ON-CHIP FABRICATION OF MICROTIPS FOR NONE-DEAD-VOLUME ELECTROSPRAY IONIZATION IN THE MS APPLICATIONS

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Abstract: This paper proposes a novel method to on-chip fabricate a none-dead-volume microtip for ESI-MS applications. The microfluidic chip and ESI tip are fabricated in low-cost plastic based materials using a simple and rapid fabrication process. A constant-speed-pulling method is developed to fabricate the ESI tip by pulling mixed PMMA glue using a 30-μm stainless wire through the pre-formed microfluidic channel. The equilibrium of surface tension of PMMA glue will result in a sharp tip after curing. A highly uniform micro-tip can be formed directly at the outlet of the microfluidic channel with minimum dead-volume zone. Detection of caffeine, myoglobin, lysozyme and cytochrome C biosamples confirms the microchip device can be used for high resolution ESI-MS applications.

Introduction

ESI-MS is a powerful technology for studying large bio-molecules and charged chemistry molecules. In the beginning, chip-based ESI nozzles are directly fabricated at the open-end of the embedded microchannel but liquid samples will accumulate at the open-end because of surface tension which will result in a poor detection resolution.[1, 2] As of today, commercial glass-based capillary has became a standard tool for ESI-MS applications.[3-6] Capillary tips can provide good surface properties and high stability for long-term electrospray performance. However, the interconnection is a problem due to the mismatched inner diameters. Many groups also fabricated ESI tips on microchips using glass[7, 8], plastic[9, 10], silicon[11] as the substrate materials.[12, 13] The performance of these devices was found to be comparable to that of conventional silica capillary tips. However, most of the approaches faced the packaging problems between the chip body and the ESI tip. Dead volume and material variations between the tip and the main channel are the major ones. Therefore, to fabricate an ESI-MS chip with single material with no dead-volume is of great important.

Many approaches have been reported for the fabrication of a no dead-volume microfluidic devices integrated with on-chip microtip for ESI application. For example, directly forming the microtip at the end of a polymer based microchannel using laser ablation had been reported by Rohner.[14] However, delicate equipment is required for the process and the quality of a laser ablating surface is an issue for practical applications. On contrast, Arscott and et. al. have also reported a method for the fabrication of micro ESI tips using standard a photolithography process.[15] This method can fabricate microtips with high precision and small inner diameter. Nevertheless, sealing the microchannel and the microchip is a great challenge such that open channel is usually used with this approach.

This paper reports a simple approach to directly fabricate on-chip microtips in front of embedded microchannel with no dead-volume within the microfluidic device. A novel constant-speed-pulling method has been developed to form the microtip via a buried stainless steel microwire. The performance of the microtips is evaluated by analyzing standard proteins of myoglobin and cytochrome C using a commercial MS machine. It is the authors' believe that the proposed method is feasible for fabricating a high quality microchip for ESI-MS applications.

Fabrication Process

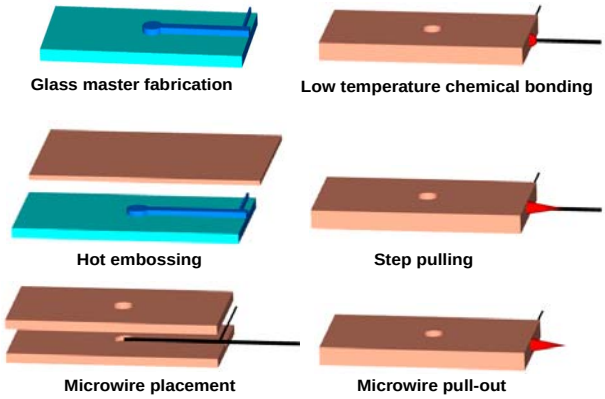

Figure 1 A simplified fabrication process for the proposed microdevice.

Figure 1 shows a simplified process for fabricating the microchip device. Briefly, a hot-embossing method, a low-temperature chemical bonding technique and a constant-speed pulling method are used to fabricate the

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microchip device. The glass master for forming the microchannel is fabricated using a fast prototyping method including a photolithography process and a glass etching procedure.[16] Once the PMMA-based microchannels have been formed, a stainless steel microwire of 30 μm is placed in the micro-trench and sealed with another drilled bare PMMA sheet while a certain length of s.s. microwire is left outside the microchannel. PMMA glue with the volume of around 50 μL is applied in front of the open-end of the microchannel and a constant-speed-pulling is adopted to form the microtip using a stepping motor with a speed of 0.5 mm/min. Finally, PMMA glue is cured at 80°C for 10 min and then the s.s. microwire is pulled out to form a microtip of the same diameter.

