NOVEL TISSUE WASHING PROCEDURE FOR THE REMOVAL OF CATION ADDUCTS PRIOR TO SELECTIVE CATION FORMATION FOR DESI AND MALDI IMAGING

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INTRODUCTION

MALDI mass spectrometry imaging is the most widely adopted technique for the molecular imaging of biological tissue. DESI has recently presented itself as a suitable alternative for the imaging of small molecules, metabolites and particularly lipids. One of the complexities of lipid analysis arises from the formation of various cation adducts such as sodium and potassium. These cations can often hinder attempts to identify the lipids due to poor MSMS fragmentation. Similarly some cations such as lithium can promote extensive fragmentation aiding in identification. Therefore it may be preferable to remove and subsite the cations present within the tissue.

METHODS

Tissue sectioning

Ethically sourced Porcine liver tissue was initially frozen at –80 °C prior to sectioning in a cryostat (Leica) at –20 °C to a thickness of 16 µm on to Polylysine coated glass slides (Sigma).

Tissue washing and cation addition

Tissue sections were removed from the freezer and desiccated for 15 mins in a vacuum desiccator. For washing, the tissue sections were submersed in a solution of 10mM Oxalic acid for 10 secs, excess removed by gentle tapping and vacuum desiccation for 15mins. This was then repeated for a total of two washes.

For cation addition, a solution of lithium chloride 35 mg/ml in 50 : 50 methanol : water was spray coated on the tissue section using a Suncollect™ (SunChrom) nebulising sprayer. A single pass at 20 µl/min, with a 1mm line spacing, using speed medium 4, a Z-height of 37.5 mm and a gas pressure of 2 bar was performed.

MALDI imaging

Pork liver sections were spray coated, with CHCA using the Suncollect™ nebulising sprayer. 12 layers of 10 mg/ml CHCA, at 20µl/min were applied with a line spacing of 0.5 mm, using speed medium 4, Z-height 37.5 mm and 2 bar nitrogen pressure.

Image experiments were defined using HDI 1.5. A Waters SYNAPT G2-S*i* HDMS with MALDI source option was used for the acquisition. Data were acquired using the HDMSe mode, where pixels were alternated between low and high energy functions. The collision energy was set at 45 V and fragmentation occurred in the transfer collision cell post ion mobility separation.

For HDMSe Acquisition the following parameters were use:

Laser:	1kHz, ND-YAG solid state
Scan time:	0.5 s
Trap Dc Bias:	60
Transfer wave velocity:	223 m/s
IMS wave start:	1000 m/s
IMS wave end:	300 m/s
Transfer CE:	2 / 45 V
Polarity:	Positive

DESI imaging

Treated / untreated tissues Pork tissues sections were imaged using a Waters Xevo G2-XS Q-ToF. Image experiments were defined using HDI 1.5, for each tissue. A low energy (CE 4 V) and a high energy (CE 45 V) imaging experiments were acquired. The scan rate was set to 0.5 s with a stage speed of 300 µm/s. The nebulising pressure was 5 bar and the capillary voltage was 3 kV for positive polarity . The DESI spray solvent was 98% MeOH, 0.1% formic acid, 1.9% Water

Data Processing

All data were processed and visualised using a combination of Masslynx 4.2 (Waters) and HDI 1.5 (Waters).

RESULTS / DISCUSSION

In MS imaging, there have been continuous methodological advancements with a view to increasing sensitivity / enhancing the performance of particular molecular species such as proteins [1] by washing of tissues.

In addition efforts have been made to remove salts from tissue particularly for negative mode [2].

Here we have explored the use of oxalic acid. The conjugate base of oxalic acid (oxalate) is a known chelating agent for metal cations (figure 1), to wash salts from tissues prior to the addition of a selected cation.

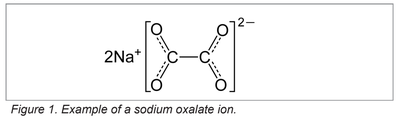

Figure 1. Example of a sodium oxalate ion.

MALDI

A combined spectrum for the normal and washed tissue sections can be seen in figure 2. A two fold increase in signal is observed for the washed sample as well as a reduction in K+ and Na+ signals.

