The Utilization of Graphene to Enhance Mass Spectrometry Imaging in Murine Brain Tissue

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Overview

Mass spectrometry imaging (MSI) is quickly gaining popularity in the field to create chemical photographs of localizations within tissues of both plants and animals. MSI not only allows for the ability to localize substances, but allows users to obtain spatial distributions without the need for target-specific labelling reagents.[1] Unfortunately, MSI when combined with MALDI suffers from inherent limitations in spatial resolution necessary to obtain cellular resolution. Therefore, we aim to utilize graphene as a co-matrix in tandem with traditional MALDI matrices to enhance both spatial resolution and ionization efficiency.

Introduction

Comparison of MSI Techniques

Although researchers have been able to test the limits of spatial resolution, the typical resolution of a commercial instrument has been summarized in Table 1.

Technique	Ionization Source	Spatial Resolution	Mass Range
SIMS	Ion Gun	< 10 µm	0 – 1,000 Da
DESI	Solvent Spray	100 µm	0 – 2,000 Da
Nano-DESI	Solvent Bridge	10 µm	0 – 2,000 Da
MALDI	UV Laser Beam	20 µm	0 – 100,000 Da

Table 1: Comparison of typical parameters in commercial MSI techniques [2]

Graphene as a MALDI matrix

- First used in 2010 by Dong et. Al at UC Riverside.[2]
- Absorbs strongly in the UV region overlapping with the Nd:YAG laser utilized in the Bruker Solarix instrument used in this study.
- Exhibits a high tolerance for salt, a common suppressant in biological tissues.
- Has a lack of background signal in mass spectra, therefore analysis of low *m/z* species is made easier then with traditional matrices.

Methods

Preparation of Samples for MALDI

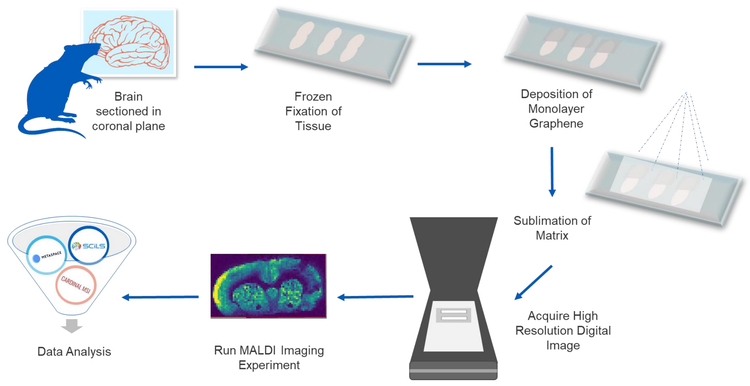

Fig 1: General overview of imaging workflow.

- Control Sprague-Dawley rats were sacrificed to obtain coronal sections of brain tissue. Brain tissue was sectioned at 12 µm and thaw mounted to ITO slides.
- Slides were stored at -20°C until imaging is completed.
- Monolayer graphene was transferred to 120°C thermal tape from copper utilizing acid etching.
- Top halves of the coronal brain sections had a 1 cm x 1 cm monolayer of graphene deposited onto them.
- Matrix was sublimated onto slides utilizing either 1,5- diaminonapthalene (DAN), 2,5- dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CHCA) in an in-house sublimation chamber.

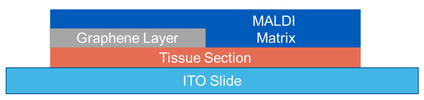

Fig 2: Illustration of the layering of matrix onto a tissue section for testing of graphene.

Instrumentation

All analysis was done by a Bruker Daltonics 12T Solarix FT-ICR MS by MALDI imaging in positive ionization mode. Scans were taken at 200 µm resolution for rat sections or 250 µm resolution for chinchilla brains with a laser repetition of 1000 Hz.

Results

Comparison of the utilization of DAN with and without Graphene

- The utilization of graphene shows higher intensities of prospective lipid signal within the motor cortex region of the brain to that without.

