. Easley CJ, Rocheleau JV, Head WS, Piston DW. Quantitative measurement of zinc secretion from pancreatic islets with high temporal resolution using droplet-based microfluidics. *Anal Chem.* 2009; 81(21):9086–9095. [PubMed: 19874061]
62. Lomasney AR, Yi L, Roper MG. Simultaneous monitoring of insulin and islet amyloid polypeptide secretion from islets of Langerhans on a microfluidic device. *Anal Chem.* 2013; 85(16):7919–7925. [PubMed: 23848226]
63. Bllaci L, Kjellstrom S, Eliasson L, Friend JR, Yeo LY, Nilsson S. Fast surface acoustic wave-matrix-assisted laser desorption ionization mass spectrometry of cell response from islets of Langerhans. *Anal Chem.* 2013; 85(5):2623–2629. [PubMed: 23384197]
64. Lazar IM, Kabulski JL. Microfluidic LC device with orthogonal sample extraction for on-chip MALDI-MS detection. *Lab Chip.* 2013; 13(11):2055–2065. [PubMed: 23592150]
65. Wang S, Chen S, Wang J, Xu P, Luo Y, Nie Z, Du W. Interface solution isoelectric focusing with in situ MALDI-TOF mass spectrometry. *Electrophoresis.* 2014; 1002/elps.201400083
66. Zhong M, Lee CY, Croushore CA, Sweedler JV. Label-free quantitation of peptide release from neurons in a microfluidic device with mass spectrometry imaging. *Lab Chip.* 2012; 12(11):2037–2045. [PubMed: 22508372]
67. Küster SK, Fagerer SR, Verboket PE, Eyer K, Jefimovs K, Zenobi R, Dittrich PS. Interfacing droplet microfluidics with matrix-assisted laser desorption/ionization mass spectrometry: Label-free content analysis of single droplets. *Anal Chem.* 2013; 85(3):1285–1289. [PubMed: 23289755]
68. Küster SK, Pabst M, Jefimovs K, Zenobi R, Dittrich PS. High-resolution droplet-based fractionation of nano-LC separations onto microarrays for MALDI-MS analysis. *Anal Chem.* 2014; 86(10):4848–4855. [PubMed: 24725135]
69. Gorbatoeva J, Borissova M, Kaljurand M. Electrowetting on dielectric actuation of droplets with capillary electrophoretic zones for MALDI mass spectrometric analysis. *Electrophoresis.* 2012; 33(17):2682–2688. [PubMed: 22965712]
70. Chatterjee D, Ytterberg AJ, Son SU, Loo JA, Garrell RL. Integration of protein processing steps on a droplet microfluidics platform for MALDI-MS analysis. *Anal Chem.* 2010; 82(5):2095–2101. [PubMed: 20146460]
71. Nelson WC, Peng I, Lee GA, Loo JA, Garrell RL, Kim CJ. Incubated protein reduction and digestion of an electrowetting-on-dielectric digital microfluidic chip for MALDI-MS. *Anal Chem.* 2010; 82(23):9932–9937. [PubMed: 21058643]
72. Luk VN, Fiddes LK, Luk VM, Kumacheva E, Wheeler AR. Digital microfluidic hydrogel microreactors for proteomics. *Proteomics.* 2012; 12(9):1310–1318. [PubMed: 22589180]
73. Lapierre F, Piret G, Drobecq H, Melnyk O, Coffinier Y, Thomy V, Boukherroub R. High sensitive matrix-free mass spectrometry analysis of peptides using silicon nanowires-based digital microfluidic device. *Lab Chip.* 2011; 11(9):1620–1628. [PubMed: 21423926]
74. Liu J, Wang H, Manicke NE, Lin J-M, Cooks RG, Ouyang Z. Development, characterization, and application of paper spray ionization. *Anal Chem.* 2010; 82(6):2463–2471. [PubMed: 20158226]
75. Ho J, Tan MK, Go DB, Yeo LY, Friend JR, Chang HC. Paper-based microfluidic surface acoustic wave sample delivery and ionization source for rapid and sensitive ambient mass spectrometry. *Anal Chem.* 2011; 83(9):3260–3266. [PubMed: 21456580]
76. Zhang Y, Li H, Ma Y, Lin J-M. Paper spray mass spectrometry-based method for analysis of droplets in a gravity-driven microfluidic chip. *Analyst.* 2014; 139(5):1023–1029. [PubMed: 24432351]

