Review

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Chip-based microfluidic devices coupled with electrospray ionization-mass spectrometry

We present the current status of the development of microfluidic devices fabricated on different substrates for coupling with electrospray ionization-mass spectrometry (ESI-MS). Until now, much success has been gained in fabricating the ESI chips, which show better performances due to miniaturization when compared with traditional methods. Integration of multiple steps for sample preparation and ESI sample introduction, however, remains a great challenge. This review covers the main technical development of electrospray device that were published from 1997 to 2004. This article does not attempt to be exclusive. Instead, it focuses on the publications that illustrated the breath of the development and applications of microchip devices for MS-based analysis.

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Contents

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Abbreviations: LC, liquid chromatography; **MEMS**, microelectromechanical systems; **PC**, polycarbonate; **PDMS**, polydimethylsiloxane; **PI**, polymide; **PMMA**, polymethyl methacrylate

1 Introduction

Despite new advances in MS instrumentation techniques, the full characterization of real samples using MS or MS/MS techniques remains a formidable analytical challenge mainly due to the complexity of interfacing sample separation and MS detection. Tedious sample preparation procedures including separation and desalting are required for complicated samples before MS analysis. Microchip techniques have been developed to facilitate sample preparation and sample introduction for MS-based analysis. This development will maximize the capability of MS as well as reduce the required sample amount, analysis time, and the costs.

Using the technology of microelectromechanical systems (MEMSs) and various chemical methods, many interconnected microchannels (10–100 μm ID) can be fabricated on a microchip, which allows analytical procedures ranging from DNA separations [1–4], immunoassays [5–7], cell manipulations [8, 9], and PCR reactions [10] to be performed on a mini-apparatus. Not only can DNA analysis be performed on the mini apparatus, but also protein pretreatment, detection, and analysis can be performed on the microdevice. The most popular microfluidic devices so far are those for microchip electrophoresis. They utilize electroosmotic flow (EOF) to drive the fluid in the microchannel and a highly efficient separation comparable to capillary electrophoresis (CE) can be achieved within just one-tenth of the time required for CE. In order to couple the functions of microfluidic devices and MS, a

robust and stable interface is necessary. Two types of ionization interfaces, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), have been developed on commercial MS instruments. Most microfluidic devices, however, are coupled with nano-ESI interfaces since the fluidics of nano-ESI are more compatible with the fluidics in the microchannel. Although the ultimate goal of coupling microfluidic chips with MS is to use the microfluidic chips for fast and one-step sample preparation and subsequent on-line sample introduction for MS analysis, most microfluidic chips discussed so far only deal with sample separation followed by electrospray or with electrospray on-chip alone. There are certainly technical challenges associated with the development of total analysis on-chip. In this review article, the design and function of electrospray interfaces built for glass (or quartz) and polymer-based chips will be summarized and discussed. Also, integration of some other functions, such as protein digestion and preconcentration on the microfluidic device, which could enhance protein identification accuracy and ESI stability for proteomics analysis, are briefly introduced.

2 Coupling of microfabricated devices with ESI-MS

The ionization of macromolecules by electrospraying was first demonstrated by Dole *et al.* [11]. In 1984, Fenn and his co-workers first successfully combined the electrospray and mass instrument to do mass detection. Following this, a breakthrough came in 1988; Fenn showed that a molecular weight accuracy of 0.01% could be obtained by applying a signal-averaging method to the multiple ions formed in the ESI process [12]. For proteins or other kinds of molecules, ESI as a sample introduction interface is very important for getting their MS signal. Not only can it ionize samples from the liquid phase into the gas phase, but it also creates the multiple charges which are required to make the high-mass-value molecules, such as proteins, become detectable in lower mass-to-charge (*m/z*) windows [13]. Recently, it has been shown that microfluidic devices for manipulating liquid solution are highly compatible with the miniaturized ESI source for use in MS detection [14–16]. With the development of microfabrication technology, a number of designs for the electrospray emitter have been developed to optimize the coupling of the microfluidic device to MS.

