Novel 2D Interfaces for Nanoelectrospray-Mass Spectrometry

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ABSTRACT

We present the evolution of a novel two-dimensional 'nib-like' interface for nanoelectrospray-mass spectrometry (nanoESI-MS) applications. The design, fabrication and testing of three generations of such sources will be outlined here. The first generation of micro-nibs was fabricated using the negative photoresist (SU-8) by employing a simple 2.5D lithographic technique for rapid prototyping. The characteristic tip dimensions of such ESI-MS interfaces were 20 × 30 µm. The second generation of micro-nibs were also formed using SU-8 but using an improved 2D lithography and fabrication method. The smallest characteristic tip dimensions for these ESI-MS interfaces were 8 × 35 µm. A third generation of micro-nibs was fabricated from low-stress polycrystalline silicon (polysilicon) using silicon-based surface micromachining techniques. These micro-nibs were highly planar and had much smaller characteristic tip dimensions of 2 × 2 µm. MS testing on an ion trap mass spectrometer with standard peptides initially showed the feasibility of the 'nib-like' approach and the subsequent improvements obtained as the design and fabrication of the interfaces evolved.

Keywords: microfluidics, interface technology, micromachining, nanoelectrospray, mass spectrometry.

1 INTRODUCTION

Today, the exploding field of Proteomics provides an increasing amount of protein samples which need to be analyzed and identified. To these ends, Mass Spectrometry (MS) is a very powerful technique. This technique requires the test sample to be present in the machine as an ionic species. Electrospray ionization (ESI) [1-4] can achieve this and has the advantage of producing multi-charged ions which can bring high molecular weight molecules into the detection range [5]. However, even though MS equipment and techniques have highly evolved, ESI is itself still a relatively cumbersome technique. ESI is often performed using 'needle-like' capillary tubes manufactured from heating, pulling and breaking techniques. This fabrication method is inherently poorly controlled, resulting in the non-reproducibility of critical tip dimensions from tip to tip. This leads to poor reproducibility in nanoESI-MS analysis. The needle-like tip is manually loaded with the sample to be analyzed and then broken at the MS inlet resulting in a characteristic tip dimension (diameter) of 1-10 µm. It is clear that such a 3D needle-like topology prevents them from being compatible with the use of robotics for analysis automation. In addition, a typical set-up to interface a microfluidic system to MS which employs such sources does not allow for optimizing the microsystem-MS coupling as the ionization tip is not part of the system. There is thus a current need for (i) stand-alone electrospray (ESI) sources with miniaturized, highly controlled tip dimensions which lead to nanoelectrospray (nanoESI) conditions, (ii) integrated nanoESI interfaces which provide world-to-chip interfaces to and from chip-based microfluidic systems and (iii) nanoESI interfaces which are compatible with robot automation for high-throughput and the inevitable reduced costs. Microtechnology can offer a solution to many of these requirements. The benefits of microtechnology for the productions of ESI sources are: (i) excellent control of critical dimensions, (ii) potential circuit-source integration, (iii) new device topologies, (iv) a greater choice of materials and (v) batch production.

The 'nib-like' approach which we present here borrows much from the topology of a simple fountain pen. Indeed, such a topology comprises the essential components required for an ESI tip: a reservoir, a capillary slot and a point-like feature. In addition to this, it is important to note that the fountain pen nib is in essence a quasi-2D object, this can be easily imagined if the nib is flattened. However, here the analogy will stop and we will proceed to present the design, fabrication and testing of three generations of novel nib-like sources intended for nanoESI-MS interfacing. The first two generations are fabricated using an epoxy-based negative photoresist (SU-8) and the third generation using Si-based microtechnology.

2 MICRO-NIB DESIGNS

2.1. First Generation Micro-nibs

Figure 1 shows a Scanning Electron Microscope (SEM) image of a first generation micro-nib nanoESI source. This first generation of sources was fabricated solely with the goal of testing the micro-nib concept. They were fabricated using the negative photoresist SU-8 2075 (Microchem, VA, USA) on a 3-inch silicon wafer. The essential features of the micro-nib are: (i) the main SU-8 support wafer, having a thickness of 400 µm, and (ii) a free-standing membrane-like point structure which contains the test liquid reservoir, a capillary slot and a nib tip. We estimated the characteristic tip dimensions to be of the order of 20 x 30 µm at the extremity of the tip. As the micro-nibs were fabricated using a simple 2.5D UV phtotolithographic method by varying the exposure doses for each feature, we have the advantage of the rapid production of prototypes.

