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Application News

MALDI-TOF Mass Spectrometry Tissue Imaging SSI-MALDI-001

Precise Lipid Localization from Tissue Sections using a Chemical Inkjet Printer for MALDI Matrix Deposition

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Introduction

The importance of lipids in cell life is demonstrated by the number of diseases related to their metabolic alteration such as in Alzheimer's, diabetes, cancer and many other disease states. The most well known roles for lipids in cell function are chemical energy storage and structural components of cell membranes. However, the whole pallet of their function is not yet totally understood partly due to their complexity and the need for new technology and tools for their study.

MALDI profiling and MALDI tissue imaging mass spectrometry are two of the most powerful techniques currently available for this type of research. These approaches allow for the direct detection and mapping of biomolecules from different types of a tissue sections. Both are based on easy but crucial sample preparation combined with performance of the mass spectrometer. One of the most important sample preparation steps is accurate and regio-specific deposition of matrix onto the tissue section. Manual matrix deposition techniques face the challenge of depositing a reproducible and uniform matrix layer yielding to efficient analyte extraction, desorption/ionization and detection. Therefore the most reliable and reproducible way to obtain accurate matrix printing on cell tissue sections and their specific cell regions would be by using automated techniques.

The objective of this MALDI tissue imaging study was to control the matrix deposition to keep biomolecules specific localization. This goal was achieved by using a chemical inkjet printer (Shimadzu ChIP 1000). The matrix microprinting involved direct and automated control of the dispensed volume of solution (in the pico liter range), and its homogenous and precise location on the tissue sample. Indeed, the solution allocation follows a grid pattern of spots that can be set up in the printer software. In this particular study an optimized methodology was successfully applied to the detection and mapping of gangliosides (Figure 1) from wild type mouse brain sections. Furthermore, phosphatidylcholines (PC) and sphingomyelins (SM) (Figure 1) were directly analyzed and mapped from a single mouse brain section model of a traumatic brain injury (TBI).

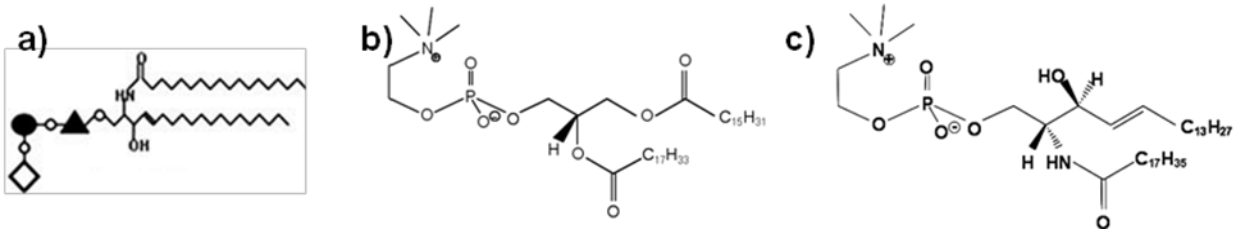

Figure 1: Chemical structure of a) a ganglioside (GM3 d18:1/C18:0; ▲represents a glucose, ● a galactose, ◊ a sialic acid), b) a phosphatidylcholine (PC C16:0/C18:1) and c) a sphingomyelin (SM d18:1/C18:0).

Materials and Methods

Tissue sections

Frozen wild-type mice brains and frozen TBI model mice brains were cut into 18 µm thickness coronal sections directly deposited onto stainless steel MALDI plates (Figure 2a). The tissue sections were left at room temperature for the plate temperature to equilibrate.

