Supporting Information

Monolithic 3D nanoelectrospray emitters based on continuous fluid-assisted etching strategy for glass droplet microfluidic chip-mass spectrometry

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EXPERIMENTAL SECTION

1. Chemicals and Materials

Acetylcholine chloride, ammonium fluoride, trichloro(1H,1H,2H,2H-tridecafluoro-n-octyl) silane, isoctane, purchased from Sigma Aldrich (Shanghai, China). Hydrofluoric acid and ammonium ceric nitrate were purchased from Macklin. Isopropanol, methanol and acetone were bought from Beijing Chemical Works (Beijing, China). Hydrogen nitrate, acetic acid and sodium were received from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). The Optima LC/MS grade methanol and Optima LC/MS grade water were from Thermo Fisher Scientific (USA). QX200 Droplet Generation Oil for EvaGreen was obtained from BIO-RAD (USA). The chrome photomasks were purchased from Shen Zhen Kelead Photoelectronic Materials Co., Ltd. (Shenzhen, China). Cr mask blanks (soda glass substrate, 75×75 mm$^2$, 0.55 mm) were the products of Dongguan Hongcheng Optical Products Co., Ltd. (Dongguan, China). Fused-silica capillaries (360 μm O.D., 10 μm I.D.) were produced by Polymicro Technologies (Phoenix, AZ, USA). No animals were used. Rat (Sprague-Dawley, Male) Cerebrospinal Fluid was provided by IPHASE Biosciences (Beijing, China).

2. Instrumentation

Expose the black mask (glass substrate) was used BG-401A mask aligner (China Electronics Technology Group Corporation No. 45 Research Institute). The channel size s measured with a stylus profile (Dektak XT, Bruker, GER). The surface cleaning and activation of glass were used plasma cleaner (PDC-002, Harrick Plasma, USA) The surface hydrophobicity of glass was tested by a contact angle meter (KINO Industry CO. Ltd., USA). Microchip cutting used a dicing machine (Model 7122 ADT Ltd., Israel). The continuous flow in the chip was controlled by the syringe pump (Pump22, Harvard, USA). To control the flow rate of the continuous phase (QX200™ droplet generation oil) and dispersed phase, two pressure pumps (Flow-EZ, LUFEZ7000, Fluigent) were used. Metallographic microscope was used to check the channel uniformity and alignment of chip bonding during chip preparation (LV100ND, Nikon, Japan). The chip structure and generated droplets were observed by an inverted microscope (Ti-E, Nikon, Japan). Digital Microscope (AM73915series, Dino-lite, Taiwan) was used to capture the frequency of droplets during mass spectrometry detection. The mass spectrometer (LTQ Orbitrap XL instrument, Thermo Scientific, USA) was used for detection of ACh standard solution. The mass spectrometer with 50 Hz scanning speed (Bruke maxis impact) was used for detection of acetylcholine droplets. The mass spectrometer (Orbitrap Eclipse Tribrid, Thermo Scientific, USA) was used to detect cerebrospinal fluid in the droplets.

3. Design and Preparation of the Glass Microchip

The droplet microchips consisted of two 0.55 mm thick soda glass slides (38×19 mm$^2$). AutoCAD was used to draw the injection channel (capillary connection), droplet generation channel, cutting channel (The cutting angle was 30°) mask layout (as shown in Figure S1). Based on our previous work[1-3], standard photolithography, wet etching, plasma-assisted alignment and high-temperature bonding were used for the preparation of glass chips, respectively for pattern transfer, depth control, substrate alignment and bonding. Finally, the sample injection channel was a circular channel with a radius of 200 μm. The droplet generation channel was approximately a circular channel with a radius of 25 μm. All channels up to the end conform to typically rounded corners from the anisotropic wet etching process.

4. Hydrophobic Pretreatment of Channels and Electrode Preparation

The hydrophobic modification of the glass chip internal channel was used of trichloro(1H,1H,2H,2H-perfluoroocctyl)silane. Briefly, all channels were rinsed with NaOH (1 mol/L) solution, DI water and
ethanol successively for half an hour, with a flow rate of 10 \( \mu \text{L/min} \). 500 psi nitrogen was used to dry all chip channels. And then 2.5% (vol) solution of trichloro(1H,1H,2H,2H-perfluorooctyl)silane in isoctane was flushed through all channels for 30 min. After nitrogen dries the channel, the hydrophobic pretreatment of channels was complete. The etched chip was cleaned and activated the surface by air plasma for 15 mins. In the low-temperature magnetron sputtering instrument, metal chromium was first plated (Process Gases: Ar, Power: 4 W, Sputtering Time: 3 min), and then a layer of metal gold (Process Gases: Ar, Power: 4 W, Sputtering Time: 8 min) was covered on it. Dustproof storage after chip preparation and waiting for use.

