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TECHNICAL NOTE

Tissue imprint imaging by desorption electrospray ionization mass spectrometry

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Cross-sections of *Myristica malabarica* (Lam) seed and mouse brain tissue were imprinted on such ordinary surfaces as printer paper and TLC plates, and successfully imaged by desorption electrospray ionization mass spectrometry (DESI-MS) at 250 μm resolution. Chemical images representing the distribution of the alkaloid malabaricone C in the seed substructures and individual lipids in the substructures of the brain were obtained. Practical implications include analysis of irregular or soft materials, easy recording, transportation and storage of the latent image, and posterior analysis of the samples by different techniques without the requirement of addition of matrices or use of specific types of surfaces.

1 Introduction

Imaging mass spectrometry (MS) is a surface analysis technique based on desorption and ionization of analytes followed by mass analysis of the resulting gas phase ions. Information on the relative intensities of the ions and their spatial distributions on the surface allows the creation of detailed 2D images specific to particular chemicals. Several MS techniques have been described for imaging of a wide range of materials.[1,2] Among these techniques, secondary-ion mass spectrometry (SIMS),[3] matrix-assisted laser-desorption ionization (MALDI),[4] and desorption electrospray ionization (DESI)[5] are most commonly used. In DESI, a spray of charged droplets is directed at the sample creating a thin film of solvent on the surface. Further spray droplets collide with the film splashing secondary droplets containing dissolved analytes into the air from which they are sucked into the mass spectrometer.[6] DESI is a member of the ambient ionization techniques family in which desorption and ionization occur in the native environment with minimal or no sample preparation.[7,8]

One important parameter in DESI imaging is the geometry of the system. It is well known that changes in the angles and the distances between the sprayer and the sample or the MS inlet and the sample during data acquisition result in changes of the signal intensity.[9] The geometry of the system prevents direct imaging of soft or irregular surfaces such as whole animal and vegetable tissue. Analysis of the spatial distribution of compounds in tissues by imaging MS is commonly performed using thin tissue sections obtained by cryosectioning bulk tissue in a cryostat. The sections are then thaw mounted onto glass slides for analysis.[10]

The use of blotting or imprint techniques where the chemicals are initially transferred to flat hard surfaces is an alternative approach which has been successfully applied in imaging MS by MALDI,[11–16] SIMS,[17] nano-assisted laser desorption-ionization (NALDI)[18] and DESI.[19–21]

Here we present examples of imaging of two biological tissue types represented by cross-sections of seeds of *Myristica malabarica* (Lam) and mouse brain tissue, both blotted onto such ordinary surfaces as printer paper and TLC plates, followed by successful DESI analysis. Since DESI does not require the addition of matrices or the use of specific types of surfaces, blots and imprints can be made on ordinary surfaces making the analysis more practical.

2 Experimental

Fruits of *Myristica malabarica* were collected from a farm located in the Uttar Kannada district of Karnataka state, India. After removal of the rind and aril, the fresh seed collected was cross-sectioned and manually blotted on ordinary printer paper by applying mild pressure for approximately 5 seconds. The imprints (Fig. 1B) were then allowed to dry at room temperature. An area of 2.5 × 2.5 cm was mapped with a spatial resolution (pixel size) of 250 × 250 μm in a lab-built 2D moving stage DESI source as described elsewhere.[22] A mixture of methanol and water (9 : 1 v/v) was used as spray solvent and delivered at the flow rate of 1.5 μL min^{-1}. Mass spectra were acquired in full scan, negative ion mode, over the mass range from *m/z* 150 to 1000

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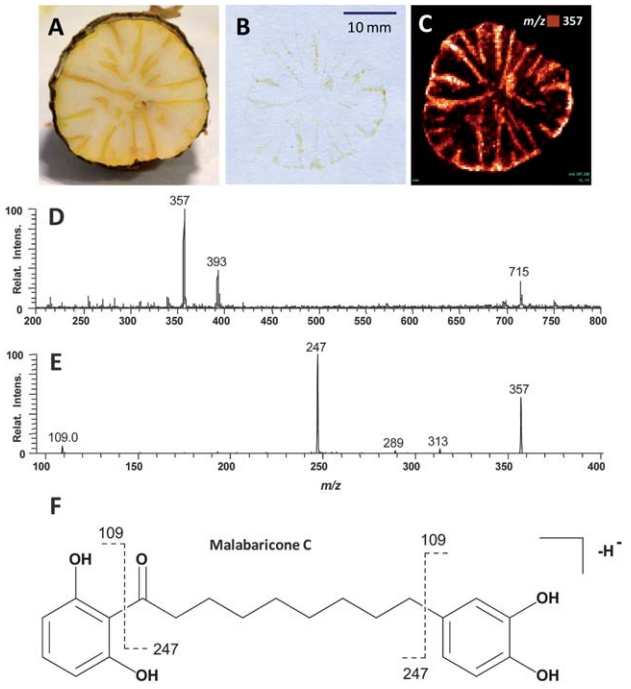

Fig. 1 (A) Cross-sectional view of a *Myristica malabarica* seed. (B) Blot onto printer paper. (C) Chemical image showing the distribution of *m/z* 357 species, deprotonated malabaricone C. (D) Average mass spectrum in negative ion mode of one line scan across the surface. (E) Tandem mass spectrum of *m/z* 357, deprotonated malabaricone C. (F) Structure of malabaricone C. Scale bar (10 mm) applies to all figures.

using a Thermo LTQ mass spectrometer (San Jose, CA). Auto-gain control (AGC) was off and each mass spectrum collected during 0.67 seconds (scan time). For more details on DESI imaging see ref. 7 and 23.

