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Journal of Chromatography A, 924 (2001) 137–145

JOURNAL OF CHROMATOGRAPHY A

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Microfabrication of polydimethylsiloxane electrospray ionization emitters

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Abstract

Microfabricated polydimethylsiloxane (PDMS) emitters for electrospray ionization mass spectrometry (ESI-MS) were implemented as tips along the edge of the PDMS device by three methods which utilize soft lithography processes. These microfabrication methods for producing PDMS emitters as an integral part of a microfluidic device will facilitate development of more complex microfluidic analysis systems using ESI-MS.

Keywords: Electrospray ionization; Instrumentation; Microfabrication; Microfluidic device; Polydimethylsiloxane; Proteins

1. Introduction

Microfluidic analytical systems, particularly for the analysis of biomolecules, are a subject of increasing interest [1–4]. The first reports of microfluidic devices employing electrospray ionization mass spectrometry (ESI-MS) utilized electrospray from channels terminating at the edge of the device [5,6] but most subsequent reports have utilized conventional electrospray emitters (e.g., tapered fused-silica capillaries) attached to the device [7–10]. Neither of these approaches is optimal. Spraying from the edge of a device was troubled by droplet formation resulting in a mixing volume that degraded the separation. Attachment of separate emitters can also introduce dead volume in the connection in addition to the disadvantage of the need for an individual attachment step for each emitter in a multichannel device. Microfabricated ESI emitters as an integral part of a microfluidic device have been reported including silicon nitride [11] and parylene [12] emitters on silicon devices, a polycarbonate emitter on an isoelectric focusing device produced using laser micromachining [13], and microfabricated silicon ESI emitters on monolithic silicon substrates [14]. These devices gave good ESI performance, but they all required relatively sophisticated facilities for production.

We report here three methods (referred to as the ‘‘trimming’’ [15], ‘‘two-layer photoresist’’, and ‘‘resin casting’’ methods) using soft lithography [16] for microfabricating ESI emitters as an integral part of polydimethylsiloxane (PDMS) microfluidic devices. These methods obviate the need for manual attachment of separate components, which is labor intensive and can cause dead volume that degrades the separation. Soft lithography provides a relatively simple microfabrication process for PDMS devices that requires less sophisticated facilities than other microfabrication processes. PDMS devices made by this method are also readily transferable to injection molding processes for larger scale production of the devices. We have developed the two-layer photoresist

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00901-3

and resin casting methods as improvements over our previously reported trimming method [15]. PDMS emitters produced by the improved methods were found to be more reproducible, and the emitter tips were more robust than those produced by the trimming method

2. Experimental

2.1. PDMS ESI emitter microfabrication by the trimming method

The previously reported trimming method [15] has been modified, and it is described in more detail here to compare it with the non-trimming two-layer photoresist and resin casting methods. The modifications to the trimming method were also applied to the non-trimming methods.

The photomask designs for the photoresist patterns (Fig. 1A and B) were drawn using a CAD program (Freehand 8; Macromedia, San Francisco, CA, USA) and printed on transparency film with a high-resolution image setter (3556 dpi resolution, Scitex Dolev 450; CreoScitex America, Bedford, MA, USA). The designs defined an angle of 60° for the sharply pointed emitter tips and a width of 100 μm and length of 4 cm for the channels.

In order to make the thickness of the photoresist pattern 30 μm, 3.5 ml of a solution of negative photoresist SU-8 50 (Microchemical, Newton, MA, USA) was dropped onto a 100 mm silicon wafer (Silicon, Boise, ID, USA) chucked in a spin coater (Model WS-200-4NPP, Laurell Technologies, North Wales, PA, USA) that was spun at 5000 rpm following acceleration at 1000 rpm s^{-1} for a total spin time of 25 s. The spin-coated wafer was prebaked at 55°C for 3 min, and then at 95°C for 25 min. After baking, the photomask image was patterned on the negative photoresist using a UV lamp in a mask aligner (Cobilt CA800; BTS, Atco, NJ, USA) for 30 s. The wafer exposed with UV was postbaked at 95°C for 15 min and developed in 2-(1-methoxy)propyl acetate (Acros Organics, Pittsburgh, PA, USA). After developing the wafer, the height of photoresist pattern on the master wafer was measured as 30 μm using a profilometer (Mitutoyo Model ID-C112C Dial Indicator; McMaster-Carr, Atlanta, GA, USA).