Matrix deposition for gangliosides imaging from a wild type mouse brain

One of the central piezoelectric heads of the ChIP was filled up with saturated DHA dissolved in 50% ethanol with 125 mM ammonium sulfate and 0.05% HFBA. Ethanol evaporation was limited by placing two iced water vials on each free well vicinal of the piezoelectric head used for matrix deposition (Figure 2b). The matrix solution was dispensed by the ChIP along a grid pattern of spots (Figure 2c and d). At each spot, 8 drops of about 100 pL were microprinted to form a first matrix layer onto the tissue sample corresponding to one cycle. This process was repeated to reach a total volume of 28 nL of matrix solution per spots. A distance of 240 µm was set in-between two spots, center-to-center. Printing conditions were optimum at 29 V for the dwell voltage, 34 µs for the dwell time and –0.25 kPa for the air flow.

Matrix deposition for PC and SM imaging from TBI model mouse brain

A solution of 30 mg/mL DHB in ethanol/water 50:50 was loaded in the liquid container of a new piezoelectric head (Figure 2b). A volume of 15 nL of matrix solution was deposited per spot with a raster of 240 µm. The matrix was iteratively microprinted using 5 drops of 100 pL per cycle. Optimum conditions were set up at 38 V for the Dwell voltage, 33 µs for the dwell time and -0.24 kPa for the air flow.

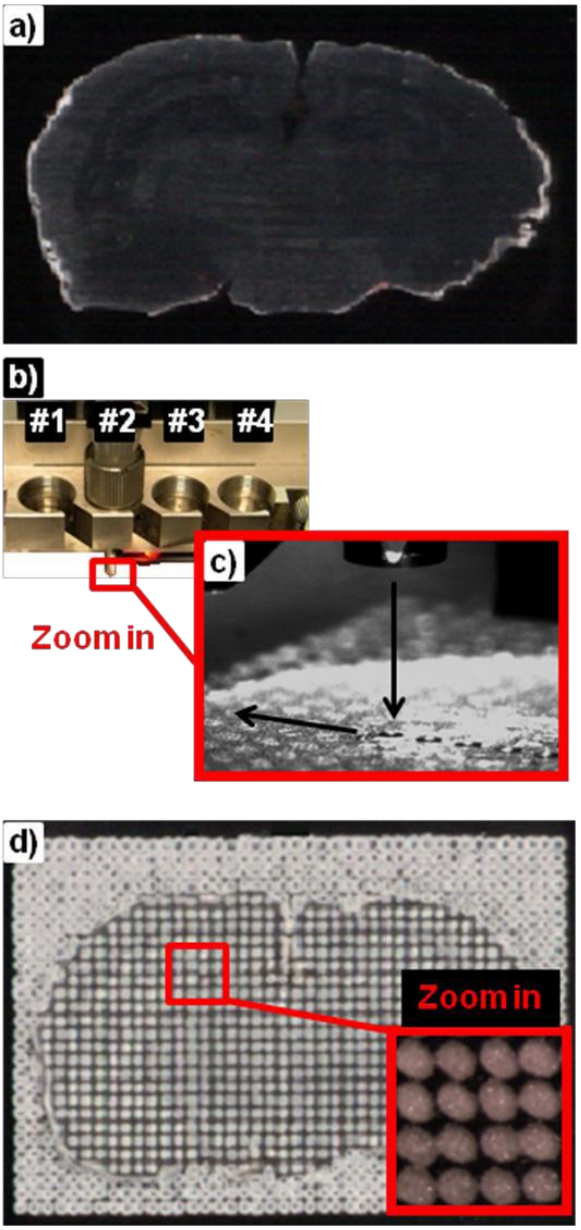

Figure 2: Matrix microprinting schematic workflow using a chemical inkjet printer ChIP 1000.

a) After sectioning, the tissue is deposited onto a stainless steel MALDI plate.

b) A ChIP liquid container (#2 in the picture) is filled up with matrix solution. Positions #1 and #3 may receive iced water vials to limit solvent evaporation from the matrix solution.

c) The matrix is microprinted through a piezohead along the (X) and (Y) directions following a grid pattern of spots.

d) After several cycles of matrix drops deposition, the tissue sample is covered of an homogenous layer of matrix and ready for MALDI imaging experiments.

