METABOLOMIC/LIPIDOMIC DESI IMAGING OF DIFFERENT CELL CULTURES

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INTRODUCTION

Lipidomics can be considered as one of the important fields of metabolomics that provides a comprehensive structural and functional characterization of various lipids in different biological samples.
A desorption electrospray ionization source (DESI) has been used for mass spectrometry imaging (MSI) analysis as well as for the identification of lipids in the cell culture.
In this study, we aimed to investigate the distribution and the localization of lipids in positive and negative ionization modes for maximum lipid coverage among three different cell line cultures: two of the gut epithelial cell lines (Caco2 and HT29-MTX cells) and one of cancerous basophil cells (RBL=Rat Basophilic Leukemia).

METHODS

Tissue sample preparation

Three cell lines were investigated which are; Caco2, HT29-MTX (gut epithelial cell lines), and RBL (basophil leukemia cell line). All cells were grown separately on coverslips. The seeding density was $1x10^5$ cell/ml, cells were cultured for 21 days (Caco2 and HT29-MTX), and 2-3 days (RBL) cells. The media was replaced every 48 hours.

Prior to DESI MSI analysis, cell grown on cover slips had the media removed and cell cultures were washed with 150 mM ammonium acetate followed by a drying step in desicator for 15 min. The cover slips were directly mounted onto microscope glass slides, using double sided tape and placed onto the DESI stage.

Mass spectrometry

DESI imaging experiments require no additional sample preparation as desorption and ionization are initiated by charged droplets (98:2 MeOH: water) impacting directly on the surface.

DESI MSI experiments were carried out on a Xevo G2-XS Q-ToF mass spectrometer (figure 1) in positive and negative ionisation modes, with a mass range of m/z 50—1,200

DESI-MS

Flow rate:	1.5 µl/min
Capillary voltage:	4.5 kV
Nebulising gas:	5 bar
Pixel sizes:	20 and 50 µm (lateral)
Stage speed:	80 and 100 µm/sec (respectively)

Data management

Samples were scanned using a flatbed scanner. Areas where cells looked confluent were selected to be imaged using the co-registered photographic image of the samples in High Definition Imaging Software (HDI) v1.5 (Waters).

DESI imaging datasets were mined using MassLynx as well as processed and visualized using HDI 1.5.

Extensive statistical analyses were carried out using Metaboanalyst (https://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml).

Regions of Interest (ROIs) defined in HDI and associated intensities were averaged and TIC normalised in the form of a .csv file which was loaded directly into Metaboanalyst .

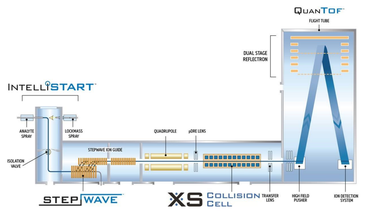

Figure 1: Schematic of the Xevo G2-XS mass spectrometer..

RESULTS

On day 21 of cell culture, the cells were subjected to a viability test using a live/dead staining assay to evaluate cell states before subjecting to DESI MSI analysis. Caco2 cultures appeared as single cells and/or as agglomerates, whereas it was difficult to differentiate the single cells in the HT29-MTX cultures, probably due the mucus secreted by HT29-MTX cells (called goblet cells), giving a cloudy appearance to the viability test images (figure 2).

The analysis area for each cell line was chosen where the confluence area was the highest by eye.

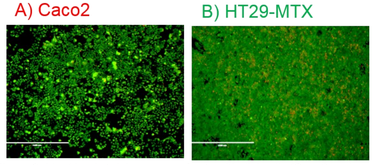

Figure 2: Live/dead staining assay of gut epithelial cells (Caco2 and HT29-MTX cells) on day 21 after seeding. Cells cultured on coverslips A) Caco2 cells, B) HT29-MTX cells (100%). Live cells (green) (Wisner) and dead cells (red).

Multivariate analysis workflow and results for POSITIVE MODE.

The simple procedure to grow, wash and freeze dry the cells is highly suitable for the DESI analysis thanks to the lack of further sample preparation and soft ionisation mechanism of DESI.

As seen in figure 3, strong signals were detected in the lipid mass range of m/z 700-1,000 for the three cell lines, with the most abundant peak being m/z 798.54, putatively identified as PC (34:1)K^+.