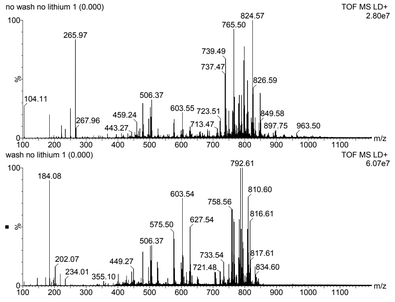

Figure 2. Upper panel normal unwashed tissue, lower panel washed oxalic acid washed tissue.

Figure 3 shows the PC head group related fragments from the high energy MALDI function. Without washing, it can be seen that the Na^+, K^+ and H^+ head group fragments (*m/z* 146.99, 162.96 and 184.08 respectively) are roughly equal in abundance. With washing however, the K^+ signal is no longer observed and the spectrum is dominated by the H^+ signal at *m/z* 184 which is now an order of magnitude more intense. Images of the various cation states of PC 34:1 from the low energy function and the head group related fragments for the washed and unwashed can be seen in figure 4. In the washed sample, the PC 34:1 K^+ is absent and the Na^+ and K^+ head group fragments are absent from the high energy washed tissue. In addition no signs of delocalisation are observed.

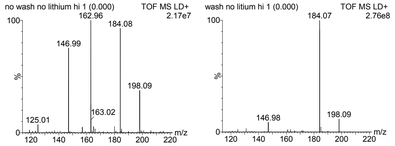

Figure 3. Combined spectra showing the PC head group related fragments for Na^+ (m/z 146.9), K^+ (m/z 162.9) and H^+ (m/z 184.0) from the high energy function for normal (left) and washed (right) tissues.

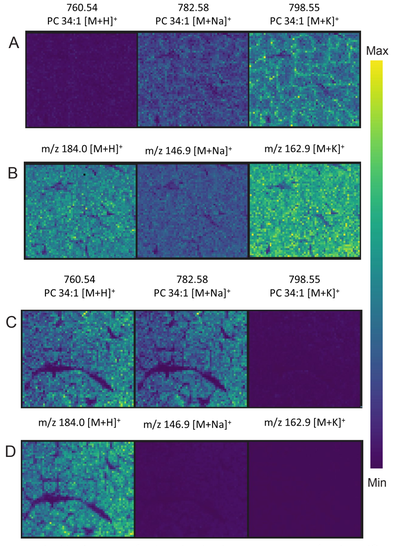

Figure 4. MALDI images for A) untreated tissue low energy function PC 34:1; B) untreated tissue high energy function PC head groups, C) washed tissue low energy function PC 34:1, D) washed tissue high energy function PC head groups. (150 µm X 150 µm)

Figure 5 shows the MALDI low energy combined spectrum of a tissue post washing and lithium addition, the peak at m/z 184 is significantly reduced. There appears to be a large degree of in source fragmentation. Figure 6 shows the combined high energy spectra for the washed tissue and the washed tissue & lithium. A larger number of intense fragments are observed in the lithium treated sample.

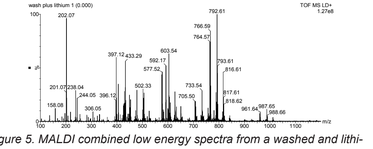

Figure 5. MALDI combined low energy spectra from a washed and lithium treated sample.

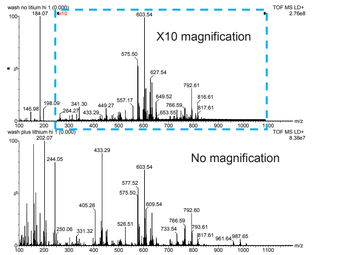

Figure 6. MALDI combined High energy spectra from a washed (top) and a washed and lithium treated sample (bottom).

DESI

A combined spectrum for the unwashed and washed tissue sections can be seen in figure 7. A x1.5 increase in signal is observed for the washed sample as well as a reduction in K+ and Na+ signals. .

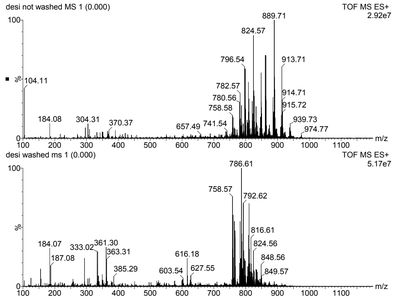

Figure 7. Upper panel normal unwashed tissue, lower panel washed oxalic acid washed tissue.