2.1 Electrospray emitter on the blunt end of a microchip

In the early ages, MEMS was the main technology to fabricate microdevices with silica-based substrates, such as quartz and glass. The first ESI microdevice was built by this mature approach. In 1997, Ramsey and Ramsey [17] built an ESI glass chip with open exits at the end of the channel as the electrospray emitters. This design extended the applicability of the microdevice from fluorescent to mass detection. At the same time, as shown in Fig. 1, Karger's group [18] developed a similar device but with

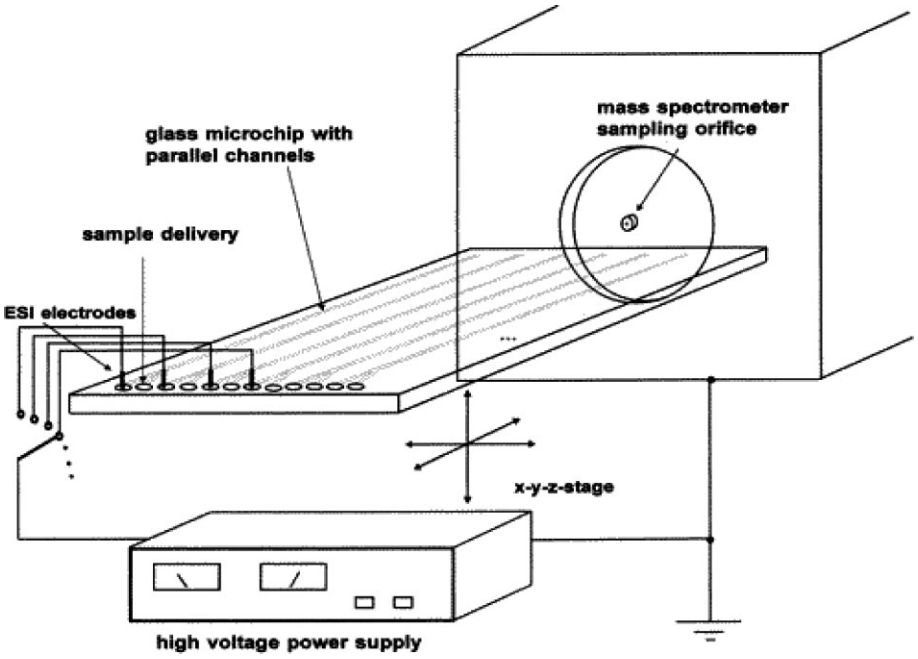

Figure 1. Schematic diagram of the microchip ESI-MS interface. The voltage for ESI was sequentially applied to one well of each channel containing buffer and a platinum electrode. The second well of each channel contained sample and was connected to a syringe pump to provide a flow rate of 100–200 nL/min. The exit ports of the microchip were aligned sequentially with the orifice of the MS using a 3-D stage. Reprinted from [18], with permission.

multichannel ESI exit to provide the potential for high-throughput analysis. Both designs eliminated complexity by using the open-end exit of the microchannel as the emitter, and the Taylor cone [19] that is essential for ESI could be directly obtained. However, some serious problems arose from this open-end exit. An open-exit electrospray emitter built on the flat edge without any fluid regulator or controlling mechanism will form a big droplet that diminishes the efficiency of the separation due to band-broadening. Moreover, there are problems associated with the ESI interface because of the hydrophilicity of glass (or quartz) emitters. The spray solution would be attracted to the microchip surface and tends to form a big droplet that interferes with stable ionization. Hence, the surface was made hydrophobic by coating [18] or derivatizing [17] to improve the situation. Although these methods reduced the wetting of the chip edge, there was still the potential for chemical stability being affected by electrical discharge. Besides, without the external pressure to generate a flow rate of 100–200 μL/min, the electrospray was not stable due to low EOF. Another attempt was adding pneumatic assistance. Nebulized gas and auxiliary liquid were added at the end of the separation channel to reduce the size of the droplet and also to increase the ionization efficiency [20]. It is an important feature for the building of miniaturized pneumatic nebulizer as a part of chip. A mixture containing five peptides was well separated and ionized for MS analysis in such an integrated device. Although attractive, the band-broadening and sample dilution may occur because of the assisted hydrodynamic flow.