Four segments of 5 cm fused-silica capillaries

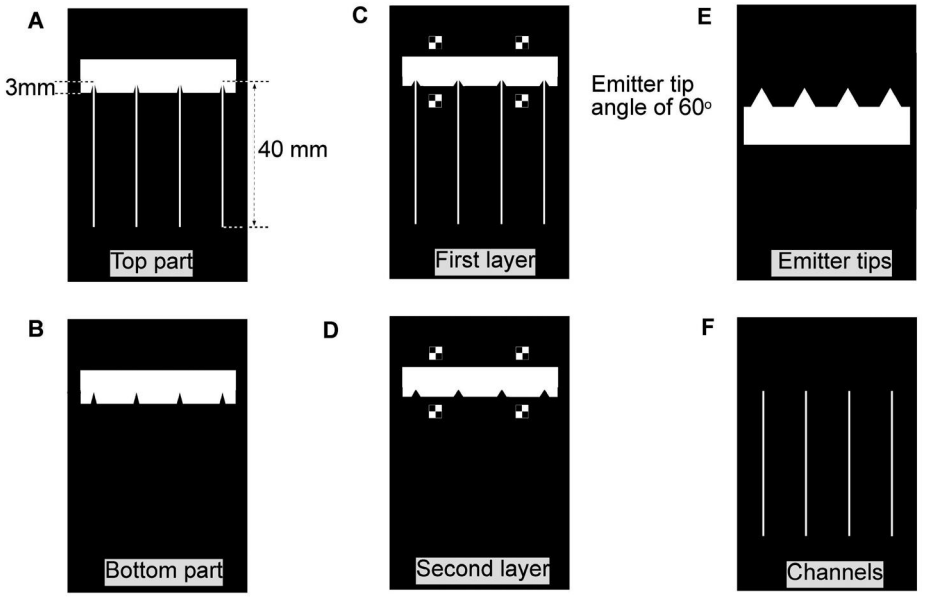

Fig. 1. Photomask images for the photoresist patterns of the PDMS emitter devices produced by the trimming method (A and B), two-layer photoresist method (C and D), and resin casting method (E and F). (The emitter tips and channels are shown wider than scale for clarity).

(FSCs, 75 μm I.D.×360 μm O.D.; Polymicro Technologies, Phoenix, AZ, USA) were attached at the end points of the channels of photoresist pattern (Fig. 1B) on the master wafer using the PDMS premixture (Silgard 184, Dow Corning) as ''glue'' in order to provide openings for connecting longer FCSs to the cured PDMS device. PDMS glue was used 5 h after the PDMS premixture was prepared and kept at room temperature. Prior to attaching the FSCs on the photoresist, 2 mm long pieces of 360 μm I.D. PTFE tubing were first attached to the photoresist, the FSC was inserted into the tubes and secured using PDMS glue, and then the assembly was heated for 1 h at 70°C to cure the PDMS.

To make the concave shape edge for the PDMS emitters, the ends of the tips on the photoresist pattern (the right side of Fig. 2A and B) was covered with a curved piece of transparency film (3M PP2500 for laser printers; 3M Visual Systems, Austin, TX, USA) which was held in place by plastic tape (Scotch Magic Tape; 3M, Minneapolis, MN, USA) and pressurized by a brass block (0.5 in.×0.25 in.; 1 in.=2.54 cm), and the rectangular photoresist pattern on the master wafer was surrounded with three brass blocks of the same size.

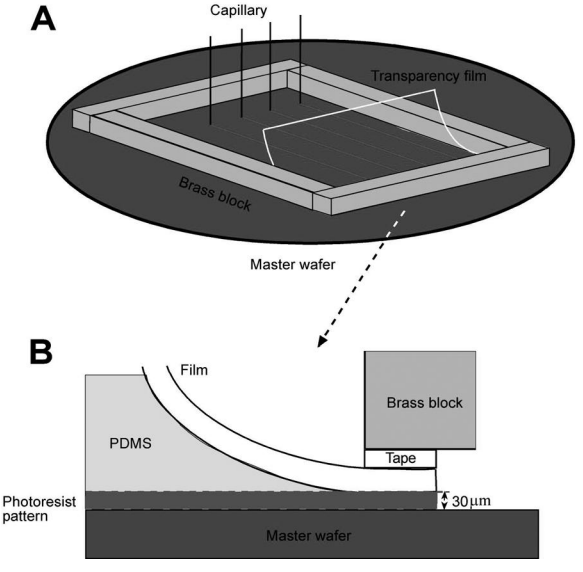

Fig. 2. Schematic illustration of the molding of the PDMS replica on the master wafer with photoresist pattern by the trimming method (A), and detail of the concave shape of the device edge leading to the PDMS emitter tip (B, side view).