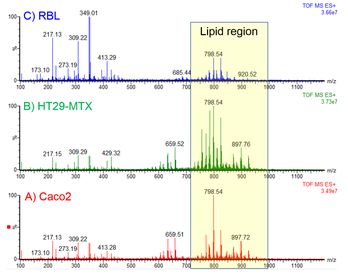

Figure 3: Positive MS spectra of cell lines grown on cover slips and directly DESI imaged. A) Caco2, B) HT-29-MTX and C) RBL cell lines..

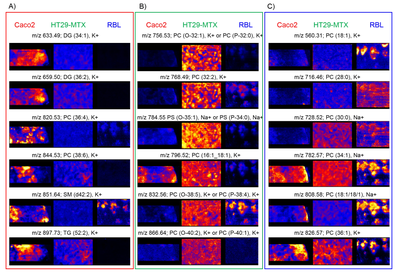

Figure 4: Ion images of the cell cover slips of Caco2 (left), HT29-MTX (middle) and RBL (right) cell lines. A) biomarkers more abundant in Caco2 (i.e. DG (34:1)K^+ and PC(36:4)K^+) B) biomarkers more abundant in HT29-MTX (i.e. PC(O-32:1) or PC(P-32:0)K^+, and PC(32:2)K^+), C) biomarkers more abundant in RBL (i.e. PC(18:1)K^+, PC(36:2), Na+). Ion images from the same m/z are TIC normalised and on the same intensity scale.

From the ROIs manually drawn on each of the ion image datasets, principal component analysis (PCA) and heatmap were carried out, using MetaboAnalyst as multivariate analysis engine (figure 5).

Caco2 and RBL cells have a wider range of lipid profiles compared to HT29-MTX which has the tightest cluster in principal component 2 due to their similarities in lipid species profile (figure 4,A). This is also confirm from the lipid distributions from Caco2 and RBL cell lines were less homogenous than the HT29-MTX cell culture.

Furthermore triglycerides (TGs) were only detected from Caco2 and HT29-MTX cell cultures but not from the RBL cell culture. Some TGs such as m/z 897.73 were more abundant in the middle area of the confluent Caco2 cell sample (figure 4A).

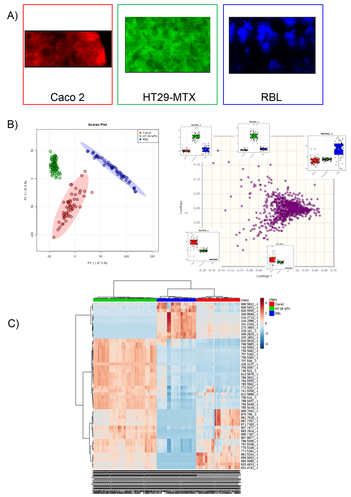

Figure 5: Positive mode MetaboAnalyst statistical analysis A) ion images m/z 798.54 PC(34:1)K^+., B) PCA score plot, Loadings plot and box plots and C) Heatmap. Red represents Caco2 cell line, Green represents HT29-MTX cell line Blue represents RBL cell line..

Cell culture high spatial resolution DESI imaging

Further analysis was carried out in positive mode at different pixel sizes, 20 and 50 µm X,Y lateral) on the HT29-MTX cell sample, without changing any DESI spray parameters. The areas that were imaged were not as confluent as the previous experiments and individual single small agglomerates could be visually observed.

The speed of acquisition for the 20 µm pixel size experiment was 4 scans/sec to minimise the time of acquisition, whereas it was 2 scans/sec for the bigger pixel size DESI experiment.

With the increase in speed of acquisition and decrease in pixel size, the overall lipid signal decreased at 20 µm pixel size as expected but only by a factor of 4 (data not shown).

Figure 6 shows the higher quality RBG overlay image (*m/z* 756.53, *m/z* 796.52 and *m/z* 923.75) at 20 µm pixel size versus 50 µm. The cell agglomerates are better defined at 20 µm.

Furthermore it can be observed that some agglomerates are more yellow in colour which suggests that the both *m/z* 756.53 and *m/z* 796.52 are co-localised, whereas some are mainly green, indicating that *m/z* 796.52 is significantly relatively more abundant. This could be due to the different stage of development of the human cells present within the culture.