The design of direct electrospraying from the end of the channel was made on a polymer-based microfluidic chip. Wen *et al.* [21] made use of polycarbonate (PC) to fabricate the microchip and they incorporated a sharp triangular nozzle at the end edge of the chip with the sheath gas to assist the ionization and some reservoirs were drilled for isoelectric focusing (IEF). A three-protein mixture was well separated by IEF in the separation channel and sequentially ionized through the emitter. With the advantage of the native property of the polymer, the complicated procedures were eliminated in the polymer chips. Although the separation efficiency was less than that for the coated capillary, it eliminates the wetting problems encountered in the glass device by the native hydrophobic property.

2.2 Capillary electrospray emitter attached to a glass microchip

The production of gas-phase ions from ESI is more efficient when using a smaller sized emitter [19, 22, 23]. It seems less complicated to attach a transfer line as an electrospray interface than fabrication by wet etching although it may obviate the advantages of microchip fabrication methods to produce multiples of functions by microfabrication. This kind of design was first introduced by Abersold's group [24]. In Fig. 2, a 12 cm long capillary was glued to the edge of the microfluidic device and connected to the mass spectrometer. This capillary transported the proteins by EOF towards the mass spectrometer. A high voltage applied to the capillary caused ionization of the samples as they entered the mass spectrometer. This work provided proof of the feasibility of interfacing micromachined devices with ESI-MS, although data on separation was not supplied by the authors. However, capillary alignment is another problem in such a design. In order to attach a capillary at the end of

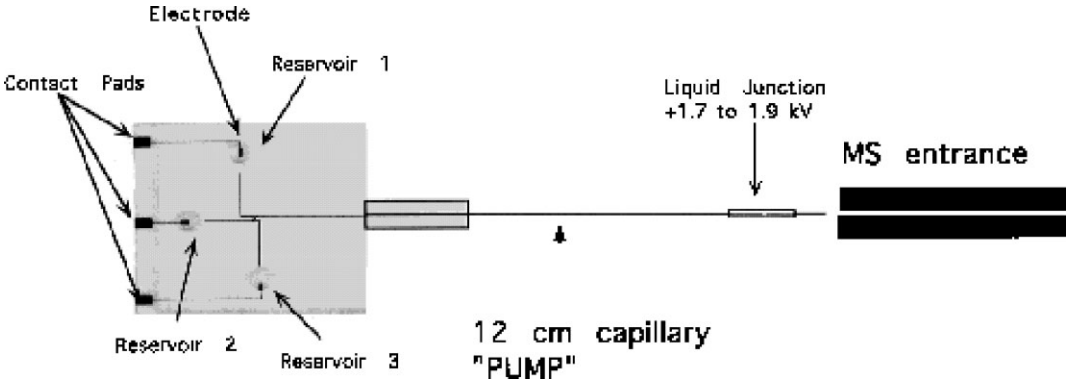

Figure 2. Schematic illustration of micromachined analytical system. Diagram of the microfabricated device, the coated capillary electroosmotic pump, and the microelectrospray interface. The microfabricated device is shown at twice its actual size. Dimensions of the channels, reservoirs, and the inside diameter of the pump are indicated in the text. The channels on the device were graphically enhanced to make them more visible. Reprinted from [24], with permission.

the hard substrate device, two main methods have been reported for this integration. First, Zhang and his coworkers [20] made use of double-etching to build a wide channel into which the capillary was inserted. The procedure was reproducible but time-consuming and a significant dead volume was produced at the interconnection. In the other design, Harrison and Thibault's groups [25] cooperated to demonstrate that a capillary could be fitted with a small dead volume ($<$ 0.7 nL) by drilling a flat-bottomed hole. This makes the chip usable with a disposable nanospray emitter. According to the results, the sample from the separation channel can be well transferred with less fluid junction, but the drilling process needs skill to get a precise channel alignment and reduce the powder contamination during chip fabrication.