Before casting the top PDMS part against the master wafer, it had been silanized with a releasing agent (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (TCS; United Chemical Technologies, Bristol, PA, USA) under vacuum for 8 h. PDMS prepolymer cast on the master wafer was cured at 70°C for 3 h in an oven, and the cured top PDMS part was peeled from the master wafer. After curing, the 5 cm FCSs in the top PDMS part were removed and replaced with new 20 cm segments of same dimension FSCs to make fluid connections to the channels. The new FSCs were attached while flowing nitrogen gas through the capillary to prevent clogging, and the FSCs were secured in the place with PDMS premixture. The bottom PDMS part, which was symmetrical to top PDMS part of the device, was cast on a silicon wafer with the photoresist pattern identical to that of the top part without the channels (Fig. 1B).

The cured top and bottom PDMS parts were surface-oxidized at same time in a plasma cleaner (Model PDC-32G; Harrick Scientific, Ossing, NY, USA) at ''medium'' power setting for 1 min at 2 Torr air pressure (1 Torr=133.322 Pa). After oxidation in air plasma, the top PDMS part was aligned to the bottom PDMS part using a thin layer of methanol between the parts, and then bonded by heating at 70°C for 4 h to evaporate the methanol. The membrane edges for the emitter tips in the bonded PDMS device were trimmed to shape using iris scissors (Roboz Surgical Instrument, Rockville, MD, USA) and a scalpel blade under a stereomicroscope (Model AO 569; American Optical, Southbridge, MA, USA) along the photoresist pattern in the cast PDMS device as a guide for the emitter shape.

2.2. PDMS ESI emitter microfabrication by the two-layer photoresist method

The photomask designs for the two-layer photoresist pattern (Fig. 1C and D) and the process for the first photoresist pattern were made as for the trimming method described above. In order to make the pattern of the second layer of photoresist (Fig. 1D) ~150 μm high, without postbaking the exposed first layer, 3.5 ml of photoresist solution was dropped on the first photoresist, and spun again at 2500 rpm following an acceleration at 1000 rpm s^{-1} for a total

spin time of 20 s. The spin-coated wafer with two-layer photoresist was baked at 55°C for 3 min, and then at 95°C for 25 min. After baking, the transparency with pattern of second layer (Fig. 1D) was aligned onto the exposed first layer photoresist using the mask aligner, and was exposed for 100 s. The silicon wafer with exposed first and second photoresist was hard-baked at 95°C for 15 min, and developed in 2-(1-methoxy)propyl acetate. The heights of photoresist pattern in each layer on the master wafer (Fig. 3A) were measured as 30 μm and 150 μm, respectively using a profilometer.

After connecting the FSCs as in the trimming method, the master wafer with two-layer photoresist pattern was surrounded with three PTFE blocks (0.5 in.×0.25 in.). The ends of the reference points in the two-layer photoresist pattern on the master wafer were covered with a convex profile of the epoxy resin block (made from EpoFix; EMS, Ft. Washington, PA, USA) for producing the emitter tips of top PDMS part (Fig. 3B). The PTFE and resin blocks were pressurized with two aluminum plates (5 in.×5 in.) held by four binder clips (Acco 72100, Large; Acco Brands, Lincolnshire, IL, USA). The degassed premixture for the top PDMS part was cast against the whole system on the master wafer which had been silanized with TCS under vacuum for 4 h.

