Understanding and decreasing visceral fat delocalisation in Imaging MS

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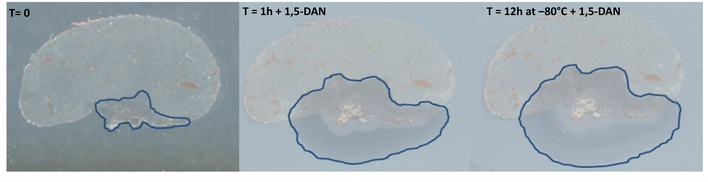
Introduction

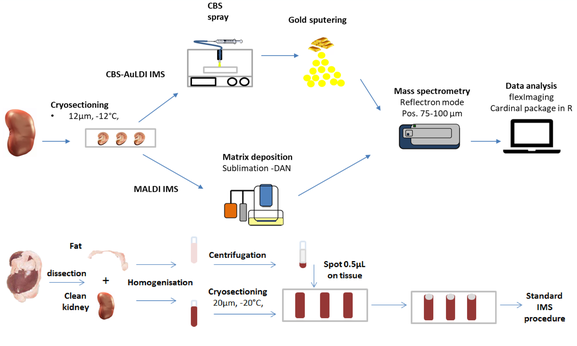
Ions suppression and preferential ionization in MALDI IMS are two well-know phenomena. Triglycerides (TAGs) have shown to delocalized horizontally across tissue sections, strongly suppressing cholesterols esters signals (1). TAGs can be found in visceral fat present on the renal capsule particularly around the hilum, a highly fatty histology where in this case significant delocalization originates. On-section lipid delocalization and the resulting ion suppression effects can lead to gross misinterpretation of IMS result. Visceral fat delocalization was first observed with optical scans after thaw-mounting of the sections on ITO slides and seems to be a **time dependant process amplified by vacuum and matrix deposition**. Lipid delocalization was monitored by MALDI and CBS-AuLDI IMS (1, 2) using fatty mouse kidney sections mounted on 13 different types of slides. We show that **porous aluminium oxide slides (POR-30) offer significant improvements** in limiting on-tissue visceral fat delocalization.



Objectives

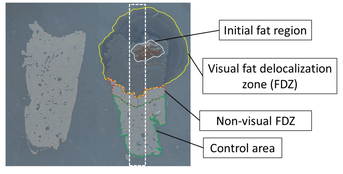
1. Characterize visceral fat delocalization across tissue sections and its effect on PLs intensity
2. Explore different types of slides in their ability to decrease visceral fat delocalization
3. Investigate how surface chemistry may affect visceral fat delocalization
4. Propose a porous-type aluminum oxide slide that greatly minimizes visceral fat delocalization
5. Validate a simple procedure to produce porous-type aluminum slides

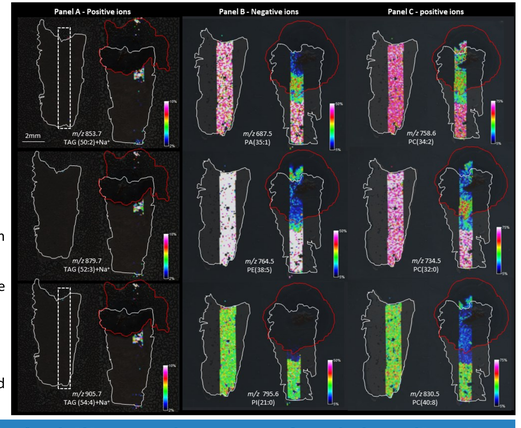
Methods



Investiging fat delocalization

Molecular characterization of the fat delocalization zone (FDZ) and effects on phospholipid (PL) IMS





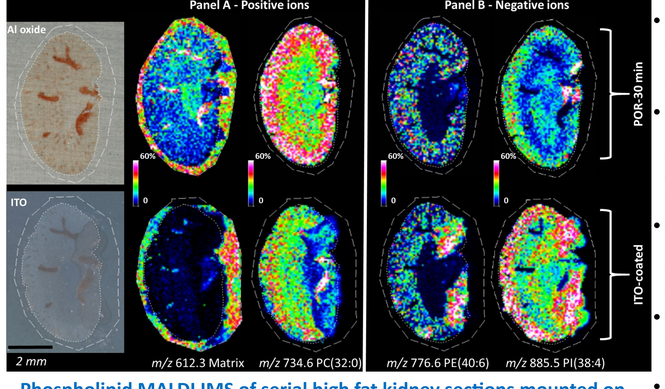
Using control and of fat doped sections from kidney tissue homogenates on ITO slides: Panel **A** shows **TAG distributions** monitored by CBS-AuLDI IMS.