Frozen coronary section of a mouse brain (Rockland Immunochemicals, Gilbertsville, PA) (Fig. 2A) was blotted and imaged on TLC plates (Silica gel HL, 250 μm from Analtech, Newark, DE) after partial thawing. The brain was manually blotted by

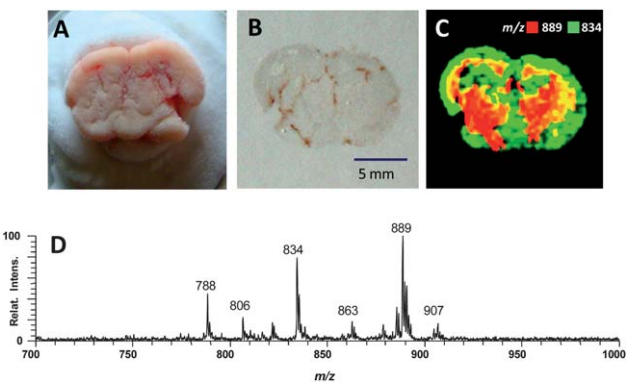

Fig. 2 (A) Cross-sectional view of a mouse brain bulk section. (B) Blot onto TLC plate. (C) Overlay of chemical images showing the distribution of the ions at *m/z* 889 (sulfatide (24 : 1) in red) and *m/z* 834 (phosphatidylserine (40 : 6) in green) present in white and gray mater, respectively. Overlapped areas are represented in yellow. (D) Average mass spectrum of one line scan across the surface in negative ion mode. Scale bar (5 mm) applies to all (A–C).

slightly pressing the tissue, which had previously been cut in a lateral section, against the surface of the TLC plate and let stand for 5 seconds. Very mild pressure was applied during this process. The imprints (Fig. 2B) were then allowed to dry at room temperature and an area of 1.2 × 0.9 cm containing the blot was mapped with the same pixel size and solvent flow rate as mentioned above. The solvent used in this experiment was a mixture of methanol : water (1 : 1 v/v) and mass spectra were acquired in full scan, negative ion mode over the mass range from *m/z* 700 to 1000. The AGC was turned off and the scan time was 1.08 seconds. The difference in the proportions of methanol in the mixtures is related to the solubility of the analytes rather than the specific surface employed.

3 Results and discussion

Cross-sections of a fresh *M. malabarica* seed revealed yellow veins in its interior (Fig. 1A). DESI analysis of the blotted material (Fig. 1B) showed intense peaks at *m/z* 357, *m/z* 393 and *m/z* 715 (Fig. 1D) at the positions of yellow veins. The peaks were identified as deprotonated malabaricone C (structure in Fig. 1F), its chloride adduct and the deprotonated dimer, respectively, based on the fragments found by tandem mass spectrometry. The main fragment ions arising from *m/z* 357 are *m/z* 247, due to the loss of neutral dihydroxybenzene, and at *m/z* 109 which is the deprotonated dihydroxybenzene (Fig. 1E). The DESI-MS and DESI-MS/MS data are consistent with previous reports in the literature of malabaricones in *Myristica crassa* observed by extraction of the plant material followed by chromatography and then ESI-MS/MS.[24] Imaging analysis by DESI-MS has the advantage of adding the information on the distribution of the compounds in the seed substructures, as seen in Fig. 1C, for *m/z* 357, *malabaricone C*. This ion is illustrated because it is the most abundant peak in the mass spectrum. The spatial distribution of other ions such as the deprotonated dimer and the chloride adduct was found to be the same as observed for malabaricone C. The ion at *m/z* 215 was found in areas complementary to the yellow veins but not on the unblotted paper (data not shown). However its ion intensity was too low to allow identification.

Phospholipids normally detected by direct DESI analysis of mouse brain were also detected after blotting. Two phospholipids, sulfatide (24 : 1) at *m/z* 889 (shown in red) and phosphatidylserine (40 : 6) at *m/z* 834 (shown in green) were mapped representing the white and grey matter regions of the brain, respectively (Fig. 2C). Note that isomers are not explicitly distinguished in these experiments unless their MS/MS spectra are different. The species found after blotting and their relative intensities were similar to those observed by direct DESI analysis.[25] The DESI lipid distributions were very similar to those observed in the corresponding MALDI data.[26]

4 Conclusions

The examples of imprinting shown here demonstrate the applicability of blotting techniques for both plant and animal tissue analysis by DESI-MS imaging. There are many advantages of imprinting samples prior to MS analysis. In terms of experimental workflow, many samples that cannot be directly accommodated in front of the MS due to its irregular/soft surface or

large size can be easily imprinted on an absorbent surface (such as paper or a TLC plate) and then imaged. Sample transportation and storage are also facilitated when using chemical imprints. For example, the need for transporting animal tissue samples in dry ice is non-existent by using chemical imprints. In terms of sample preparation, the use of chemical imprints makes the use of cryosectioning dispensable when that equipment is not available. Furthermore, posterior analysis of the blotted material can be done after imaging MS by different techniques. If paper is used as the imprint surface, a recently introduced technique, paper spray ionization,[27] could be used for further analysis of the remaining material on the paper without need of further sample preparation. Tissue imprinted into TLC plates could be sequentially separated after DESI-MS imaging, and imaged again with the goal of obtaining more extensive chemical information.[28,29] The resolution (pixel size) used in our experiments was 250 × 250 μm and it is comparable to the resolution used in the most DESI imaging applications found in the literature. Some resolution is lost by blotting but this is offset by the gains in the convenience of performing experiments.

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