Over the years, capillary attachment has become the main design on the ESI chip because of easy fabrication and excellent ESI performance. However, this setup is not easily controlled and gives poor reproducibility. Some attempts have been made to improve the performance. Henion *et al.* [26, 27] built a removable coupled microsprayer, which included a make-up flow and a nebulizing gas to enhance ionization efficiency and the emitter tubing was made of stainless steel (SS). A liquid junction between the chip and the coupler (Fig. 3) was specifically fabricated to increase the stability of ESI. Not only does it offer the possibility to apply the voltage for ESI but also functions as a buffer zone, which means that the separation is not affected by the nebulized flow. All conditions could be fine-tuned in the separated emitter for each different microfluidic device. In this design, the operation of the ESI performance was optimized outside the device and the sprayer could be reattached to different chips, which extends the lifetime and eliminates the contamination problem. Results indicate that the separation efficiency of such microfluidic chips could reach as high as 2860 plates for small molecules like carnitine and acylcarnitines using this coupled microsprayer electrospray device. In this design, however, the dilution effect arising from the pneumatic flow could reduce the sensitivity of the detection.

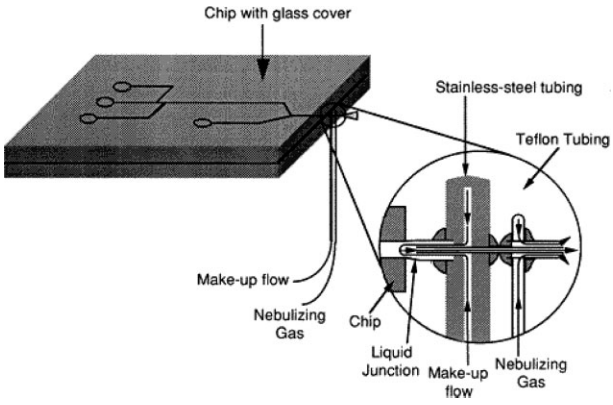

Figure 3. Schematic drawing of the glass chip-based CE/MS apparatus and the expanded view of the coupled microsprayer. Reprinted from [26], with permission.

2.3 Sheathless capillary tip attached to a glass microchip

To eliminate the problem of dilution effect caused by nebulized flow, a sheathless electrospray emitter becomes an alternative to use on the ESI microchip. In order to obtain stable ionization without pneumatic assistance, the shape, the inner size, and the surface properties of the emitter, will need to be considered. Usually a narrow capillary [19] with a reduction in the outer diameter could attain a Taylor cone with a lower voltage before the onset of a corona discharge. On the other hand, some coating material, like gold [23, 28] and carbon-black [29] on the emitter surface, could increase the stability of the ionization. However, under a steady operation condition of the electrospray, the intensive electrolysis was preceded to balance the charge induced by the applied voltage, which causes bubbles on the high-voltage electrode. Hence, in order to eliminate the bubbling effect and get stable ionization performance, a suitable voltage applied to ESI plays an important role in the sheathless design.

Karger's group [30] built a liquid junction on the chip and the voltage applied for electrophoresis could also be used for electrospray through the junction, by which the CE circuit is also completed. In order to successfully transfer the sample through the junction to the ESI emitter, a guiding channel was fabricated to precisely align with the exit of separation channel. From the results obtained, a high separation efficiency of 40 000 plates was achieved with a low detection limit in the attomole range. A similar design was developed by Harrison's group [31–33]. They created another sheathless design by adding a side channel for applying ESI voltage. The side channel extended from the connection point between the ESI tip and the separation channel. The analytes eluted from the separation channel could be directed to the tip immediately by the force generated by the liquid spray. Such a design could also lower the band-broadening effect caused by the liquid junction. Also, a high sensitivity of up to 7 fmol/μL could be achieved for peptide digests by using such devices [33].