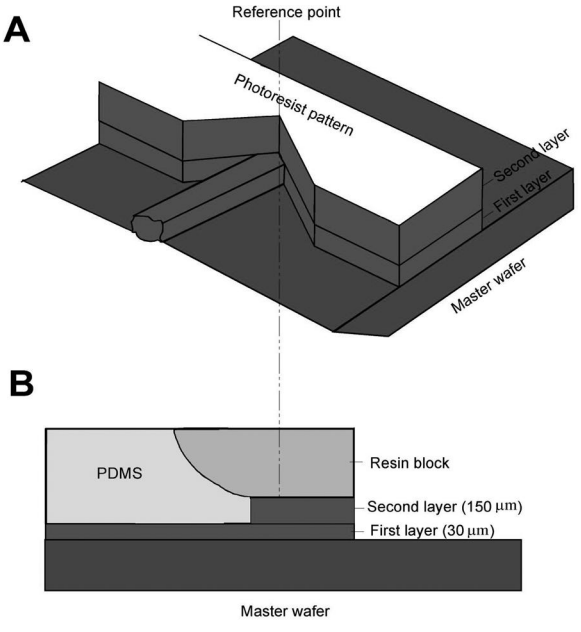

Fig. 3. Schematic illustration of the molding of the PDMS replica on the master wafer with the pattern for the two-layer photoresist method (A), and detail of the concave shape of the device edge leading to the membrane PDMS emitter tip (B, side view).

After casting the top part, the processes for curing, casting the bottom PDMS part, and bonding the top and bottom parts were followed as for the processes in the trimming method; however, the two-layer method avoided the need for trimming the emitter tips.

2.3. PDMS ESI emitter microfabrication by the resin casting method

The production of the photomask designs for the photoresist patterns (Fig. 1E and F) and the processes for producing the photoresist patterns were the same as for the trimming method.

To make a mold for a more robust emitter tip of the PDMS device, ca.1 μl of the epoxy resin (EpoFix) was dropped on each emitter tip position in the photoresist pattern (Fig. 1E) of the master wafer held at the slope of 20° to allow the resin to flow to the end of the emitter tip photoresist pattern (Fig. 4A), and then allowed to cure at room temperature overnight. As a second step in the process, 10 μl resin was placed immediately adjacent to the cured resin at each tip position of the photoresist pattern, and then cured at room temperature for 5 h (Fig. 4B). In the third step, a concave shaped PDMS support block (the shape produced on the PDMS device edge in the trimming method), which had been fabricated the same size as the molding size (Fig. 1E), was aligned onto the cured resin pattern (Fig. 4B and C), and then more resin was filled between the PDMS support and the master wafer (silanized with TCS under vacuum for 2 h) which had been surrounded with three PTFE blocks (0.5 in.×0.25 in.). After curing overnight at 40°C, the resin imprint was easily peeled off from PDMS block and PTFE blocks. The resin premixture had been mixed in a 16:1 ratio of resin and hardener, and was degassed to remove bubbles under vacuum for 30 min prior to the casting.

After connecting the FSCs as in the process for the trimming method, the resin imprint was aligned to the end points of four channels of the photoresist pattern on the master (Fig. 1F), and PDMS pre-

