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전기 분무 이온화를 이용한 단백질 질량분석용 마이크로 유체 소자의 제작 및 실험

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Sheathless electrospray ionization with integrated metal emitter on microfluidic device

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Abstract - In this study, sheathless electrospray from PDMS/glass microchips with conducting metal emitter tip is described. A chip-based capillary electrophoresis/mass spectrometry (CE/MS) system has advantages of the CE separation and on-line electrospray detection of peptide solution. We have fabricated a new electrospray ionization(ESI) device composed of the metal emitter tip and CE separation channel monolithically in a glass microchip. The separation channel and metal emitter tip are fabricated using a glass wet etching and gold electro plating process, respectively. The fabricated micro electrospray chip was tested by spraying peptide sample for mass spectrometric analysis. Singlely-charged peak and doublely-charged peak of peptide were detected and further MS/MS fragmentation was performed in each peak. Direct comparisons with conventional glass or fused silica emitters showed very similar performance with respect to signal strength and stability.

1. Introduction

The use of mass spectrometric techniques for protein analysis has received growing interest over the past few years [1] due to the selectivity and the low detection limit provided by the new generation of mass spectrometers and their capacity to work in tandem mode. In their turn, microfabricated fluidic devices (μTAS) have been developed for a large range of bioanalytical applications such as CE, PCR analysis, immunoassays, DNA separation and hybridization. Miniaturized total analytical schemes have also been applied in the area of proteomics protein profiling technologies. As a result, new tools are essential for MS analysis and especially MS analysis based on ESI. ESI-MS is a powerful analytical tool that has been broadly applied to biomolecular structure analysis mainly because of its ability to detect large biomolecules with great sensitivity and accuracy. ESI also enables liquid separations to be coupled to MS with high ionization efficiency.

Several approaches have been explored to connect in a convenient mount both μTAS and ESI-MS [2-3], and two main chip-MS coupling orientations can be distinguished. In the first approach, the interface based on a microfluidic device is coupled to capillary sprayer in electrospray or nanoelectrospray mode [4]. However, even if this configuration allowed the development of various designs of sheathless electrospray emitters, all the systems previously presented require a gold conducting coating of the tip which may lead to deterioration of the spray stability due to the poor adhesion of the metallic layer. In the second approach, a few groups have described direct electrospray form microchips made in glass without any tip at the exit channel [5]. The problem they encountered was to establish a well-defined Taylor cone at the exit of the microchannel. Extensive wetting of the emerging liquid over the flat edge of the microchip resulted in poor cone formation. Various attempts have been considered to minimize this spread effect, such as coating and derivatization of the exit orifice of the microchannel [6], but the problem of aligning the Taylor cone in front of the microchannel exit still existed.

In this work, sheathless electrospray from PDMS/glass microchips, allowing the generation of an efficient nanospray for protein detection is described. As a mass spectrometry electrospray source, a triangular-shaped gold emitter tip was formed by lithography and electroplating on a glass substrate. It is more easily fabricated by MEMS technology and it is more robust than that of silica or polymer recently reported.

2. Experimental

2.1 Microchip fabrication

The separation channel and metal emitter tip are fabricated using a glass wet etching and gold electro plating process, respectively. As a wet-etching mask, a amorphous silicon layer was deposited on both side of the 500 μm thick glass wafer. A positive photoresist (AZ1512; Clariant Corp., Somerville, NJ, USA) was then spin-coated, and the column design was transferred to the substrate using a film photomask (Ppm technology Corp., Seoul, Korea). After amorphous silicon patterning, the microfluidic channel, 70 μm wide at half-depth, 20 μm deep, was then isotropically etched in 49% HF for 3 min (etching rate: ~7μm/min) at 20°C. A triangular-shaped gold emitter tip was formed by lithography and Au electroplating process on a glass substrate after second standard photolithographic, wet etching process

for emitter part. The metal tip was aligned with the channel at the end of the glass channel, so microchannel is expanded to the end of emitter tip. Fabricated emitter structure was released to establish a well-defined Taylor cone by wet etching process. This glass plate having microchannels and emitter structure was irreversibly bonded with PDMS plate for closed channel. Fig. 1 shows the fabrication process of the proposed device.