2.4 Capillary electrospray emitter attached to a polymer microchip

Compared to glass substrates, plastics have the advantage of low costs and can be fabricated on a large scale, which makes them more attractive and suitable for com-

mercial production. Several methods, ranging from hot embossing [34], UV laser machining [21, 35, 36], X-ray lithography [37], and replica molding [38, 39], have been developed to make microchannels on polymer-based microdevices. Until now, many polymer substrates have been used for ESI chip fabrication (*e.g.*, polymethylmethacrylate (PMMA), polydimethylsiloxane (PDMS), polycarbonate (PC), *etc.*). Two critical properties make the polymers suitable as materials for fabrication of ESI source. First, compared to glass or quartz material, the softness of the polymer makes it moldable to any shape. In this way, the use of plastic material becomes a satisfactory alternative for complicated MEMS technology. Another key property of plastic devices is their hydrophobicity. Because of their inherent hydrophobic character, the plastics are suitable for use as MS emitters without any further modification procedures. Moreover, low-temperature production is an attractive feature of polymer substrates, which makes it easy to fabricate the microchips in a common laboratory.

Similar to the glass chip, capillary tip attachment was used to fabricate the polymer-based ESI device. However, the polymer substrate used for ESI chip fabrication is limited by their softness. In order to avoid chip swelling during ESI ionization, Kameoka *et al.* [40] built a polymer microfluidic chip with an open exit at the end of the channel and the chip was coupled with the microsprayer. The CE sample separation was done on the chip and the subsequent ionization was achieved by the sprayer, which demonstrated that a polymer-based microfluidic device could be coupled on-line for MS detection.

For sheathless design, however, Chen's group [41] made use of PDMS as the chip substrate. A liquid junction was set at the end of the channel to connect the pulled capillary tip and the separation channel [41]. Based on MS detection, results show that four peaks corresponding to three peptide standards and acetylated by-products were well resolved by the modified PDMS chip and the deduced sequences were consistent with those expected [41]. Compared to glass substrates, plastic chips have the advantages of low cost and easy fabrication procedures and thus the fabricated chips were more suitable for disposable use.

2.5 Infusion ESI device fabricated on silicon substrate

Anther type of devices was initially designed for direct infusion to eliminate complicities of integrating separation steps and thus this type of device can be built as in dense array formats for multiple analyses. Although direct infusion is likely to suffer from ion suppression [42] or interference effect [43] during MS analysis, direct MS analysis eliminates the time required to run the liquid chromatography (LC) or electrophoresis separation, and that allows multiple cycles of ion fractionations to be executed by the MS instrument in a fast speed to minimize interference or suppression effects. Most of the direct infusion devices were fabricated by MEMS technology using silicon substrates. Lee and co-workers [44] described a process to fabricate a parylene nozzle on the edge of a silicon microchip. A nozzle was extended from the channel of the silicon chip. The stable ion emission can be achieved at low nL/min flow rates without the use of a neubulizing gas. Although it needs relatively high voltage to form an electrospray, the use of parylene material for microtip fabrication overcomes the fragility problem associated with the capillary tip and thus the device becomes more robust for commercialization.

Another infusion design was made by Zhang *et al.* [45–49], which has been commercialized for real-sample bioanalysis. The authors used a deep reactive ion etching (DRIE) process [45] or MEMS [50, 51] to build a miniaturized ESI plate. A channel was etched across the silicon plate and a nozzle (10 μm ID × 30 μm OD) was connected beyond the end of the channel which is shown in Fig. 4. The reservoir etched in the backside of the plate could hold the analyte for direct ionization. It was also possible to couple other separation devices because of the low dead volume. By making use of MEMS technology, multiple analyses could be achieved by aligned high-density nozzle arrays, 96, 384, 1536 in a small plate [46, 47]. Because of the fast sampling speed of the device, a tandem MS instrument can be conveniently used for ion fractionation in the first mass analyzer and then detection in the second mass analyzer to compensate the lack of the separation. This microchip is also compatible with MALDI-MS interface. When coupled with 2-D gel sample preparation, the sample from in-gel digestion can be sequentially analyzed by MALDI-MS and ESI-MS instruments to increase the amount of information obtained. Although some drawbacks arose from the direct infusion [42, 43], several unique features associated with the de-

