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

Emily R. Sekera1, Kevin J. Zemaitis1, Kayla E. Mascaro1, Alexis C. Thompson2, Troy D. Wood1

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. 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.

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

- 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-diaminonaphthalene (DAN), 2,5-dihydroxybenzoic acid (DHB) or o-cyano-4-hydroxycinnamic acid (CHCA) in an in-house sublimation chamber.

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 signals within the motor cortex region of the brain to that without.

Enhancement of Derivatization Agent by Graphene
- Ionization of DPMP is enhanced when paired with graphene as opposed to CHCA alone.

On Tissue Derivatization to Enhance Lipid Signal
- Derivatization agent, DPMP, and graphene utilized in tandem have enhanced the ionization of lipid species with putative matches to phosphocholines, sphingomyelins, and multiple others.

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
5. Esteve, C.; et Al; Metabolomics 2016, 12.

Acknowledgements
We gratefully acknowledge the financial support of the NIH through the National Center for Research Resources (Grant # S10-RR029517-01) for providing funding used to obtain the FT-ICR instrument. We thank Dr. Muthiaha of the Department of Rehabilitation Science at UB for his donation of chinchilla brains for this work. We are also grateful to MSACL for providing an MSACL Young Investigator Grant to attend the conference.