Two simple photomasks were required for the fabrication. The process can also be defined a 'low temperature' as the maximum processing temperature was 95°C.

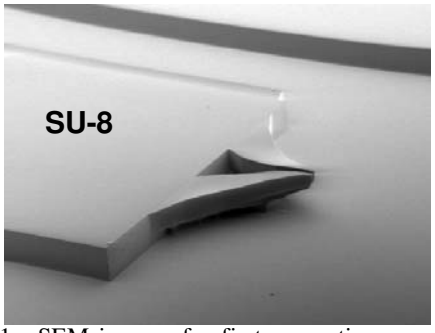

Figure 1 : SEM image of a first generation nanoESI-MS micro-nib. These sources were fabricated in SU-8 using a 2.5D photolithography The characteristic tip dimensions were 20 × 30 µm

In addition to this, device dicing was easy as the tip was not in proximity to the wafer surface following photoresist development. However, as the thickness of the membrane-like feature was difficult to control during UV dosage and photoresist development, the critical tip dimensions were thus difficult to control. Close inspection of tip dimensions proved that the effect of this was to reduce the device yield. In addition, the high tensile stress incurred whilst employing thick films of SU-8 tends to reduce the planarity of free-standing structures. The fabrication of these micro-nib prototypes has been described in detail elsewhere [6,7].

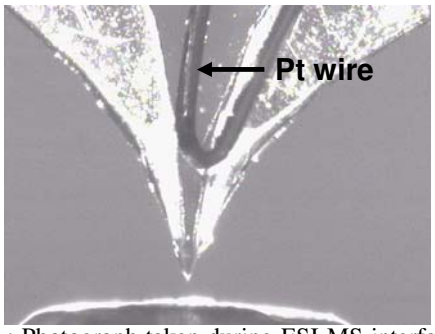

Figure 2 : Photograph taken during ESI-MS interfacing of a first generation micro-nib using a platinum wire to apply the high voltage (HV) to the test liquid.

The nib tips were tested in mass spectrometry on an ion trap mass spectrometer (LCQ Deca XP+, Thermo Finnigan). The nib tip was mounted on a tip holder which was then introduced in the mass spectrometer inlet. The high voltage (HV) was applied to the micro-nib via a platinum wire which was inserted in the reservoir feature of the nib tip, as shown on figure 2. MS tests were carried out using standard peptide samples which were prepared using a H_2O/MeOH 50:50 solution acidified with 0.1 % HCOOH. Gramicidin S was mainly used as standard peptide for these experiments; it was tested at 50 down to 1µM. HV supply was in the 1.2-2.5 kV range.

Firstly, a camera allowed us to directly observe the tests; thus, the flowing of the liquid in the capillary slot could be observed as well as the formation of a Taylor cone (see figure 2) as soon as the voltage was applied to the liquid. Secondly, the signal (ionic current) in MS was observed to be stable, reflecting thus the stability of the spray using these micro-nib tips (data not shown).

Figure 3 presents a typical mass spectrum obtained using one of first generation nib tips with two peaks at *m/z* 571.3 and 1141.7 corresponding to the Gramicidin S $(M+2H)^{2+}$ and $(M+H)^{+}$ species respectively. Nonetheless, it should be noted that the slightly more intense peak corresponds to the singly charged species.