77. Gasilova N, Yu Q, Qiao L, Girault HH. On-chip spyhole mass spectrometry for droplet-based microfluidics. *Angew Chem Int Ed Engl.* 2014; 53(17):4408–4412. [PubMed: 24643919]
78. Kuoa S-M, Yanga C-C, Shieab J, Lina C-H. A post-bonding-free fabrication of integrated microfluidic devices for mass spectrometry applications. *Sens Actuators B.* 2011; 156(1):156–161.
79. Ding X, Li P, Lin S-CS, Stratton ZS, Nama N, Guo F, Slotcavage D, Mao X, Shi J, Costanzo F, Huang TJ. Surface acoustic wave microfluidics. *Lab Chip.* 2013; 13(18):3626–3649. [PubMed: 23900527]
80. Yeo LY, Friend JR. Surface acoustic wave microfluidics. *Annu Rev Fluid Mech.* 2014; 46:379–406.
81. Tian Y, Higgs J, Li AL, Barney B, Austin DE. How far can ion trap miniaturization go? Parameter scaling and space-charge limits for very small cylindrical ion traps. *J Mass Spec.* 2014; 49(3):233–240.

Highlights

- Review of coupling and applications of microfluidic-MS
- Covers the years 2010–2014
- Subdivides by types of ionization methods and microfluidic systems

Figure 1. Hybrid capillary LC microfluidic CE-ESI

A capillary LC system is connected to the microfluidic device as shown by the orange line. The electrical connections to control the microfluidic CE-ESI system are shown by the green lines. To compensate for flow rate differences between the LC and CE system, the majority of the LC eluent was split to waste via the split flow channel. Reprinted with permission from [17]. Copyright (2013) American Chemical Society.

Figure 2. Microfluidic system for high-resolution chiral separations

The separation channel was filled with sulfated cyclodextrins (Sulfated CD) and as analytes migrated through the plug they became separated. Cells could be cultured above the sample inlet reservoir using a 0.22 μm membrane. Reprinted with permission from [23]. Copyright (2013) Elsevier.

Figure 3. Images of the microchip with integrated low melting point alloy electrode
 (A) Channel lay out on the photomask with 160° turning angle on the electrode channel. (B) Charge coupled device was used to image the ESI emitter tip showing the integrated electrode. (C) Photograph of the fabricated device where the electrode channel was connected to the power supply through the red wire. Adapted with permission from [24]. Copyright (2010) Elsevier.

Figure 4. Schematic of fabrication of PDMS membrane based ESI emitter

(A) The four layers of PDMS are shown with the PDMS slabs (labeled 1 and 4) and membranes with and without microstructures (labeled 2 and 3). (B) Enclosing the fluid channel with the two inner membranes and bonding to the top PDMS slab. (C) The extruded membrane is cut into a triangular shape. (D) The bottom PDMS slab is bonded to provide further support to the PDMS membrane. (E) Top view and (F) side view of the emitter tip. Reprinted with permission from [26]. Copyright (2011) American Chemical Society.

Figure 5. Three-dimensional glass emitter tips
(A) Zoomed in and (B) side view of the emitter tip. (C) Schematic of the CE-ESI microchip (dimensions not to scale). Adapted with permission from [27]. Copyright (2012) American Chemical Society.

Figure 6. Microchip with dual channel ESI emitter

(A) The schematic of the microfluidic polyimide device. (B) Cross section images of different inter-channel separation distances (D_s) between two microchannels at the emitter tip: (a) $D_s = 29 \pm 1 \mu\text{m}$; (b) $D_s = 15 \pm 1 \mu\text{m}$; (c) $D_s = 9 \pm 1 \mu\text{m}$. Adapted with permission from [28]. Copyright (2013) Springer.