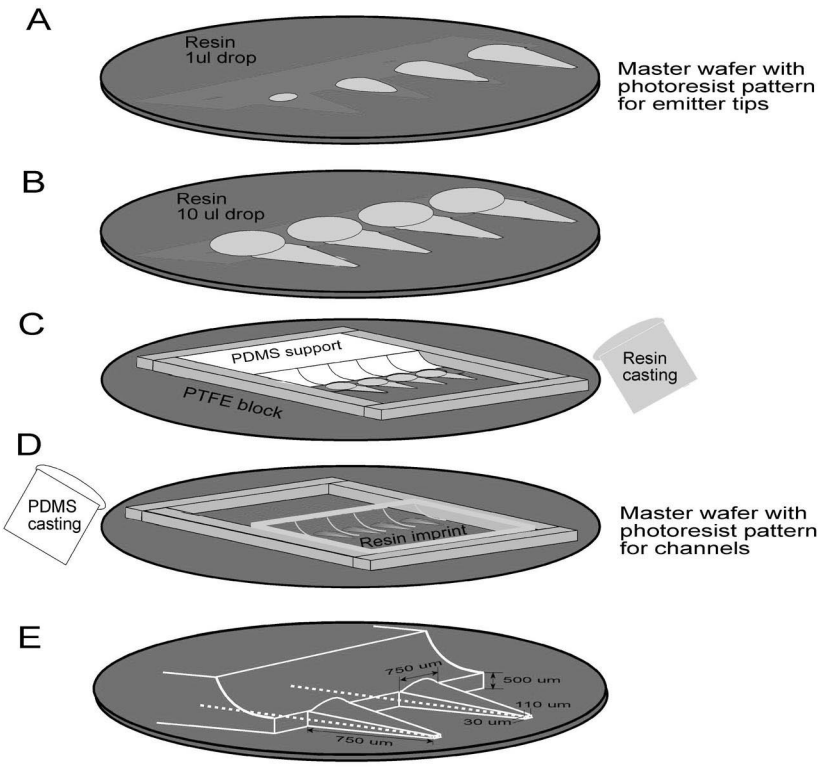

Fig. 4. Schematic illustration of the fabrication of the mold for making the PDMS replica using the resin casting method (A, B, and C), the casting of the device (D), and the convex shape of the resulting emitter tip (E).

mixture was cast against this imprint and master wafer (Fig. 4D) with curing overnight in a 40°C oven. The process for replacing the FCSs, preparing the bottom PDMS part, and bonding the two parts were the same as for the processes in the trimming method. The shape of the emitter tips and the channel shape and size are shown in Fig. 4E.

2.4. Electrospray mass spectrometry data acquisition

A Mariner ESI time-of-flight (TOF) MS instrument (PE Biosystems, Framingham, MA, USA) was used to acquire MS data. The instrument was modified by adding a *z*-axis adjustment made from an acrylic plate that attached edgewise to a microscope mechanical stage mechanism (Fisher Scientific) mounted to the existing *xy* adjustable ESI mount.

The channels of the microfabricated PDMS device were washed with methanol and water using a syringe pump (Model 11; Harvard Apparatus, South Natick, MA, USA). The FCSs in the PDMS device were connected with a metal union (Model ZU.5T; Valco, Houston, TX, USA) to which ESI high voltage was applied. A standard solution of 1 mg ml^{-1} each of angiotensin I and bradykinin (Sigma, St. Louis, MO, USA) in a water–methanol (1:1, v/v) solution, to which 0.1% acetic acid was added, was diluted to 10 μM with same solvent. The sample was injected into the PDMS device by the syringe pump with flow-rate of 1–20 $\mu l\ min^{-1}$. The distance of the emitter tip for the single channel and the four channel PDMS devices was varied from 5 to 10 mm in front of the orifice of the ESI-TOF-MS system using the *xyz* translational stage. To generate electrospray from the emitters, the ESI potential from the ESI power supply of the instrument was applied to the metal union connecting the FSC to the syringe pump. The flow-rate of nitrogen curtain gas varied

from 500 to 2000 ml min^{-1}, and the interface was heated to 120°C.

3. Results and discussion

3.1. Design of the PDMS electrospray emitters

Fig. 1 shows the photomask images for the design of the microfluidic channels and ESI emitters of PDMS devices composed of top and bottom PDMS parts bonded together. In the trimming method (Fig. 1A and B), the photomask image for the bottom PDMS part had only the emitter shape that was used to trim the emitter tip on the concave shaped membrane edge (see Fig. 2B). The photomask images for the first and second photorsist layers in the two-layer method are shown in Fig. 1C and D (the square marks were used for alignment of the two layers with the mask aligner). The lengths of the emitter tips were varied from 0.5 to 2.5 mm. The photomask for the second photoresist layer was also used for the bottom PDMS part. The photomask images for the top and bottom PDMS parts for the resin casting method are shown in Fig. 1E and F. The channels in all the methods were designed with a width of 100 μm and a length of 4 cm. The channel width of 100 μm was used to minimize clogging problems in the prototype devices, but the method can be used to produce more narrow channels limited only by the minimum feature size of the soft lithography method (about 20 μm) [17].

3.2. Microfabrication of the PDMS emitters

3.2.1. Trimming method

Fig. 2 shows how, in the trimming method, the concave shape ending in a thin membrane along the edge of the PDMS device was formed by casting the PDMS against a piece of curved transparency film. The 0.5 in.×0.25 in. brass block provided sufficient pressure to the film to control the membrane thickness at the channel openings of the emitters to less than 100 μm using the tape to fix the film on the end of channel openings on the master wafer.