5. **Numerical Simulation in the Continuous Fluid-assisted Etching Process**

The COMSOL software simulates the fluid state of the methanol protection solution at the tip to understand the etching process of the tip. A map of the velocity distribution of methanol at the exit of the emitter was obtained. The established model parameters were as follows: the channel I.D. size was 50 \( \mu \text{m} \), the tip angle was 30\(^\circ\), the flow rate of methanol in the channel was 10 \( \mu \text{L/min} \), and the outlet of the tip was located 1 mm below the gas-liquid interface.

6. **Standard solution MS detection**

The coupling mode between the microchip and the MS: instead of the commercial ESI source, the microfluidic chip was positioned in front of the MS orifice on an XYZ-positioning stage. There were a 1.5 mm gap between the microchip and the MS inlet. The capillary temperature was 350 °C and the mass range was 100-200 m/z. All experiments were undertaken in positive ionization mode.

The continuous flow samples detection: the standard solution is 50 \( \mu \text{mol/L} \) ACh dissolved in methanol-water 50:50 (containing 1% formic acid). The flow rate for continuous flow testing of standard samples in the chip was controlled by the syringe pump. The mass spectrometer (LTQ Orbitrap XL instrument, Thermo Scientific, USA) was used for detection of ACh continuous flow standard solution. Application of electric potential between the syringe stainless steel needle and the entrance plate of the MS. The instrument was calibrated using commercially available calibration solutions.

The droplet samples detection: T-junction structure was used to generate the picoliter-volume droplets. Two pressure pumps (Flow-EZ, LUFEZ7000, Fluigent) were used to control the flow rate of the oil phase (QX200™ droplet generation oil) and aqueous phase (50 \( \mu \text{mol/L} \) ACh in 1% FA in methanol-water 50:50) for droplet detection. The pressure pump of the oil phase was 80 mbar, and the pressure pump of the aqueous phase was 40, 60 and 80 mbar. The mass spectrometer with 50 Hz scanning speed (Bruke maxis impact) was used for detection of ACh droplets. A copper wire was connected to the metallic coating of the chip emitter as electrodes to apply the electrospray voltage.

7. **Cerebrospinal Fluid MS detection**

Rat (Sprague-Dawley, Male) CSF was provided by IPHASE Biosciences (Beijing, China). CSF solution filtered prior to use. Two pressure pumps (Flow-EZ, LUFEZ7000, Fluigent) were used to control the flow rate of the oil phase and CSF solution. The pressure pump of the oil phase was 80 mbar, and the pressure pump of the CSF was 60 mbar. The mass spectrometer (Orbitrap Eclipse Tribrid, Thermo Scientific, USA) was used to detect cerebrospinal fluid in the droplets. The coupling mode between the microchip and the mass spectrum was the same as above. A copper wire was connected to the metallic coating of the chip emitter as electrodes to apply the electrospray voltage.
SUPPLEMENTARY REFERENCES:

SUPPLEMENTARY FIGURES

Fig. S1 Mask design for the microchannels on glass microfluidic chips (A and B). Photomicrographs of glass microfluidic chip tips (C) Before and (D) After cutting.
**Fig. S2** Photograph of the monolithic 3D nESI etching system.

**Fig. S3** (A) Photomicrographs of glass microfluidic chip tips without and (B) with protection solution after etching for 5 minutes.

**Fig. S4** Simulation of continuous fluid-assisted etched 3D nESI emitters on glass microfluidic chips. The corresponding side view and top view under different flow rates.
**Fig. S5** Photomicrographs of glass microfluidic chip with flat exit (A) Before and (B) After etching. (Methanol flow rates: 10 μL/min, Etching time: 5 min).

**Fig. S6** Methanol with black watercolor paint as continuous flow and water instead of etching solution to explore the effect of stirring speed.
Fig. S7 Photomicrograph of 3D nESI emitters monolithically integrated on glass microfluidic chips: top view (A) before etching and after etching in (B) 20% Vf, (C) 30% Vf, (D) 40% Vf of HF; side view (E) before etching and after etching in (F) 20% Vf, (G) 30% Vf, (H) 40% Vf of HF. (I) The relationship between chip thickness and etching time in etching solution with 20%, 30% and 40% Vf of HF.