Figure 7. Operation of the CE-ESI dual emitter microchip

Images of electrosprays (green plumes) generated from (A) analyte emitter and (B) reference emitter. Adapted with permission from [29]. Copyright (2012) American Chemical Society.

Figure 8. Scanning electron microscope image of MSF

The silica MSF shown here has 54 parallel channels and was used as both a frit to retain the LC packing material and for electrospray. Adapted with permission from [30]. Copyright (2013) Springer.

Figure 9. Microfluidic devices for proteomic applications

(A) A photo of the microfluidic reactor for on-chip protein digestion. The inlet is split into multiple channels which flow into the microreactor containing the pepsin-agarose beads. Eluent is then delivered to the MS inlet via the capillary shown on the right. Reprinted with permission from [32]. Copyright (2010) John Wiley and Sons. (B) Schematic of the device used for HDX pulse labeling. Cartoons of chemical structures at each stage are shown at top. Processes of inhibitor binding, D₂O pulse labeling, acid quenching, mixing of the reagents, pepsin digestion, and ESI are shown from left to right. The central capillary can be pulled

back to allow different mixing times of the protein with inhibitor prior to pulse labeling.
Reprinted with permission from [34]. Copyright (2013) Royal Society of Chemistry.

Figure 10. Multifunctional protein processing chip

The top image shows the microfluidic device used for performing tryptic digestion, SPE, electrophoresis and ESI-MS. A zoomed in view of the integrated digestion and SPE bed is shown in the lower part. Both beds were 150 μm deep and 800 μm wide loaded with 40–60 μm diameter beads. Adapted with permission from [35]. Copyright (2010) John Wiley and Sons.

Figure 11. Integrated microfluidic devices for drug evaluation and cell signaling

(A) A schematic design of a microfluidic device integrated with a concentration gradient generator, cell culture, and SPE columns. Reprinted with permission from [37]. Copyright (2012) American Chemical Society. (B) Two cell culture chambers connected via a STP for controlling cell-to-cell communication. Reprinted with permission from [39]. Copyright (2013) American Chemical Society. (C) A Matrigel-coated polycarbonate membrane sandwiched between two PDMS layers. (D) Side view of the microfluidic device shown in

(C). A cell culture well was integrated in the top layer with microfluidic flow in the bottom layer. Reprinted with permission from [40]. Copyright (2013) Royal Society of Chemistry.

Figure 12. On-chip electromembrane extraction system

Voltage was applied across the SLM sandwiched between the sample and acceptor channels for selective extraction of metabolites allowing metabolism products to be monitored. Reprinted with permission from [42]. Copyright (2012) Royal Society of Chemistry.

Figure 13. Droplet analysis system integrated with ESI emitter

A droplet generation system was integrated with a droplet extraction interface via a hydrophilic tongue. The segmented flow channel was made hydrophobic by modification with OTS while the hydrophilic tongue and ESI carrier flow channel were hydrophilic. Reprinted with permission from [45]. Copyright (2010) American Chemical Society.

Figure 14. Schematic diagram of the protein analysis microfluidic platform

Fractions from an HPLC separation (inlet A) were mixed with trypsin (inlet B) and PFD oil (inlet C). The pH was adjusted through inlet D to modify the sample for ESI-MS detection. Reprinted with permission from [49]. Copyright (2012) Royal Society of Chemistry.

Figure 15. Fabrication and operation of a hybrid DMF-microchannel device

(A) Schematic of the channel layout showing the three layers used for fabrication. (B) Droplet movement during analysis of amino acids in dried blood spots: (a) blood sample derivatization and extraction, (b) extraction re-solubilization in MS solvent, (c) sample solution moved to spray microchannel. Adapted with permission from [51]. Copyright (2011) Royal Society of Chemistry.

J Chromatogr A. Author manuscript; available in PMC 2016 February 20.

Figure 17. Nanospray ESI-MS coupled to DMF

(A) Droplets were actuated by applying AC electric potentials between the top and bottom substrates. (B) NanoESI was generated by applying a DC electric potential between the top plate and the MS orifice. Adapted with permission from [53]. Copyright (2012) American Chemical Society.