When making the bond between the top and bottom PDMS parts of the device by plasma oxidation, the edge surface of the membrane between the top and bottom parts was aligned along the profile formed by the photoresist pattern (Fig. 2) using a thin layer of methanol between the parts [18]. The methanol allows precise alignment of the two parts. The emitter tips were trimmed to points at each channel opening following along the photoresist pattern profile as a guide using iris scissors. An emitter tip length of 3 mm was found to be optimal for easy trimming and for stability of the electrospray. After trimming, the PDMS device was heated at 70°C to remove the prepolymer residue and to evaporate the methanol in the device.

3.2.2. Two-layer photoresist method

In the two-layer photoresist method of producing PDMS emitters, the first layer of photoresist was spin-coated onto the master wafer and exposed to UV with the transparency of the photomask image for the first layer (Fig. 1C). The second layer of photoresist was spin-coated on top of the undeveloped first layer. After spin-coating the second layer, the photomask for the second layer was aligned to the cross-linked photoresist pattern in the first layer using the alignment marks, and then exposed for 100 s (more than the 30 s in the first layer because the thickness of the second layer was 150 μm high). Fig. 3A shows the resulting profile of the two layers of photoresist that were developed at the same time. Although the developing time for two layers (total 180 μm) was longer than that for single layer, the developed first and second patterns on the master wafer were stable because the negative photoresist (SU-8) has a high mechanical strength.

After development, the master wafer photoresist pattern was surrounded with three PTFE blocks and a resin block to make the replica molding for top PDMS part (Fig. 3B). In order to make the resin block, resin was cast against a concave-shaped PDMS block like that made in the process for the bottom PDMS part in the trimming method. The resin block does not adhere to the PDMS surface even without treatment with the releasing reagent (TCS). The use of EpoFix resin cast against PDMS has been previously reported [10]. The convex shaped resin block was fixed on the reference points (Fig. 3) using tape in order to cover the ends of the two-layer photoresist pattern of the master wafer for top PDMS part, and then the PTFE and resin blocks were pressurized with two aluminum plates, which have a hole in the area for FSCs connecting, by four

binder clips. After casting PDMS premixture against this assembled molding system, the PDMS replica peeled off easily, and the membrane of emitter tips was robust and very reproducible. The optimal emitter tip length was found to be 0.75–1 mm. The thickness of emitter tips in the bonded device with top and bottom PDMS parts was ~400 μm. If the thickness of the emitter tip membrane was made less than 180 μm in either the top or bottom PDMS parts, the emitter tip membranes were prone to distortion when peeling off the PDMS replicas.

3.2.3. Resin casting method

To form the convex emitter tip master to produce the mold for making emitters by the resin casting method, 1 μl of EpoFix resin was dropped on the positions of each emitter tip (Fig. 4A), the master wafer was held at the slope of 20° for 10 min, and then the wafer was placed on a horizontal surface in order to allow the resin to flow to the end of photoresist pattern. The resin was cured overnight at room temperature because the resin overflows from the photoresist pattern when curing at high temperature for fast curing. Next, 10 μl of resin was dropped immediately adjacent to the cured resin to form a convex shape for the emitter tip and serve as a barrier to prevent the overflowing of additional resin which was filled between the PDMS support and the master wafer held at a slope of ~50° (Fig. 4B). The cured resin imprint was easily peeled off from the silanized molding system after overnight curing at 40°C (Fig. 4C). The resulting resin imprint mold was aligned to the photoresist patterns on another master wafer that had been made using the photomask image for channels (Fig. 1F). Efforts to make the imprint mold using PDMS instead of EpoFix resin were unsuccessful because it was difficult to align a PDMS imprint mold along the patterns for the channels due to the elastomeric properties of PDMS and the slight shrinkage that occurs upon heating. Emitter tips from 0.5 to 2.5 mm long were tested; all of the sizes were robust because of convex shape in spite of the 70 μm thickness of the emitter tips.