Mass Spectrometry

MALDI mass spectra were obtained using a conventional MALDI TOF/TOF mass spectrometer with a 355-nm Nd:YAG laser (200 Hz, ~100 µm) in reflectron mode. Images were acquired using a 4000 series MSI image acquisition software. A distance of 80 µm was set for the target plate stepping in both the X and Y directions. Gangliosides analysis was conducted in negative ion mode whereas phosphatidylcholines and sphingomyelins analysis was conducted in positive ion mode. A number of 50 shots were used for each data set acquired per image pixel. Images were generated by TissueView 1.0 software.

Results and Discussion

Special cares in matrix microprinting

Attention was especially paid to each type of matrix solution preparation and handling. Importantly saturated matrix solutions easily obstructed the narrow print head (internal orifice of 55 µm) due to matrix recrystalization. Two matrix preparations were successfully employed to solve this issue. When using saturated matrices, iced water vials placed on each side of the print head in use limited solvent evaporation from the matrix solution. Decreasing the matrix concentration was also another solution to avoid piezo head clogging. However, it was used in cases where the limited amount of matrix was still enough to get an efficient desorption/ionization of biomolecules from the tissue section. Iterative printing cycles was possible when using lower concentrations of matrices allowing for homogenous matrix crystallization on target.

The number of printing cycles was another important parameter to deposit as much matrix solution as possible in a minimum time period while avoiding vicinal spots to coalesce due to too much solution deposited at once. On the one hand, the highest volume of matrix deposited by cycle yielded to the highest spread of the matrix solution onto the tissue surface. Limiting this spreading was critical to maintain biomolecule localization integrity. On the other hand, the lowest volume of matrix solution resulted in the longest delay of solution microprinting. A compromise was needed to print the matrix solution within the smallest time period but with the lowest matrix spots coalescing. This was achieved for each matrix solution by setting an appropriate number of deposited drops per cycle and an appropriate number of cycles.

Gangliosides mapping from a wild-type model

Every previous prescription taken into account, gangliosides (Figure 1) mass spectra were acquired (Figure 3) from a coronal brain section at level 46 from Allen's Mouse Brain Atlas as a function of their anatomical localization. They were obtained from discreet spots of matrix from the cerebral cortex (Figure 3a), left lateral ventricle (Figure 3b) and center of the midbrain (Figure 3c). An additional mass spectrum extracted from an off tissue spot displayed matrix ions and chemical noise only (Figure 3d) as expected. This set of data shows the strength of such a methodology. Controlling matrix deposition avoids molecules spreading allowing for their intact localization.