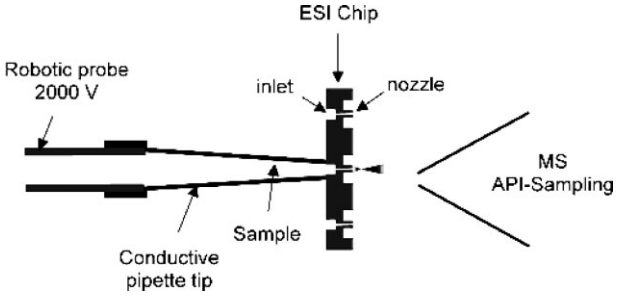

Figure 4. Diagram of the layout of multiple ESI chip. Reprinted from [46], with permission.

vice, such as the miniaturized format and fast analysis speed, provide a high-throughput platform at low costs for proteomics analysis.

2.6 Infusion ESI device fabricated on polymer substrate

Direct electrospray from plastic microfluidic devices has been described in several reports. In order to get stable ESI performance, the channel exit has always been sharpened to reduce the liquid-spreading phenomenon and to decrease the voltage required to establish the Taylor cone. Polymer substrates, such as PDMS [52], PMMA [53], and polyimide (PI) [54], were used for this investigation. The multichannel spraying devices were also developed for multiple sample analysis. Yuan and Shiea [55] built a star-shaped sequential analyzer composed of a sharp tip at the exit of an open channel, which was manually cut by a knife on PMMA substrate. The liquid can be trapped in the deep channel without the usage of cover plate. Although the chip fabrication is attractive, the device requires a large-scale reservoir to minimize the evaporation effect from the open channel and a relatively high voltage (3–4 kV) is needed to get ESI. For this, as shown in the Fig. 5, Rolando *et al.* [56, 57] developed a micronib design source that functions in nanoelectrospray condition. They fabricated a planar on-chip nib-like structure by using the epoxy-based negative photoresist SU-8, which offers high quality and reproducibility in the fabrication process. The function of the micronib is much akin to a fountain pen such that it contains a reservoir and an open capillary slot leading the analytes to the nib point. With the help of the slot design, the electrospray potential is successfully reduced to 1.5 kV, which makes it more compatible with other channel-based microfluidic devices.

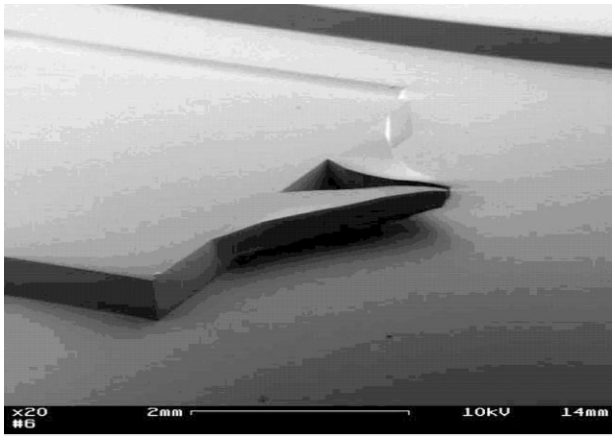

Figure 5. Photograph of a nib structure (first generation) fabricated in the epoxy-based negative photoresist SU-8 and supported on a silicon wafer. Reprinted from [56], with permission.

For most manually sharpened tips, the position of the Taylor cone is not stable, which would cause cross channel contamination. In order to reduce the contamination phenomenon, as shown in the Fig. 6, Kameoka *et al.* [58] inserted a triangular parylene sheet between the two wafers so as to confine the liquid at the system outlet. A dense array device was fabricated with an 80 μm distance between each tip. From the results shown, the system showed good electrospray stability and reproducibility with different tips. No system carryover was detected when used for sequential analysis. Besides, the benefit from the point-like design is in its application for drug discovery, which requires a high-throughput qualitative and quantitative analysis of drugs. The linear dynamic range of methylphenidate (0.4–800 ng/mL) fortified in human urine gave a lower limit of quantization by employing this infusion device [59].