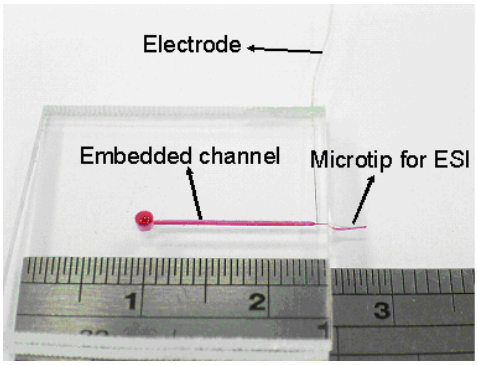

Figure 2 A picture of the micro ESI-MS chip after assembly.

Figure 2 shows a photo image of the fabricated microESI chip. A copper wire is buried in the microchip device to contact the sample fluid for sample driving. Since PMMA glue will slightly reflow into the microchannel during fabrication, the surface tension between the PMMA glue and the microchnnel will form a curvature and result in a no dead-volume connection. Note that the microchip device is filled with red dye for a better observation. Figure 3 shows the close-up views of the fabricated micro tips. A perfect volcano shape microtip was directly formed in front of microchip due to the force balance of the surface tension in glue and the adhesion force between the s.s. microwire and glue. The dimension of the tip is 1500 m in height, 30 m for the inner diameter and 150 m for the outer diameter. The reproducibility of the microtips is acceptable for measuring tens of tips.

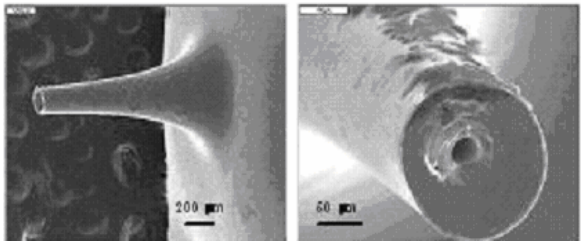

Figure 3 Close-up images of the on-chip fabricated microtip for electro-spray.

Experimental

Figure 4 presents an experimental image of the Taylor cone formed in front of the microtip (Fig. 4a) and the relationship between the applied voltage versus the height of the induced Taylor cone.(Fig. 4b) Note that the electrospray phenomena happened at the voltage of 2000V. In order to test the long-term ESI stability of the fabricated microtip, the ion intensity emitted from the microtip was measure using a commercial MS equipment (Esquire 3000 plus, Bruker, Germany). Figure 5 shows the measured ion intensity and the result indicate the proposed microchip can provide good ionization stability for more than 10 min. Furthermore, the feasibility of the microchip device was evaluated using a standard low molecule sample composed of 10^{-5} M caffeine with trace amount of acetic acid to enhance the sample ionization level. Figure 6 presents the MS spectra of the standard sample. A significant peak for caffeine sample with molecule weight of 194.9 can be observed in the figure and two peaks with the molecule weights of 61.4 and 120.9 can also be clearly identified for acetic acid molecules and the acetic acid dimer, respectively. The result indicate that the develop microtip is feasible for high performance MS analysis.

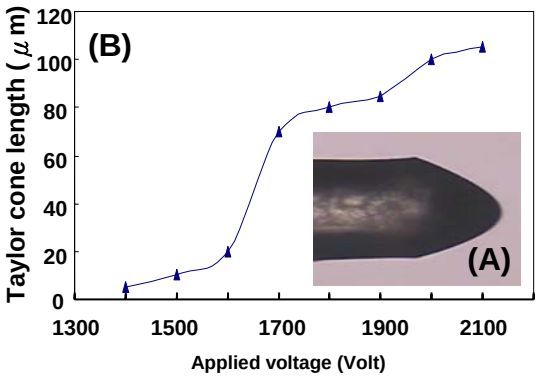

Figure 4. (A) An experimental image of the Taylor cone formed at the front of the microtip. (B) The relationship between the applied voltages versus the height of the Taylor cone.

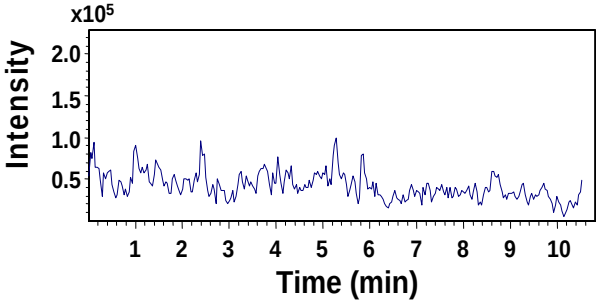

Figure 5. A long-term measurement for the ion intensity of the electrospray ionization using the proposed microchip.