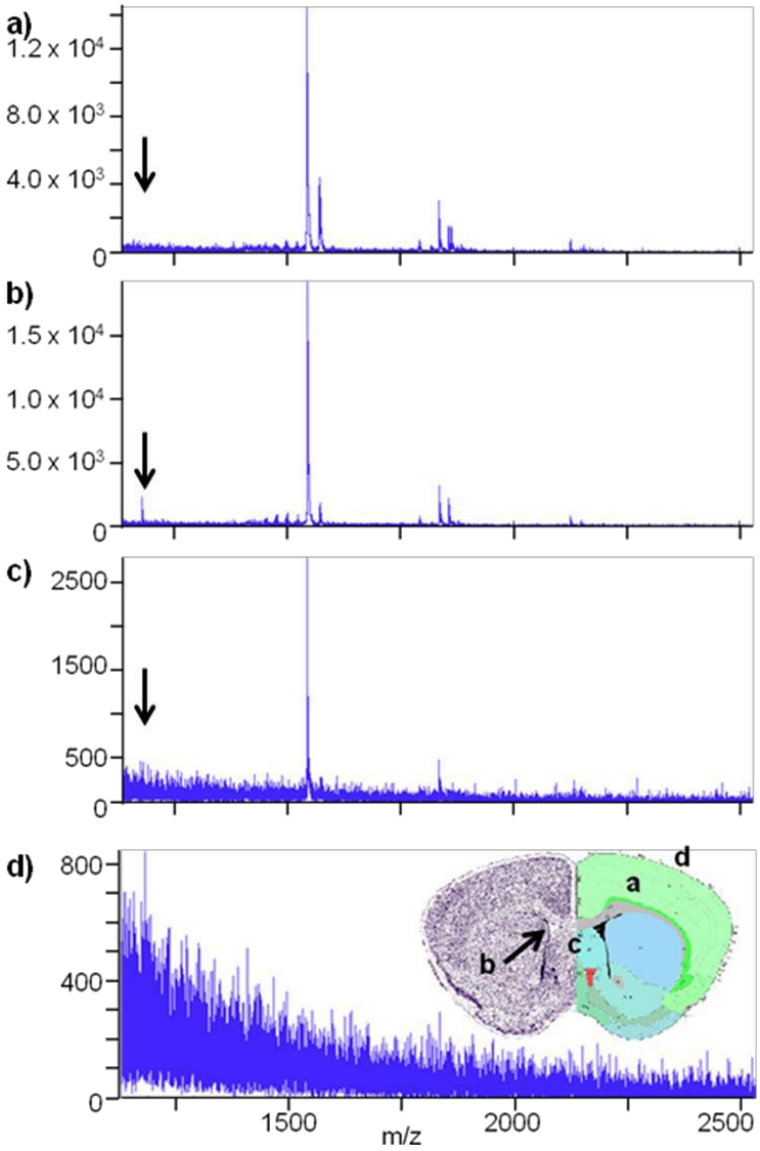

Figure 3: MALDI mass spectra of gangliosides using saturated DHA/ammonium sulfate 125 mM/HFBA 0.05% in negative ion mode from a wild type mouse brain section (levels 46 of coronal locations in Allen's Brain Mouse Atlas).

Mass spectra represent data obtained from matrix spots on tissue a) from the cerebral cortex, b) from the left lateral ventricle and c) from the midbrain center, and off tissue d).

Arrows follow m/z 1179.7 (GM3 d18:1/C18:0),an example of the presence or absence of ions as a function of the anatomical area of interest.

This was evidently more important when imaging $[M-H]^-$ ganglioside species. Four coronal regions of one mouse brain were successfully imaged from level 46, 67, 80 and 82, respectively (locations from Allen's Mouse Brain Atlas). Images of GM3 d18:1/C18:0, GM1 d18:1/C18:0 and GM1 d20:1/C18:0 are displayed in Figure 4 as an example of the image quality which can be achieved using the chemical inkjet printer for matrix deposition.
(Note the anatomical localization which arise from these images and can be recognized from the mouse brain atlas images.)