Another multiple ESI emitter array was designed by Smith's group [60]. The authors used the laser ablation method to fabricate the cross channel (30 μm ID) through

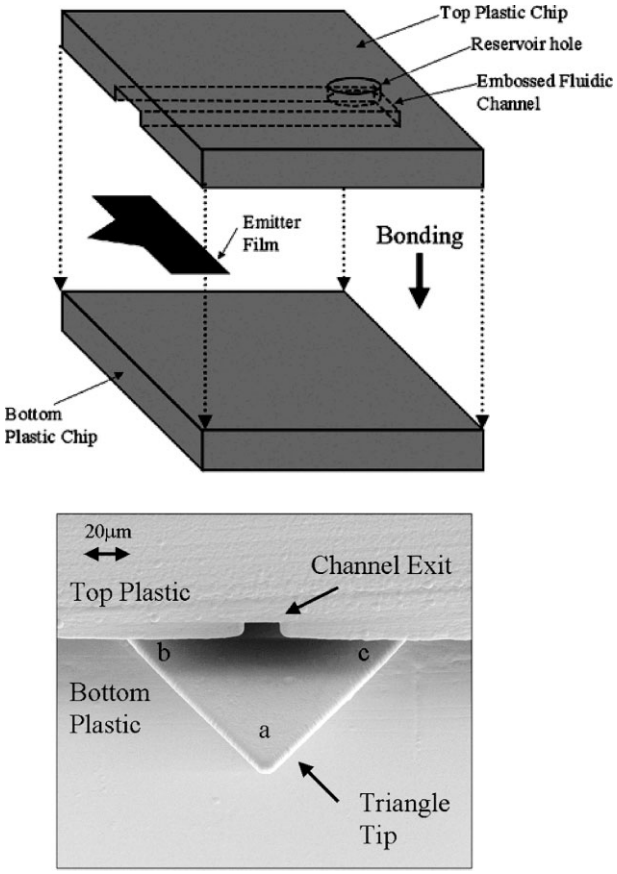

Figure 6. Fabrication process of the single triangular tip electrosprayer integrated with the microfluidic channel. The parylene C film was sandwiched between two plastic chips to stabilize the ionization position. Reprinted from [58], with permission.

the PC plate and built as array format. Unlike the sequential electrospray application [50, 51], the emitters on the plate were spraying simultaneously to produce a multielectrospray ion source. Hence, the signal intensity can be enhanced by collecting the total ions generated from all the emitters at the same time. In order to maintain stable ESI ions, a higher flow rate system, like LC, should be a suitable coupling system to get fast sample ionization.

3 Functional integration towards micrototal analysis for proteomics

For protein analysis by MS, sample pretreatment involves many tedious steps. Pretreatment steps, such as enzymatic digestion, increase the sequence coverage while others like desalting increase the detection sensitivity. Integration of these steps is especially important for large-scale proteomics analysis, in which the complexity of the sample is extremely high. Microfluidic systems are expected to offer a powerful means for method integration, such as automatic protein pretreatment and analysis.

For proteomic analysis, protein digestion plays an important role in the sample pretreatment process. The first trypsin digestion strategy on an ESI chip was built by Xue *et al.* [61]. The solution of trypsin and sample was put in the reservoir, then directly infused for MS analysis. Although the strategy is simple, the digestion rate was limited by the low trypsin-to-protein ratio. In order to enhance the rate of digestion, Harrison and the co-workers [31] built a microfluidic chamber containing a trypsin immobilized bed (2.4 μL) as shown in Fig. 7 and the protein could be cleaved into small peptides when flowing through the reaction bed. Moreover, the reactor bed could be removed for use without enzyme digestion or be replaced with a new one. From the results shown, the amino acid coverage can reach 92% for standard proteins, which makes the integrated device a convenient platform for automated sample processing.