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In order to further investigate the performance of the microtip developed using the proposed method, a high resolution MS machine of Bio TOF-Q (Bruker, German) was used to run a bio-sample of 10^{-5} M lysozyme using commercial available capillary tip and the proposed microtip. Figure 7 shows two MS spectra of the experimental results. A Gaussian distribution of the sample peaks for myoglobin can be clearly seen in both figures. However, as shown in the figures, there were satellite peaks appeared with the main signals of myoglobin in the spectra obtained using commercial tip and resulted in a lower signal to noise ratio.

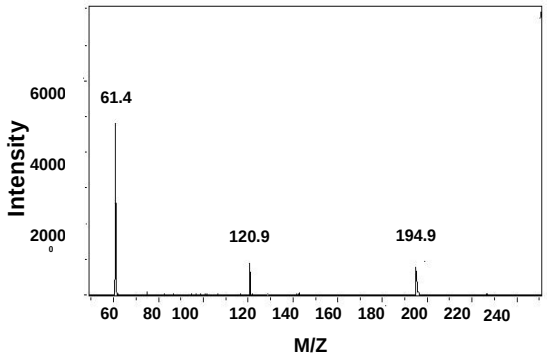

Figure 6. Positive ESI mass spectra of a 10^{-5} M caffeine sample after 20 min of continuous ESI spray.

Dead volume is the major factor that reduces the detection resolution in the MS applications. A good detection result can only be obtained while feeding samples without leakage effect due to the existence of dead volume. Therefore, a mixed protein sample of 10^{-5} M myoglobin and 10^{-5} M cytochrome C was also used to confirm the performance of the proposed none-dead-volume microchip device. Figure 8 shows the positive MS spectra of the mixed protein sample. The peaks labeled with blue M/Z ratios represent the signals from myoglobin and the peaks labeled with red M/Z ratios represent the signals from cytochrome C. The result indicates that the signals of both samples with different charges were well separated and confirms the feasibility of the microchip device for high performance MS applications.

Conclusions

This paper reports a novel constant-speed pulling method to fabricate high-quality micro tips for ESI-MS applications. The dead-volume problem can be eliminated while connecting the micro tip and the microchannel since the tip was directly fabricated in front of the microchannel. A long-term electrospray was successfully demonstrated using the proposed microchip. Furthermore, detections of protein samples including myoglobin, lysozyme and cytochrome C were presented in this study to confirm the feasibly of the microchip. The proposed method provides a very simple but reliable way to produce high performance ESI chips in the MS research field.

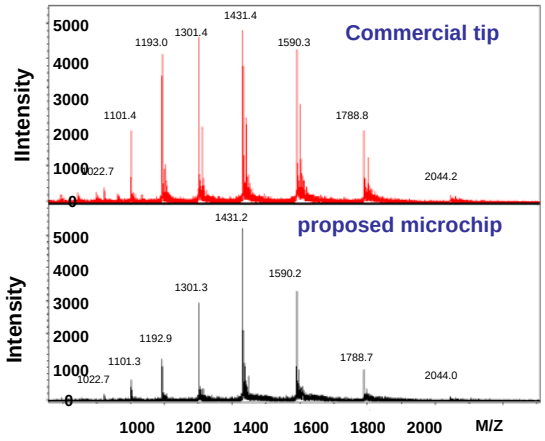

Figure 7. Positive ESI mass spectra of a 10^{-5} M lysozyme in obtained using commercial tip and the proposed microtip.

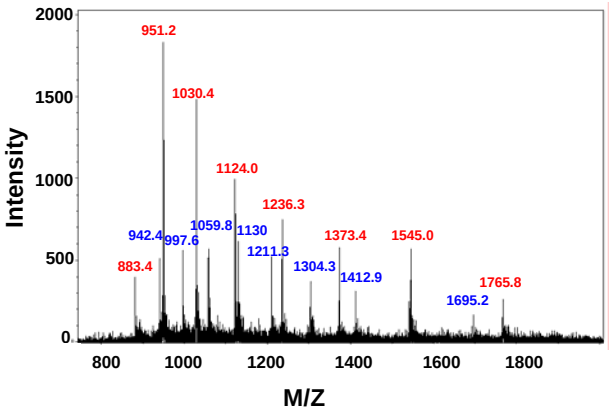

Figure 8. Positive ESI spectra of a mixture of a 10^{-5} M myoglobin and a 10^{-5} M Cytocrome C obtained using the proposed microtip.

Acknowledgments

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