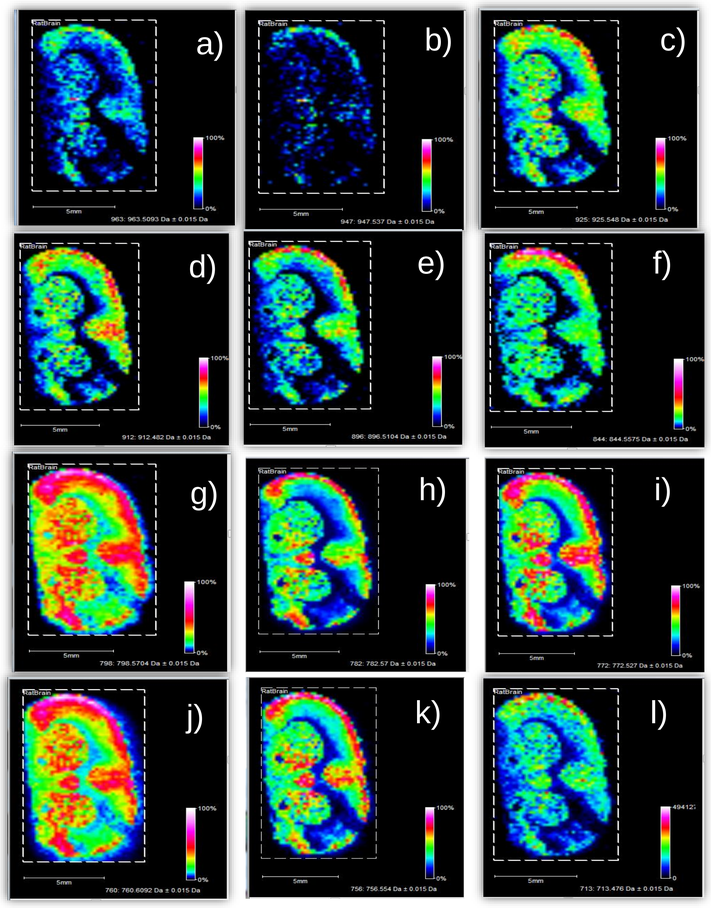

Fig 3: Intensity maps by *m/z*, where the top half of the images are coated with 2D graphene and a sublimed DAN over layer, and the bottom half of the images are coated with DAN.
a) 963.51 b) 947.54 c) 925.55 d) 912.48 e) 896.51 f) 844.56
g) 798.57 h) 782.57 i) 772.53 j) 760.61 k) 756.55 l) 713.48

On Tissue Derivatization to Enhance Neurotransmitter Signal

- Adapted from previously reported methods. [4,5]
- 2,4-diphenyl-6-methylpyrylium Tetrafluoroborate (DPMP) was utilized as a derivatization agent to determine the potential for increasing signal from neurotransmitters such as GABA.
- Due to the necessity of fresh samples to observe neurotransmitters, control chinchilla brains from the auditory cortex were utilized.

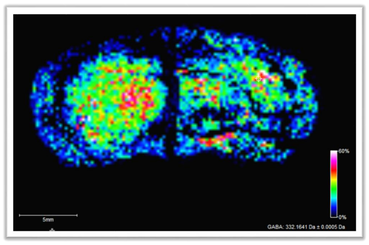

Fig 4: Intensity map of GABA in chinchilla, where the left half of the image is coated with 2D graphene, CHCA, and DPMP, and the right half of the image is coated with CHCA and DPMP.

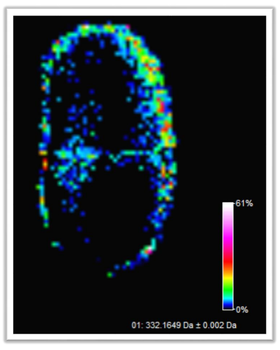

Fig 5: Intensity map of GABA in an older rat sample, where the top half of the image is coated with 2D graphene, CHCA, and DPMP, and the bottom half of the image is coated with CHCA and DPMP.

Enhancement of Derivitization Agent by Graphene

- Ionization of DPMP is enhanced when paired with graphene as opposed to CHCA alone.

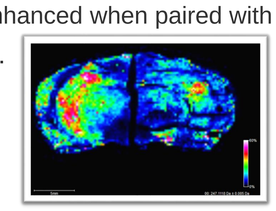

Fig 6: Intensity map of DPMP

On Tissue Derivatization to Enhance Lipid Signal

- Derivatization agent, DPMP, and graphene utilized in tandem have enhanced the ionization of lipids species with putative matches to phosphocholines, sphingomyelins, and multiple others.

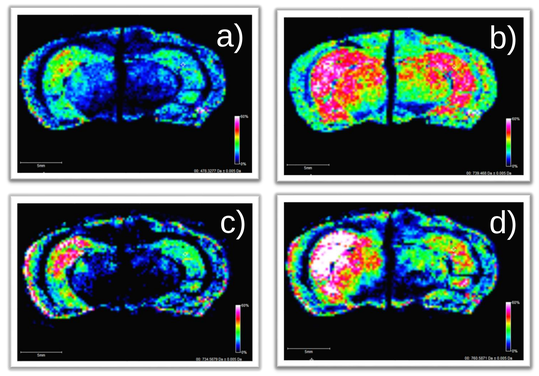

Fig 7: Intensity maps by *m/z* with graphene deposition on the left hemisphere.
a) 478.33 b) 739.47
c) 734.57 d) 760.59

Conclusions

- The utilization of graphene enhances the ionization efficiency of both lipids and neurotransmitters as opposed to traditional matrices alone.
- The appearance of enhanced spatial resolution has been observed in samples with graphene but further tests utilizing AFM are needed to validate these observations.
- Although neurotransmitter degradation occurs quite rapidly in stored brain tissue, the utilization of both graphene and DPMP appears to enhance ionization efficiency.

Future Work

- Completion of the further development of methods to be utilized for the analysis of neuropeptides within tissue samples.
- Evaluation of different formulations of 2D graphene on the performance of MSI.
- Completion of AFM experiments to determine the actual size of ablation craters created in samples with and without graphene.
- Determination of compounds with enhanced ionization due to the utilization of graphene as a co-matrix.

References

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