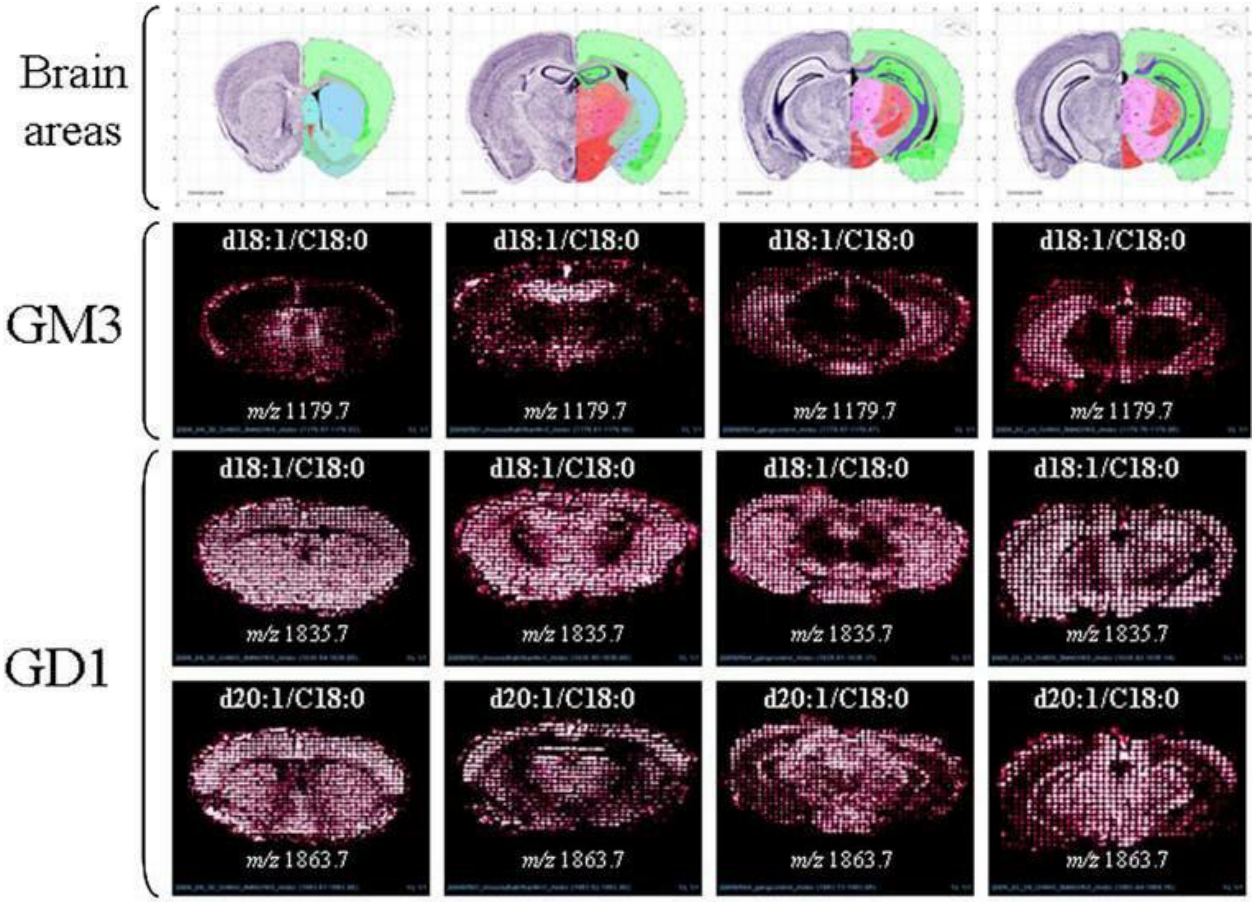

Figure 4: MALDI images of gangliosides (GM3 d18:1/C18:0, GD1 d18:1/C18:0 and GD1 d20:1/C18:0) using saturated DHA/ammonium sulfate 125 mM/HFBA 0.05% in negative ion mode from coronal levels 46, 67, 80 and 82, respectively (locations from Allen's Mouse Brain Atlas). Diverse ganglioside localization are highligthed as a function of the species headgroup chemical structure. Figure reproduced from Colsch B, Woods AS. *Glycobiology. 2010. 661-667* by permission of Oxford University Press.

PC and SM mapping from a TBI model

Phospho- and sphingolipids (**Figure 1**) were imaged from a single TBI mouse brain section (coronal level 67 from the Allen's Mouse Brain Atlas). Images of two PCs (16a:0/16:0 and 16a:0/18:1) and one SM (d18:1/C18:0) are presented in **Figure 5**. Each $[M+H]^+$ form was interestingly localized in the same brain areas such as the cerebral cortex and hippocampus. Images of their $[M+Na]^+$ and $[M+K]^+$ forms were additionally extracted from the MSI data set to check for the specificity of these localizations. The coincidence of their respective $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ images proved this specificity as well as the reliability of the matrix deposition automation.