- Massive **ion suppression effects** are observed within the **fat delocalization zone** (FDZ) essentially comprised of TAGs.
- TAG signals are also observed outside of the visual FDZ.

Panels **B** and **C** show **PL distributions** after 1,5-DAN sublimation and IMS.

- Intense ions suppression effects are observed within the visual FDZ and beyond.

Porous-type Aluminum slides (POR-30) ability to decrease fat delocalization

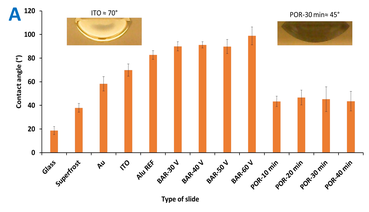


Phospholipid MALDI IMS of serial high fat kidney sections mounted on POR-30 aluminium oxide and ITO coated slides.

Excess visceral fat was carefully removed before mounting the kidney on the sectioning block. Approximatively 0.15mg/cm^2 of 1,5-DAN was sublimated on the section and IMS was acquired at 125µm of spatial resolution

- Visual fat delocalization observed on ITO coated slide photomicrograph is confirmed by matrix signals in positive ion mode (*m/z* 612.3) while not being significant on Al oxide.
- Extensive ion suppression caused by fat delocalization is observed on ITO coated slide for multiples signals as shown by PC(32:0) in positive ion mode (*m/z* 734.6) while no ion suppression is detected on POR-30 slide.
- Many signals are overexpressed in the area corresponding to FDZ on ITO coated slide as shown by PE(40:6) and PI(38:4) in negative ion mode (*m/z* 776.6 and *m/z* 885.5).
- Many PLs like PI(38:4) migrate along with TAGs and show off-section delocalization on ITO coated slide. This phenomenon is not observed of POR-30.
- POR-30 drastically decrease visceral fat delocalization and allow high fidelity IMS of PLs and a better correlation with the corresponding histology for organs surrounded by visceral fat.

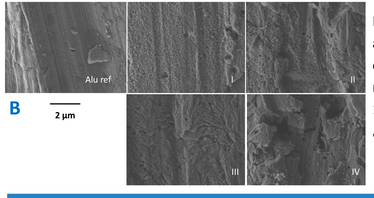
Surface characterization



Contact angle and SEM measurements of various surfaces analysed

Inset **A** shows contact angle measurements of 1µL of HPLC water for all the surfaces tested. Errors bars represent standard deviation of 10 independent measurements. BAR: barrier type and POR: porous type aluminum oxide.

- Results clearly indicate that surface hydrophilicity is a key parameter in decreasing visceral fat delocalization.



Inset **B** shows representatives SEM measurements of aluminum slides without anodization (Alu ref) and 4 different porous aluminum oxide. Captions I to IV represent various anodization times from 10 to 40min with 10min increments.

- SEM results indicate that surface morphology, specifically high rugosity, helps reduce visceral fat delocalization.

Conclusion

- Extreme delocalization and ion suppression was observed for high fat sections mounted on ITO coated slides resulting in intense ion suppression for various phospholipid classes as shown by MALDI IMS analyzes.
- Surface topography and hydrophilicity are two important parameters to combat visceral fat delocalization.
- Our proposed porous aluminum oxide slide (POR-30) is easy to produce (4) and a very effective solution to minimize visceral fat delocalization as demonstrated by IMS analyses of mouse kidney and a fat marbled steak samples (not detailed here).
- Special care should still be taken during all sample preparation steps to minimize visceral fat delocalization.

This study has recently been published in Analytical chemistry: DOI : 10.1021/acs.analchem.9b0566

References and aknowledgments

The authors would like to acknowledge Pr. Jean-François Masson (Department of Chemistry, Université de Montréal) for unlimited access to the metal sputtering system, Pr. Antonella Badia (Department of Chemistry, Université de Montréal) for granting us access to a custom instrument for contact angle measurements and Dr. Nidia Lauzon (Drug discovery Platform at the Glen Site of the Research Institute of McGill University Health Center) for granting us access to the MALDI TOF/TOF MS instrument. The authors would also like to acknowledge funding from the Natural Sciences and Engineering Research Council of Canada (NSERC).

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