Figure 8 shows the PC head group related fragments from the high energy DESI image without washing, it can be seen that the H^+ fragment is the dominate fragment with significantly less Na^+ and K^+ in comparison to MALDI. It is postulated this is due to the extraction effect of the MALDI matrix application drawing salts from deeper in the tissue.

For the washed sample the Na^+ and K^+ related fragments are not observed and the H^+ fragment has increased by an order of magnitude. Images of the various cation states of PC 34:1 from the low energy function and the head group related fragments for the washed and unwashed can be seen in figure 9. In the washed sample, a reduction of the PC 34:1Na^+ and K^+ peaks is observed is and the Na^+ and K^+ head group fragments are absent from the high energy washed tissue. In addition no obvious signs of delocalisation are observed.

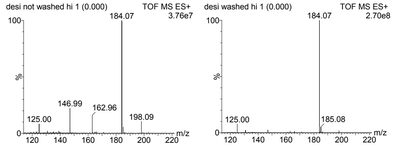

Figure 8. Combined DESI spectra showing the PC head group related fragments for Na^+ (m/z 146.9), K^+ (m/z 162.9) and H^+ (m/z 184.0) from the high energy function for normal (left) and washed (right) tissues.

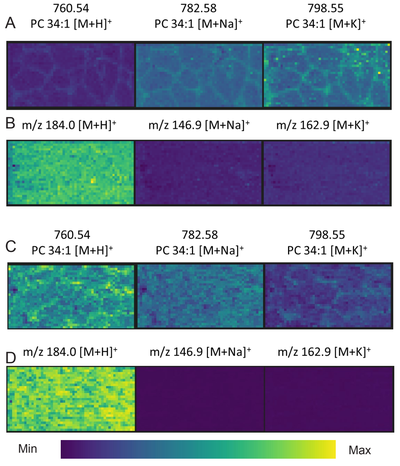

Figure 9. DESI images for A) untreated tissue low energy image PC 34:1; B) untreated tissue high energy function PC head groups; C) washed tissue low energy function PC 34:1; D) washed tissue high energy function PC head groups. (150 µm X 150 µm)

Figure 10 shows the DESI low energy combined spectrum of a tissue post washing and lithium addition. Whilst more potential fragmentation is apparent in the low mass region compared to the untreated sample, there doe not seem to be any obvious signs of lithium cation formation and there is an overall reduction in sensitivity. Figure 11 shows a comparison of the high energy images for a washed tissue and a washed & lithium tissue. In both cases the spectra are dominated by the m/z 184 H^+ PC head group peak which whilst this is much more intense in the washed sample compared to the washed & lithium, the overall levels of fragmentation are very similar. This is also indicative that the lithium introduction for DESI has failed.

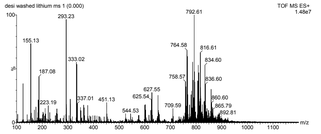

Figure 10. MALDI combined low energy spectra from a washed and lithium treated sample.

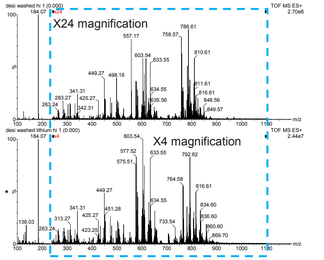

Figure 11. DESI combined High energy spectra from a washed (top) and a washed and lithium treated sample (bottom).

CONCLUSION

- **Oxalic acid wash was successfully employed to enable the removal of sodium and potassium cations from tissue sections.**
- **The washing procedure did not appear to cause wide spread disruption to the tissue or severe delocalisation.**
- **The washing procedure increased the overall sensitivity by a factor of 1.5 to 2.**
- **Inclusion of lithium after washing increased the levels of fragmentation by MALDI in a HDMSe experiment.**
- **Using the same method for lithium inclusion for DESI was not successful**

References

1. Seeley, E. H.; Oppenheimer, S. R.; Mi, D.; Chaurand, P.; Caprioli, R. M., Enhancement of protein sensitivity for MALDI imaging mass spectrometry after chemical treatment of tissue sections. J Am Soc Mass Spectrom 2008, 19 (8), 1069-77.

2. Angel, P. M.; Spraggins, J. M.; Baldwin, H. S.; Caprioli, R., Enhanced sensitivity for high spatial resolution lipid analysis by negative ion mode matrix assisted laser desorption ionization imaging mass spectrometry. Anal Chem 2012, 84 (3), 1557-64.

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