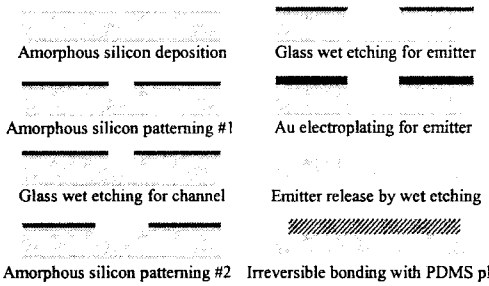

Fig.1 Fabrication process of the micro emitter with the microfluidic channel

2.2 Mass spectrometry

Test sample is a fragment peptide of bradkynin 2-9 (Mr, 903.4603) purchased from Sigma(St. Louis, US). ESI-MS was performed in the positive-ion mode using an LCQ Deca ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with a syringe pump. For the emitting test, the sample is introduced via syringe pump and carrier solvent, i.e. water -acetonitrile (1 : 1), was used at a flow-rate of 0.5 μl/min. The operational parameters for the ESI source and ion transfer optics were as follows: spray voltage 2 kV supplied at the emitter, through an embedded microelectrode, capillary temperature 200 °C, capillary voltage 31.5 V, tube lens offset 80 V, multipole 1 offset 7.25 V, lens voltage 20 V, multipole 2 offset 9.5 V, multipole RF amplitude 425 V peak-to-peak (pp) and entrance lens voltage 40 V. Spectra were scanned in the range m/z 5002000 (Fig. 2).

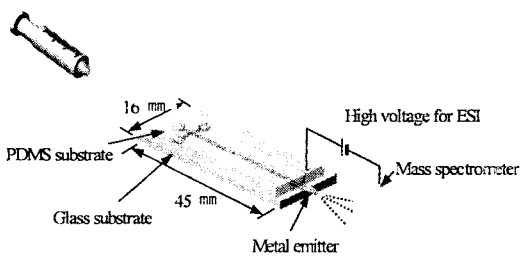

Fig.2 Experimental setup with the electrospray device

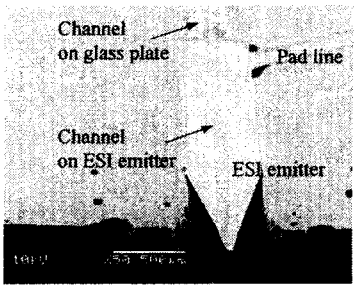

Fig.3 Corresponding scanning electron micrograph of the emitter structure

3. Results and Discussion

3.1 Fabrication result

A scanning electron micrograph of the spraying emitter is presented in Fig. 3. The ESI chip has been characterized by scanning electron microscopy with a JEOL 5600 instrument (Peabody, MA, USA). The cross section of the channels shows the typical isotropic etching of HF solution. It was reported that a well-defined Taylor cone couldn't be formed when the microfluidic emitter device is too thick [7]. It is remarkable that the thickness of the tip edge is only 10 μm which favors the generation of the spray, and avoids droplet formation at the outlet. Needlelike structures provide the ideal geometry for an electrospray emitter. A sharp tip has much less surface area for solvent to accumulate, and excess liquid is readily removed by electric field gradient. If the emitter is at least 1 mm long, there is no tendency for liquid to flow back to the supporting structure when the tip is at high potential [8]. Those two characteristics contribute simultaneously to the onset of the spray as well as to its stability.

3.2 Tests with MS

3.2.1 MS performances

The micro emitter were tested on an ion trap mass spectrometer; They were introduced into the mass spectrometer on an xyz moving component. The electrical contact was achieved using a metal wire.

