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Single Layer Graphene Assisted Transmission Mode Continuous Wave Laser Desorption for Micrometer Spatial Resolution Atmospheric Pressure Mass Spectrometry Imaging

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When a fresh tissue slice is placed on a graphene layer substrate and irradiated with a 532 nm continuous wave laser, the transmission mode desorption of biomolecules is found to be greatly enhanced enough to facilitate the mass spectrometry analysis. The subsequent ionization process with nonthermal atmospheric helium plasma jets enables production of sufficient amounts of molecular ions from a fresh hippocampal tissue, such as fragments of glycerolipid and sphingolipid, adenine, and cholesterol. Micrometer spatial resolution mass spectrometry imaging of the hippocampal tissue, which enables to clearly identify the spatial distributions of small molecules, is also achieved using 532 nm continuous wave laser and a single-layer graphene as an energy transporter.

1. Introduction

Atmospheric pressure mass spectrometry (AP-MS) is a technique to make small substances separated from a specimen into charged particles using desorption and ionization sources at atmospheric pressure and to introduce them into a mass analyzer [1, 2]. Because a specimen is not in direct contact with the vacuum, AP-MS significantly reduces the possibility of specimen deformation and damage as compared with mass spectrometry in vacuum environment [3]. Here, we are demonstrating a new method that can facilitate the transmission (TM) mode laser desorption process without nanoparticle treatment to the specimen. The use of graphene layer substrate is excellent platform to induce the desorption process using 532 nm-continuous wave (CW) laser. By using this method, high spatial resolution atmospheric pressure mass spectrometry (AP-MS) imaging of humid tissues can be obtained.

2. Experimental Setup

We used the lab-built AP-MS imaging system previously reported [4]. The AP-MS imaging system consists of a mass analyzer, a sampling stage, a 532-nm CW laser, an atmospheric pressure (AP) plasma device, and airflow-assisted ion transport equipment. A graphene layer substrate was prepared by transferring commercial chemical vapor deposition (CVD) graphene on Cu foil (SLG, Graphene Platform Corp, Japan) to slide glass (**Fig. 1A**). The 532 nm CW laser beam was introduced into the inverted microscope by a dichromic beam splitter to focus precisely on the specimen through the objective lens (**Fig. 1B**). All the MS images in this study were obtained using a 20× objective lens. For AP-MS imaging, first, the fresh tissue slice of adult-mouse hippocampus (transverse directed chopping with a thickness of 200 μm or more) was aerated with 95% O_2 and 5% CO_2 in the artificial cerebral spinal fluid (ACSF) for 1 h. After aeration, the tissue was placed on graphene layer substrate and positioned on the scanning stage for imaging.

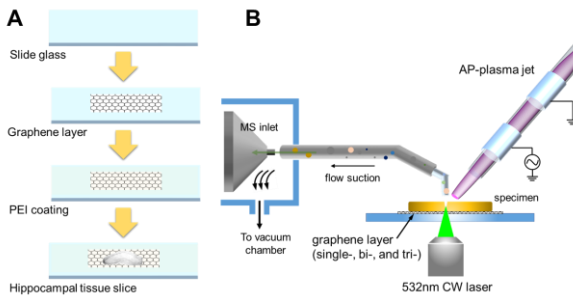

Fig. 1. Visible-light absorbing graphene layer substrate and TM mode CW-laser based mass spectrometry imaging system for fresh tissue slice. (A) Specimen preparation on visible-light absorbing graphene layer substrate and (B) atmospheric pressure mass spectrometry analysis with transmission mode CW-laser.

3. Results and Discussion

In the hippocampal formation, pyramidal cells in the cornu ammonis (CA) are known as a principal neuronal type. Thus, CA1 and CA3 regions of the hippocampus were analyzed. We applied CW-laser AP-MS system to obtain hippocampal tissue imaging with a pixel size of 1.4 μm × 5.0 μm covering an area of 600 μm × 500 μm (433 × 100 pixels). Total acquisition time was 117 min. Since the nonthermal AP-plasma jet was used as a separate ionization source, the mass spectra from the specimen can be obtained by removing the background spectra generated by the plasma. After removing the plasma background, more than 200 specimen-related MS spectra were obtained and some of them were assigned as metabolites, lipids, and their derivatives by a strict comparison of the measured masses with the calculated chemical formulas. By transforming each MS spectra with BioMAP software, AP-MS imaging for hippocampal tissue were obtained as shown in **Fig. 2**. After MS analysis with CW laser, the scanned area showed destruction indicating efficient desorption and ionization process occurred in this area (**Fig. 2A**). The MS images with selected ion-species for mono-acylglycerols (16:1, 16:0, 18:1, 18:0), adenine, cholesterol, sphingosine, and sphinganine were displayed in **Fig. 2B, 2C**. The imaged ion peaks were mainly in the form of H_2O subtracted from the base ions, $[M+H-H_2O]^+$. The intensity of the ion peaks of $[M+H-H_2O]^+$ was generally observed to be higher than that of $[M+H]^+$ except cholesterol ion.

4. Conclusion

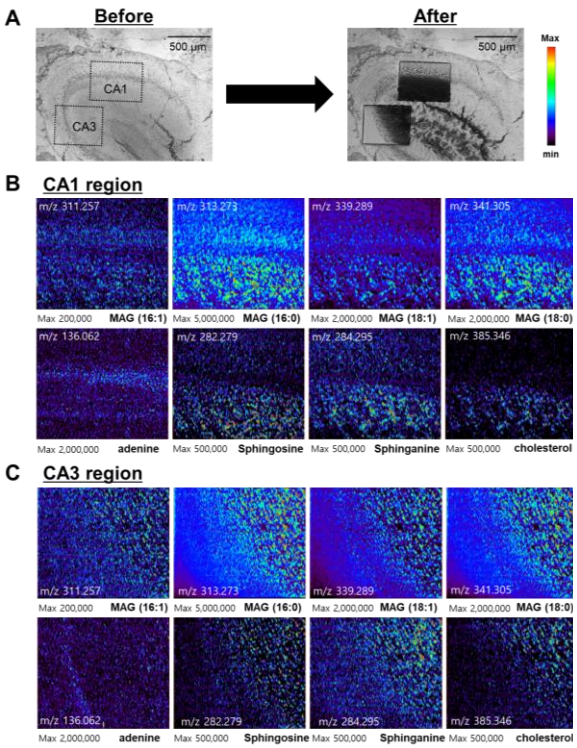

Fig. 2. (A) Photo of adult-mouse hippocampal tissue before and after AP-MS analysis. Ion images for CA1 region (B) and CA3 region (C) of hippocampus.

We demonstrate the TM mode CW-laser based AP-MS imaging is possible with the use of graphene layer substrate. The graphene layer onto the slide substrate was essential to induce efficient desorption of wet-state hippocampal tissue. Compared to the preparation of the nanoparticles treated biological specimen, while the nanoparticle treatment of the specimen was omitted, the process of transferring the graphene layer was added to the slide glass substrate. The graphene layer substrates can be prepared and stored in advance, resulting in a simplified specimen preparation and a great advantage in preparing fresh tissues faster.

5. References

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