Fig. S8 Photomicrographs of the etched nESI emitters at methanol flow rates of (A) 5 μL/min, (B) 10 μL/min, and (C) 15 μL/min.
μL/min, and (C) 15 μL/min.

Fig. S9 Photomicrographs of the etched nESI emitters at different etching time. The flow rate of methanol was 10 μL/min and the Vf of HF was 30%.

Fig. S10 The reproducibility of the 3D nESI emitter size for five repeated experiments before etching, etching without continuous fluid, and etching with continuous fluid.

Fig. S11 The signal intensity of ACh at different electrospray voltage and the photographs of the electrospray plumes at 1.4, 3.4, 4.0 and 4.6 kV.
Fig. S12 The signal intensities of ACh at different injection flow rates.

Fig. S13 The signal intensities of ACh for 30 mins. The electrospray voltage was 4.0 kV and the sample flow rate was 500 nL/min.
Fig. S14 Mask design for the microchannels on glass microfluidic chips with a T-junction structure.

Fig. S15 The microscopic images of the connection between the external capillary and the glass microfluidic chip channel observed under different focusing conditions. Focused on the droplet inside the chip channel (A), the top of the capillary (B), the sidewall of the capillary (C) and the top of the connecting capillary channel (D). (The droplets remaining in the dead volume cavity were indicated by yellow dashed lines.)
The investigation of the impact of voltage on signal intensity. The voltage dropped by 100 V every two minutes.
**Fig. S17** The effect of droplet size on ACh MS signals. Different droplet volume of (A) total ion chromatogram (TIC), (B) microscope image of droplets and (C) extracted ion chromatogram (EIC) of ACh. In each set of experiments, 100 consecutive drops were used for statistical analysis.

**Fig. S18** Stability testing of droplets detected by established glass droplet microfluidic chip-MS system. (B) A partially enlarged view of the rectangular marked area of (A).
**Fig. S19** Electrospray plume diagram of continuous oil phase (Left) and continuous aqueous phase (Right).

**Fig. S20** The EIC of ACh in (A) continuous aqueous phase and (B) droplets. Mass spectra of (C) continuous aqueous phase (D) and droplets.

**Fig. S21** The effect of ACh concentration on signal intensity of ACh in droplets and EIC of ACh of 25, 100 and 500 nmol/L.
Fig. S22 The MS/MS spectra of ten neurochemicals.

SUPPLEMENTARY TABLE

Table S1 Ten neurochemicals assigned from CSF.

<table>
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<tr>
<th>Analyte</th>
<th>Measured (m/z)</th>
<th>Theoretical (m/z)</th>
<th>Mass Accuracy (ppm)</th>
<th>Format</th>
<th>Characteristic product</th>
<th>Mass bank number or HMDB number</th>
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<tr>
<td>Choline</td>
<td>104.106894</td>
<td>104.1069906</td>
<td>695789158</td>
<td>Chol'</td>
<td>58, 59, 60</td>
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<td>Acetylcholine</td>
<td>146.117381</td>
<td>146.1175553</td>
<td>1192875145</td>
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<td>Glutamine</td>
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<td>147.0771105</td>
<td>870023368</td>
<td>[Glu + H]'</td>
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<td>Serine</td>
<td>106.054963</td>
<td>106.0550969</td>
<td>2183878316</td>
<td>[Ser + H]'</td>
<td>70,88</td>
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<td>3,4-Dihydroxybenzoic acid</td>
<td>159.049754</td>
<td>159.0495353</td>
<td>1293705645</td>
<td>[DOPAC + H]'</td>
<td>151</td>
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<td>Tyrosine</td>
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<td>182.0811699</td>
<td>1010931088</td>
<td>[Tyr + H]'</td>
<td>123,147,165</td>
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<td>Histamine</td>
<td>112.088561</td>
<td>112.0889239</td>
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<td>[His + H]'</td>
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<td>3-methoxytyramine</td>
<td>186.109233</td>
<td>186.1091905</td>
<td>917789563</td>
<td>[3-MT + H]'</td>
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<td>Phenyldiamine</td>
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<td>[Phe + H]'</td>
<td>121, 149</td>
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<td>Dopamine</td>
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<td>154.0862555</td>
<td>3666778416</td>
<td>[DA + H]'</td>
<td>119,137</td>
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