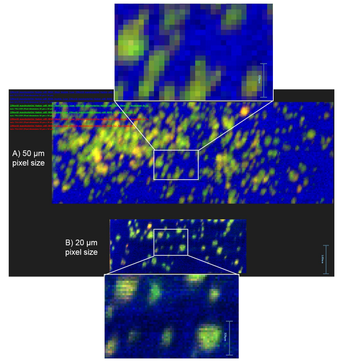

Figure 6: RGB overlay of m/z 756.53, m/z 796.52 and m/z 923.75 at 20 µm pixel size versus 50 µm.

Multivariate analysis workflow and results for NEGATIVE MODE.

Oleic acid (m/z 281.25) was the most intense signal in negative mode for all three cell line samples. As seen from the MS spectra in figure 7, the lipid profiles were quite significantly different with PE (36:2)H^- the most abundant lipid in Caco2 cell line and RBL, whereas PI(36:1)H^- is highly abundant in HT29-MTX and RBL cell lines.

From the putative identification based on *m/z* , the more differentiated lipid species in Caco2 in the PCA plots are the phosphoethanolamines (PEs) whilst in HT29-MTX there are a subclass of PE, either 1-alkyl,2-acylphosphoethanolamines or 1-(1Z-alkenyl),2-acylphosphoethanolamines.

From figure 8 and 9, it can be seen that a number of small metabolites such as Taurine were the main differentiators between the three cell lines, with Taurine being more abundant in RBL cell line.

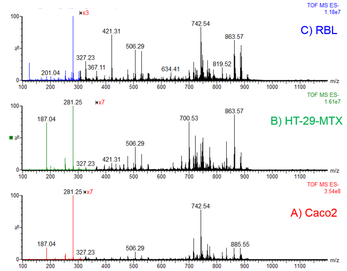

Figure 7: Negative MS spectra of cell lines grown on cover slips and directly DESI imaged. A) Caco2, B) HT-29-MTX and C) RBL cell lines..

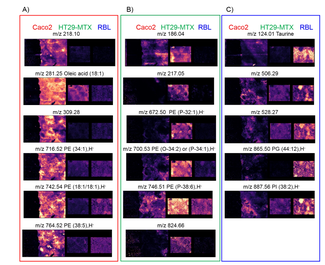

Figure 8: Ion images of the cell coverslips of Caco2 (left), HT29-MTX (middle) and RBL (right) cell lines. A) biomarkers more abundant in Caco2, B) biomarkers more abundant in HT29-MTX and C) biomarkers more abundant in RBL.Ion images from the same m/z are TIC normalised and on the same intensity scale.

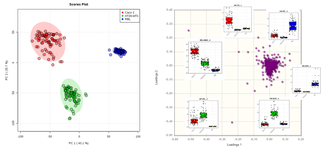

Figure 9: Negative mode MetaboAnalyst statistical analysis. Left : PCA score plot. Right: Loadings plot and box plots and C) Heatmap.

A) Positive mode

m/z	Lipid identification	Ion
756.53	PC (P-16:0/16:0)	K+
770.51	PC (16:0_16:1)	K+
782.55	PC (16:0_18:1)	Na+
784.56	PC (P-34:0) or PC (O-34:1)	K+
796.53	PC (16:1_18:1)	K+
798.55	PC (16:0_18:1)	K+
808.59	PC (18:1/18:1)	Na+
810.51	PC (17:1/18:1)	K+
824.56	PC (18:1/18:1)	K+

B) Negative mode

m/z	Lipid identification	Ion
738.51	PE (16:0_20:4)	H-
742.54	PE (18:1/18:1)	H-
766.54	PE (18:0_20:4)	H-
773.54	PG (18:1/18:1)	H-
819.52	PG (18:1_22:6)	H-
861.55	PI (18:1/18:1)	H-
863.57	PI (18:0_18:1)	H-
885.55	PI (18:0_20:4)	H-

Table 1: Lipid identifications from MS/MS experiments. A) positive mode; B) negative mode

CONCLUSION

- **Direct analyses of three different cell lines were successfully achieved by DESI MS imaging, without further sample preparation in positive and negative mode.**
- **In positive mode, high phospholipid signals were detected from the three cell lines. Triglycerides were only detected from the gut epithelial cell lines (Caco2 and HT29-MTX) and not basophil leukemia cell line (RBL).**
- **In negative mode, Oleic acid was the most abundant endogenous species detected from all three cell lines.**
- **The DESI imaging experiment at 20 µm gave better quality ion image quality than 50 µm.**

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