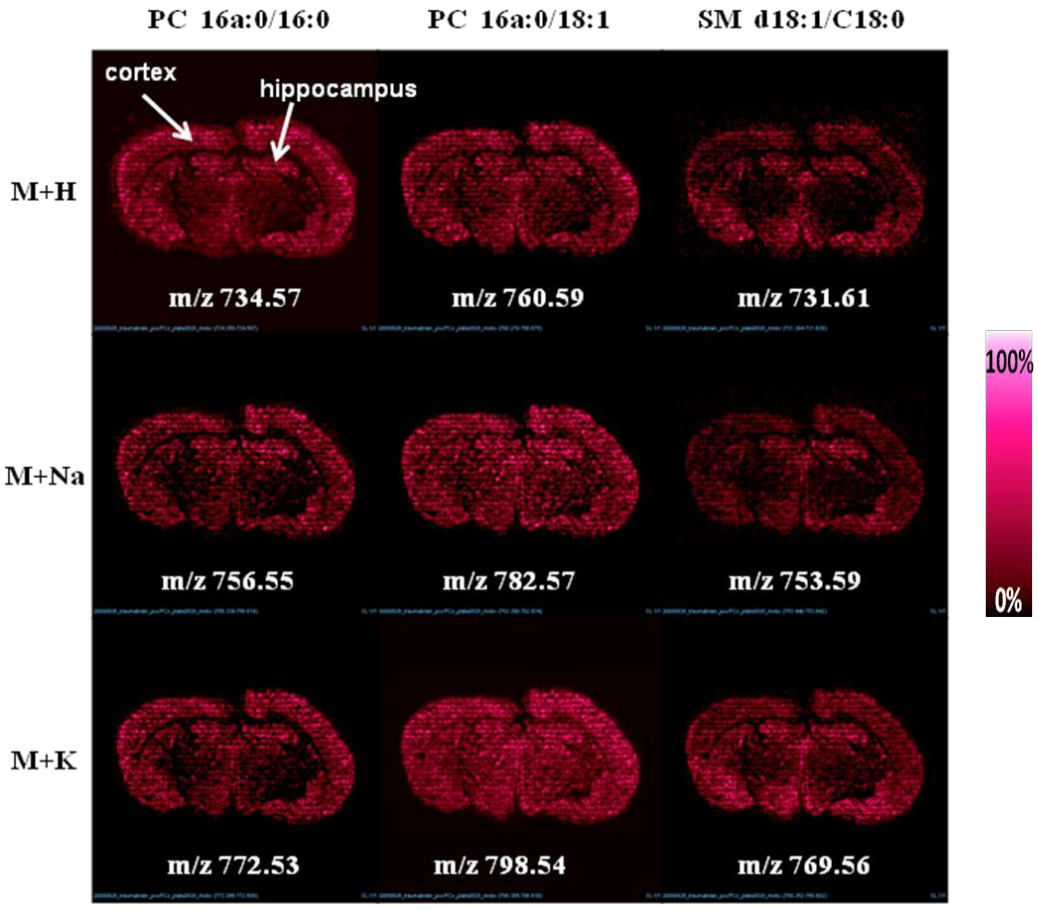

Figure 5: MALDI images of lipids detected from a TBI model mouse brain section in positive ion mode using 30mg/L DHB in ethanol/water 50:50 (coronal level 67 in Allen's Brain Mouse Atlas). Images of two PCs and one SM are displayed as a function of their chemical structure (rows) and as a function of their cationized forms (columns).

Conclusion

Microprinting of matrix solutions combined with imaging using a conventional MALDI TOF-TOF provided accurate mapping of lipids from healthy and diseased tissue sections. This work proved the efficiency of such a methodology when choosing the appropriate printing parameters. Indeed, its automation generated a controlled and reproducible printing process as well as a uniformity of the matrix layer. Both allowed for the efficient detection of gangliosides, PCs and SMs extraction, desorption/ionization and thus detection.

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