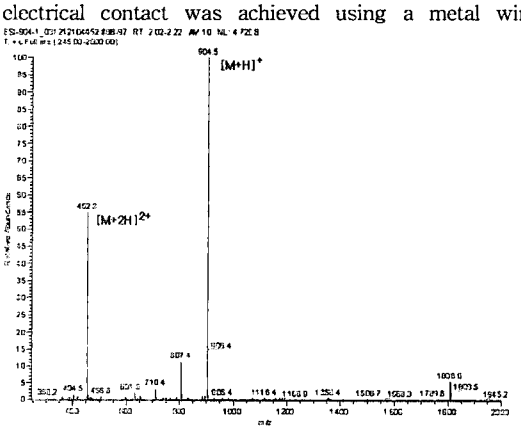

Fig.4 (a) Mass spectrum of Bradykinin(fragment 2-9)

Once the emitter was introduced into the mass spectrometer, the reservoir feature was loaded with the liquid sample using a micropipette; this liquid was seen moving readily towards the emitter structure. As described in the experimental section, the electrospray signal was recorded. Fig.4 shows the mass spectrum obtained for a bradkynin 2-9 sample using the micro emitter. With the help of the released emitter structure and the hydrophobicity of the gold material, which avoids solution spreading at the outer walls, the device has shown an efficient ionization performance providing a stable MS signal constantly. Singlely-charged peak (m/z 904) and doublely-charged peak (m/z 453) of peptide were detected, respectively.

We observed that the sample consumption of our emitter sources was rather high, e.g. a low 0.5 μl/min. We believe that the open structure favored the evaporation of the liquid sample in the mass spectrometer inlet as it is in direct contact with air. Appropriate surface treatment could avoid the liquid dispersion around the outlet part and thus could limit the contact surface area with air.

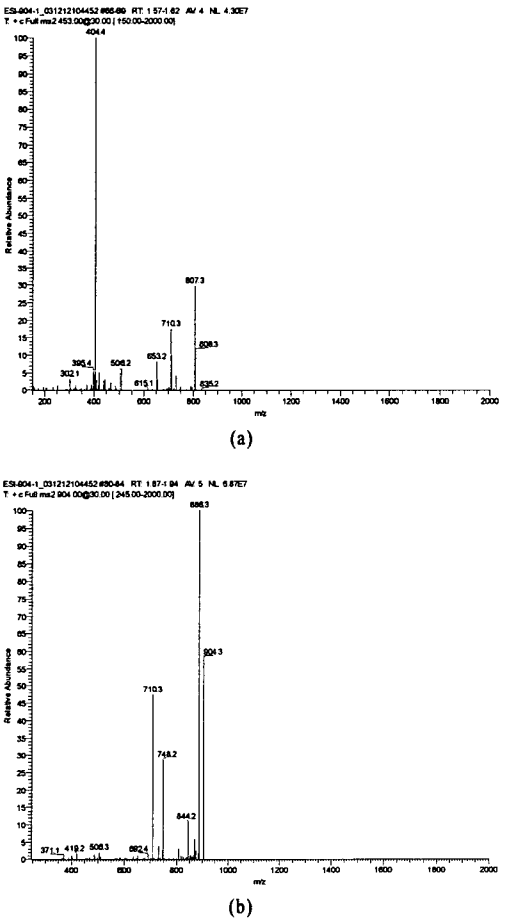

Fig. 5 (a) MS/MS fragmentation spectrum of doublely -charged peak (m/z 453), and (b) singlely-charged peak (m/z 904)

3.2.2 MS/MS experiments

Finally, we carried out fragmentation experiments in each peak as described in the experiment section. Fig. 5 shows the MS/MS spectrum resulting from the fragmentation of the bradkynin 2-9 sample under a 2 kV HV supply and using a micro emitter structure source. The performance was similar with conventional glass or fused silica emitters with respect to signal strength and stability.

4. Conclusions

We have designed and demonstrated a sheathless ESI-MS interface with conducting metal emitter tip on PDMS/glass based microchips. The separation channel and metal emitter tip are fabricated using a glass wet etching and gold electro plating process, respectively. This approach is less involved than applying a conductive coating to the exit end to establish electrical contact. As such, the interface is less dependent upon the longevity or durability of such coating, factors that have been consideration in the sheathless interfaces. The fabricated micro electrospray chip was tested by spraying peptide sample for mass spectrometric analysis. Singlely-charged peak and doublely-charged peak of peptide were detected and further MS/MS fragmentation was performed in each peak. The performance of the metal emitter sources were seen to be similar with conventional glass or fused silica emitters in signal strength and stability.

Acknowledgements

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