For proteins present at low concentrations, such as those extracted from 2-D gel spots or post-translationally modified proteins, preconcentration steps are extremely important to obtain satisfactory results. Solid-phase extraction (SPE) is an important sample pretreatment technology. Jianjun *et al.* [33, 62] developed a microsystem in which C18 reversed-phase beads or metal affinity media were packed on chip to enrich analytes. This investigation demonstrated the capability of chip-based affinity selection for trace-level target peptides (20 ng/mL) spiked in human plasma. Furthermore, the compatibility of a modified PDMS microfluidic device with other miniaturized devices [41] to integrate the whole process for flow-through sampling [63], separation, and ESI-MS/MS analysis was also explored by connecting a miniaturized enzymatic digestion cartridge and a desalting cartridge in series to the sampling inlet.

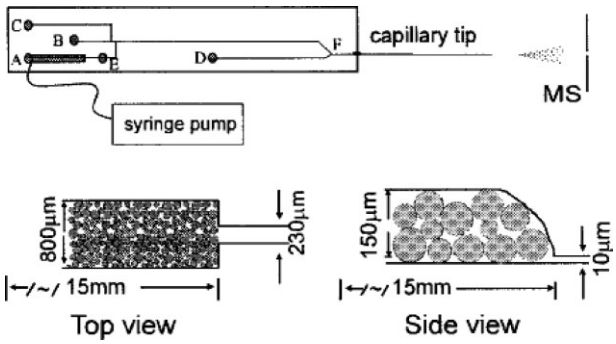

Figure 7. Schematic representation of the integrated enzyme reaction bed and CE chip. Top and side views show a blow-up of the packed trypsin bead bed. The other channels on the chip are 230 μm wide and 10 μm deep near each fluid reservoir, then narrow to 30 μm wide and 10 μm deep for the majority of their length. Reprinted from [31], with permission.

Unlike the packed beads strategy in the chip, Tan *et al.* [64] fabricated an array of eight porous monolithic columns for direct MS analysis. The *in situ* polymerization owns several advantages, like frit-free construction, easy preparation with good control of porosity and varied surface chemistry, and with these advantages, *in situ* polymerization may eventually replace the packing strategy. From the results shown, high recovery and good reproducibility was achieved between the eight monolithic columns. Another application of *in situ* polymerization was demonstrated by Lazar *et al.* [65] for electrochromatographic chips in complex sample separation in the microfluidic channel before MS analysis. The positively charged monolithic polymer had the hydrophobicity, which improves the separation performance and the charge to generate EOF, which increases the elution efficiency. For standard protein digests, 70–80% amino acid sequence coverage was obtained and separation efficiency was approximately 3000–4000 plates in 5–6 cm long channels.

There is a commercial microfluidic ESI device now on the market [66]. The channel was built by laser ablation on the PI film and a platinum electrode was deposited at the end of the channel to apply the voltage for ESI. Unlike the electrophoresis chips mentioned above, a reversed-phase column was built inside the polymer-based chip to perform liquid chromatographic separation by coupling the nanofluidic pumping system. In this integrated device,

the chip is sandwiched between the stator and a rotor valve, which can direct the sample to flow through the enrichment and separation column built in the chip. Besides, the chip requires no fittings between the separation and nanospray tip while still allowing complex valve port switching, which eliminates the fluidic leakage and blockage problems and shortens the time when compared to the traditional LC-ESI system. With these advantages, higher sequence coverage can be obtained when coupling with strong cation exchange (SCX) columns and thus this microfluidic chip bears a great potential for proteomics applications.

4 Conclusions

It is clear that MS is one of the most important analytical techniques for many applications in recent years. The microfluidic ESI chip provides an improved front-end technology to greatly improve the analytical capability of MS instrument. The infusion chip seems to provide an easier starting strategy for direct and massive MS analysis while the integration of multiple steps for achieving total analysis remains as a great challenge. If technical hurdles discussed here can be solved, microfluidic-based ESI chip will not only be regarded as a state-of-the-art technology, but also really make MS instruments user-friendly due to the simple and superior interface between the chip and the instrument.

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