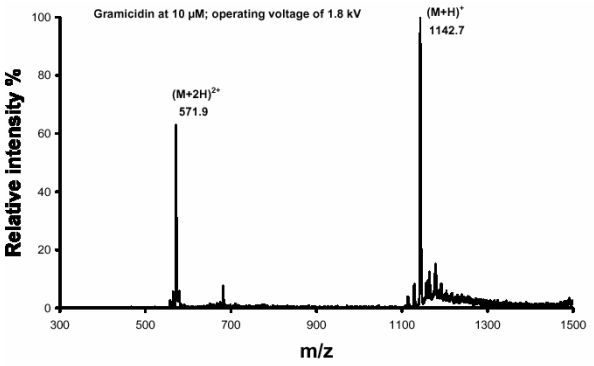

Figure 3: Mass spectrum obtained using a first generation nanoESI micro-nib having characteristic tip dimensions of 20 × 30 µm (Gramicidin S at 10 µM and an HV of 1.8 kV)

In conclusion, these MS tests using standard peptides allowed us to validate the idea of an ESI micro-nib tip, as these tips performed well also for MS/MS experiments. Nonetheless, the optimal functioning conditions for this first generation of micro-nibs did not satisfy us, as these were a 1.8 kV ionization voltage and a peptide sample concentration of around 10 µM. Thus, we proceeded to improve the design to produce a second generation of micro-nibs.

2.2 Second Generation Micro-nibs

Figure 4 shows an SEM image of a second generation nanoESI micro-nib.

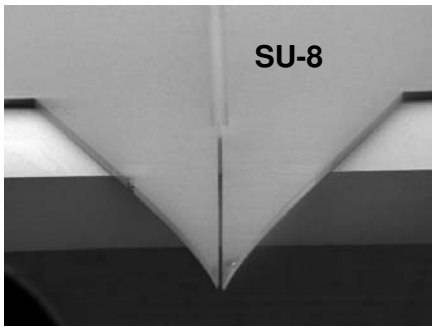

Figure 4 : SEM image of a second generation nanoESI-MS micro-nib. 2D photolithography was used to fabricate the micro-nibs in SU-8.

These micro-nibs were fabricated in a 2D topology using the negative photoresist SU-8 2035 on standard 3-inch silicon n-type substrates orientated (100). Firstly, a 200 nm nickel etch release layer was deposited onto the Si wafer surface. This nickel layer was then patterned using a photomask to form localized etch-release pads. Micro-nibs were then fabricated from SU-8 to have a thickness of 35 µm. Again, standard UV photolithographic techniques were

employed in order to form the nibs in SU-8 which were composed a reservoir and a capillary slot, having an aspect ratio of >4, leading to the nib tip and microfluidic channels linking a main test liquid reservoir to the input of the capillary slot. The design contained a smallest characteristic tip dimensions of 8 µm × 35 µm. After development of the photoresist, the nickel etch-release layer was removed. Following this, the individual systems were diced using a technique which ensures that the nib tips remained unaffected by the wafer cleaving and thus gave a good fabrication yield. The fabrication details have been presented elsewhere [7,8]. It can be noted that the use of SU-8 gives rapid prototyping and low temperature processing. However, the main advantages of the second generation micro-nibs over the first generation are (i) improved control of critical tip dimensions due to the more 2D nature of the fabrication, (ii) smaller critical dimensions , i.e. 8 µm, (iii) improved cantilever planarity due to reduced stress of a thinner SU-8 film and (iv) improved yield due to improved fabrication process.

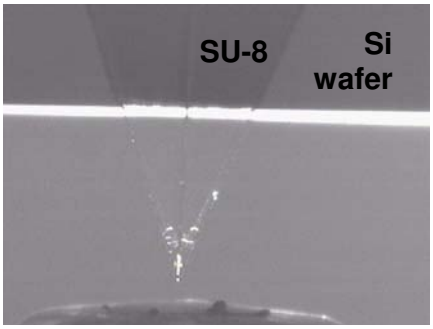

Figure 5 : Photograph of a second generation micro-nib during the nanoESI-MS tests. The high voltage (HV) was applied directly to the rear surface of the silicon.

The MS tests of these second generation nib tips were first carried out under the same conditions as described previously with the application of the ionization voltage on a Pt wire inserted in the nib reservoir, figure 5. The capillary slot width of the nib tip was either 16 µm or 8 µm. The test conditions consisted here of lower ionization voltage values, typically 0.8-1.5 kV and less concentrated peptide solutions, i.e. 1-5 µM.