3.3. Electrospray performance and stability of the total ion current (TIC)

Fig. 5 shows the signal intensity as a function of time for electrospray data acquired with various

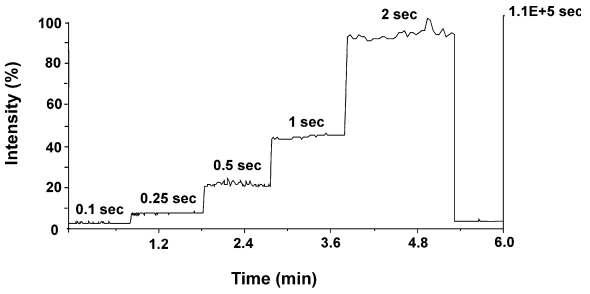

Fig. 5. Signal stabilities for the total ion current observed using a 10 μ*M* peptide mixture solution with spectral acquisition times of 0.1 to 2 s. The mass range of the TIC was from 200 to 700 m/z with a flow-rate of 3 μl min^{-1} and emitter–orifice distance of 10 mm.

spectrum acquisition times (spectral summing times for TOF spectra) from a typical emitter prepared using the trimming method. The average of the relative standard deviations for the different acquisition times was 3.2%. These data demonstrate that the emitters are capable of producing a stable electrospray. Fig. 6 shows a typical electrospray spectrum acquired using the acquisition time of 0.5 s.These results illustrate the type of electrospray mass spectral data that can be obtained using the microfabricated emitters. A more extensive description of the performance of emitters prepared by the trimming method has been reported [15].

3.4. Comparison of the three emitter microfabrication methods

No difference was observed in electrospray performance of tips prepared by the trimming method with a 30° or 60° tip angle. However, the 60° angle was found to be preferable for tips prepared by the other methods to facilitate the bonding between the top and bottom parts of the device. The thickness of the emitter tips was thinnest in the resin casting method (ca. 70 μm) compared to the trimming method (ca. 100 μm) and the two-layer method (ca. 400 μm). The microfabrication process was simplest for the trimming method (although a separate labor intensive manual trimming was required) compared to the more complex resin casting method and the most complex two-layer method. The two-layer method eliminated the manual trimming step, but required more equipment in that a mask aligner is

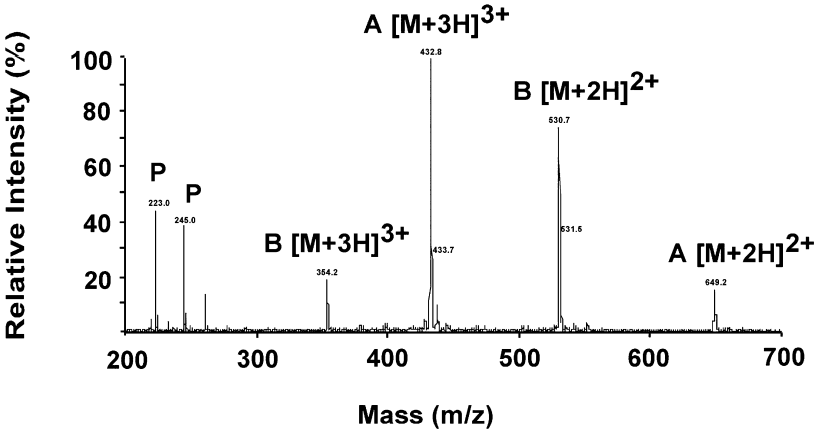

Fig. 6. Electrospray mass spectrum for the angiotensin I [A] and bradykinin [B] mixture with acquisition time of 0.5 s using a concentration of 10 μM with flow-rate of 3 μl min^{-1} (P indicates PDMS background signal of 223 and 245 m/z).

needed to align the first and second photoresist layers. The two-layer method provided the most accurate and reproducible tips in this work. The accuracy and reproducibility of the tips produced by the trimming method is limited by the manual trimming step, and the resin casting method was limited by the reproducibility of the method of forming each tip in the mold. Efforts are ongoing to improve the methods to facilitate production of devices with larger numbers of channels and with different device geometries.

4. Conclusions

The methods described here permitted the microfabrication of ESI emitters as an integral part of a PDMS microfluidic device. The trimming method provided a means to demonstrate the basic approach, and results from this method showed that the resulting devices gave adequate electrospray performance. The subsequently developed two-layer and resin casting methods provided more efficient processes for producing the emitters without the manual trimming step. It is expected that the basic design of these emitters can now be further refined in terms of channel dimensions and geometry to optimize and enhance ESI sensitivity. This approach to microfabricating ESI emitters will facilitate production of microfluidic analytical devices that utilize mass spectrometric sample detection.

Acknowledgements

This work was supported by NSF grant number DBI-9906662 and NASA grant number P101007. We thank B.L. Karger and F. Foret for helpful information on the use of EpoFix resin.

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