Figure 18. DMF-ESI/MS device with an integrated emitter

Schematic of the DMF device with the two-plate region performing microscale chemical processes, and the one-plate region delivering droplets to the emitter. Adapted with permission from [54]. Copyright (2013) Royal Society of Chemistry.

Figure 19. PMMA microfluidic device coupled to MALDI

A PMMA device containing an integrated trypsin bioreactor was used to automate MALDI deposition via either a continuous spotting mode (A) or a spot deposition mode (B).

Reprinted with permission from [57]. Copyright (2011) John Wiley and Sons.

Figure 20. Microfluidic device with SAW atomizer

SPUDT electrodes are shown as grey and orange blocks on the light grey piezoelectric substrate. **(A)** The setup is shown in non-operational mode with the sample droplet deposited on the substrate. **(B)** Application of voltage to the SPUDT electrodes generated SAWs shown as red waves. The produced aerosol was collected by a MALDI plate placed above a pinhole. Adapted with permission from [63]. Copyright (2013) American Chemical Society.

Figure 21. Microfluidic LC device with orthogonal extraction for MALDI imaging
EOF pumps generated a flow through the reversed phase separation channel. Separated components were then extracted orthogonally into collection reservoirs for MALDI detection. Adapted with permission from [64]. Copyright (2013) Royal Society of Chemistry.

Figure 22. MALDI-MS imaging of cell secretions

(A) The length of channel where AP was detected is shown as yellow. Each image shows a different concentration of AP as indicated. (B) Mass spectrum of AP detected in the channel. (C) Calibration curve for relating the channel coverage length to the concentration of analyte. Adapted with permission from [66]. Copyright (2012) Royal Society of Chemistry.

Figure 23. Droplet to MALDI coupling

(A) A schematic of the method used to form and deliver aqueous droplets to a MALDI plate.

(B) The difference in MALDI-MS signals from the start of the enzymatic reaction to the end of the monitoring period. (*angiotensin I, ‡ angiotensin II). Adapted with permission from [67]. Copyright (2013) American Chemical Society.

Figure 24. Hydrogel enzyme reactor on a DMF device

The figure is taken from a movie of the digestion reaction on a DMF device. **(A & B)** Delivering an intact protein sample to the hydrogel reactor. **(C)** Actively mixing the droplet over the hydrogel reactor by moving the droplet as shown by the red arrow. **(D)** Dispensing the droplet containing the digested proteins away from the hydrogel reactor. **(E)** Moving the digested proteins down the device for further processing. Adapted with permission from [72]. Copyright (2012) John Wiley and Sons.

Figure 25. Paper based device for interfacing SAW-MS

(A) SAWs are delivered from the left and interact with the sample from the paper-based delivery device on the right, producing aerosol droplets of sample. (B) Mass spectra of caffeine (left) and fluorouracil (right) detected from whole blood. Adapted with permission from [75]. Copyright (2011) American Chemical Society.

Figure 26. Droplet based microfluidic system coupled offline to MS using paper spray ionization (A) Schematic illustration of the experimental design. Droplets were collected at the bottom of the device by the paper substrate and analyzed by MS. (B) Photograph of microdroplet device with 2 reactants and 1 quenching solution. (C) Mass spectra of acetylcholine hydrolysis reaction with the relative amounts of the reactant, acetylcholine, and product, choline, shown. Adapted with permission from [76]. Copyright (2014) Royal Society of Chemistry.

Figure 27. Illustration of selective ionization of droplets

The aqueous sample droplet (blue circles) passing the spyhole were ionized by applying a high voltage pulse (0 to 8 kV) on the electrode below the spyhole while the carrier oil continued to the outlet. Reprinted with permission from [77]. Copyright (2014) John Wiley and Sons.

Figure 28. An integrated microfluidic system generating plasma for real-time MS detection
The picture shows the two sample inlet channels, the mixing channels, and the inlet for the He gas. The generated plasma is shown in the picture below. Reprinted with permission from [78]. Copyright (2011) Elsevier.