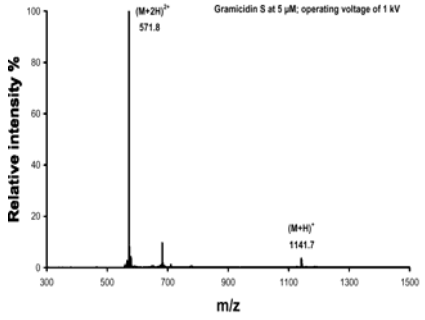

Figure 6 : . Mass spectrum obtained using a second generation nanoESI micro-nib having characteristic tip dimensions of 8 × 35 µm (HV supply of 1 kV, Gramicidin S at 5 µM).

Figure 6 presents the typical mass spectrum obtained with the 8 µm slot width nib tip with, as before, two peaks. However, it should be noted here that the mass spectrum pattern was different with a very intense peak for the doubly charged species and almost none for the singly charged one. Thus, the influence of the nib slot width was further investigated; 2 nib tips with a slot width of 8 or 16 µm were tested in the same HV and concentration sample conditions. This allowed us to observe how the slot width could influence the ionization phenomena and as a consequence, the intensity ratio for the singly and doubly charged species; the narrower the slot, the higher the intensity of the $(M+2H)^{2+}$. These MS results were compared to those obtained with a commercial tip (Proxeon). Using this latter tip, the mass spectrum pattern was seen to be the same as with a 8 µm slot width nib tip; this confirms that the ionization performances were enhanced using a nib tip having smaller dimensions.

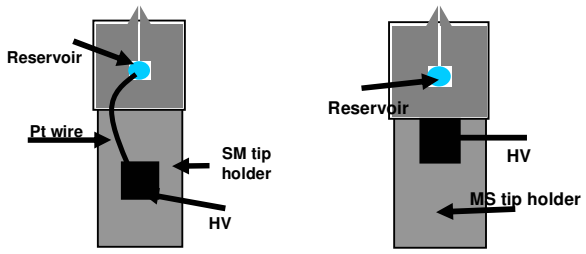

Figure 7: Set-ups used for testing the nib tips in mass spectrometry: (left) using a Pt wire that allows to apply the HV on the test liquid, (right) using the semi-conducting properties of the supporting Si wafer.

Lastly, we performed a last series of MS tests, whereby the ionization voltage was directly applied onto the supporting silicon wafer (see Figure 7). This greatly simplifies the test set-up as no manual step was required anymore to prepare the test. Once the tip mounted on the tip holder, the test liquid was simply dropped using a deposition robot into the reservoir feature and the tip introduced in the ion trap inlet. For this second series of tests, various samples were employed; standard peptides as before: Gramicidin S, Glu-Fibrinopeptide B and a Cytochrome C digest (figure 8).

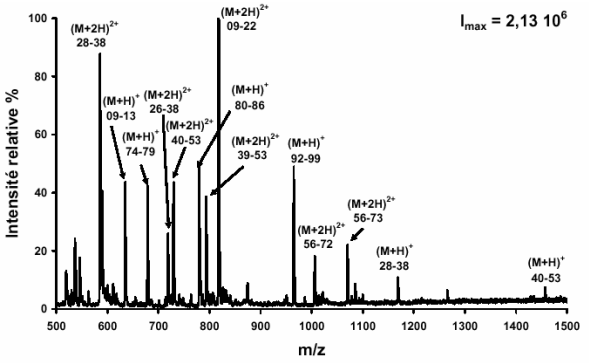

Figure 8: mass spectrum obtained using a 8 µm slot width nib tip for a biological sample, i.e. a Cytochome C digest (mixture of around 12 peptides); sample concentration of 1 µM and HV of 1.2 kV.

Irrespective of the nature of the sample, the nib tips were seen to perform even better than by applying the HV on a Pt wire. For example, in what we called 'limit conditions' using a Pt wire, the mass spectrum pattern did not look like the Proxeon one, i.e. there were two peaks with comparable intensities for both Gramicidin S ionic species. However, using this novel simplified set-up, better ionization performances could be yielded and the mass spectrum pattern was close to the Proxeon pattern.

As a conclusion, this second series of nib tip prototypes revealed that a decrease of the tip dimensions went along with better ionization performances. The nib tips with a 8 µm slot width performed as well as commercial nanotips. In addition, these nib tips were tested in a set-up fully compatible with analysis automation. Thus, we achieved our double goal with these nib tips; their geometry is fully appropriate for automation through the use of robotics and these microfabricated tips could be used in stand-alone conditions as well as being integrated as part of a microsystem. As the smallest critical dimensions for these micro-nibs were 8 × 35 µm, we have developed a process using silicon-based micromachining in order to fabricated micro-nib having critical dimension approaching 1µm.

2.3 Third Generation Micro-nibs

Figure 9 and 10 show SEM images of the polysilicon based micro-nibs produced for the third generation of nanoESI-MS interfaces. We fabricated this third generation of micro-nibs using Si-based microtechnology. The micro-nib cantilevers were made from polysilicon. A series of three photomasks were employed to produce: (i) pre-defined cleaving lines, (ii) localized sacrificial etch release pads (SiO_2) and (iii) micro-nib structures from polysilicon which contain: a reservoir, a capillary slot and a pointed cantilever. A very high planarity was achieved for the cantilevers as the deposition of the polysilicon was optimized in order to reduce the stresses to near-zero. In addition to this, a very high length/thickness ratio is obtainable due to the high Young's modulus of polysilicon compared to SU-8. The critical tip dimensions for this generation were 2 × 2 µm, much smaller than those obtainable with the second generation micro-nibs. Using Si-based microtechnology we achieved an excellent control of layer deposition. Figure 11 shows a global view of a stand-alone Si-based micro-nib (3rd generation). The details of the fabrication will be published elsewhere.

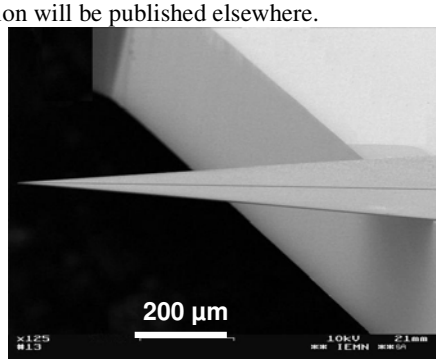

Figure 9 : Microscope photograph of a third generation of nanoESI-MS micro-nibs made from polysilicon. The characteristic tip dimensions were 2 × 2 µm

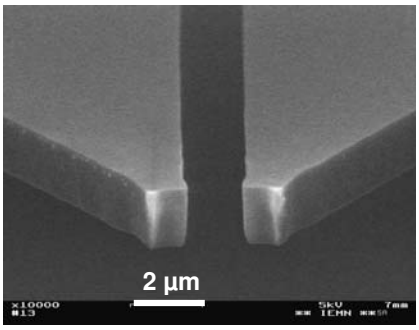

Figure 10: Close-up SEM image showing the critical dimensions (2 × 2 µm) of the capillary slot at the tip on the micro-nib made from polysilicon.

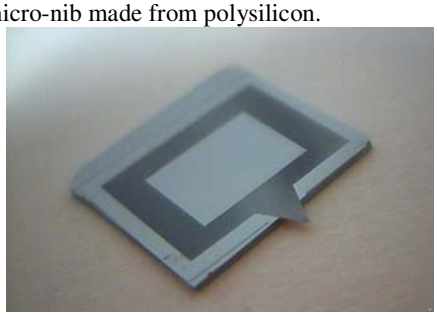

Figure 11: View of a third generation nanoESI-MS micro-nib fabricated from polysilicon using Si-based micromachining techniques.

3 CONCLUSIONS AND FUTURE WORK

We have presented a novel 2D electrospray source based on the idea of a nib. Such emitter tips have been successfully fabricated using the negative photoresist SU-8 and were seen to perform in nanoESI-MS mode. A third generation of micro-nibs has been successfully fabricated using Si-based microtechnology. The sources have several advantages over existing standard ESI sources: (i) excellent control of critical dimensions through microtechnology, (ii) simple easy-to-use stand-alone use (iii) integration with microfluidic circuitry and compatibility with the use of robotics for high throughput.

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