Single Cell Metabolite Detection Using Inertial Microfluidics-Assisted Ion Mobility Mass Spectrometry

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INTRODUCTION

Project Aim:

To develop a technology improving existing methods to analyse single-cell metabolites by increasing the sensitivity and resolution of products obtained via mass spectrometry

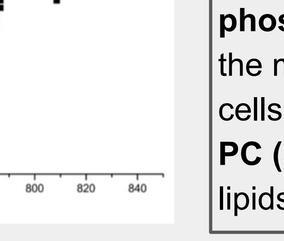
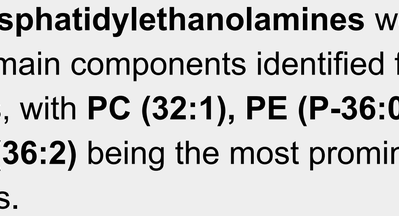
Single cell metabolite detection is important for translational medicine and biomarker discovery.

The pre-existing technique is **electrospray/nanoelectrospray ionisation mass spectrometry (ESI-MS):**

- Generates intact gas-phase biomolecular ions for analysis

We propose **microfluidics-assisted ion mobility mass spectrometry (IM-MS)**:

- Separates ions according to the gas-phase mobility of ions
- Microfluidics employed to ensure high-throughput

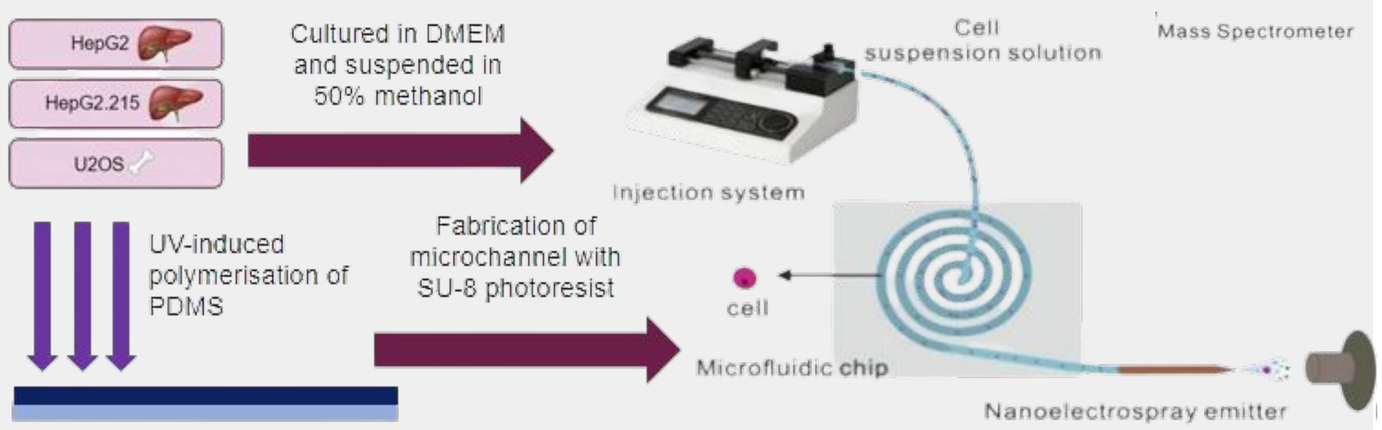
Properties	ESI	IM
Generates intact biomolecular ions	✔	✔
Provides collision cross section (CCS) measurements	✖	✔
Facilitates efficient, accurate annotation	✖	✔
		

MATERIALS AND METHODS

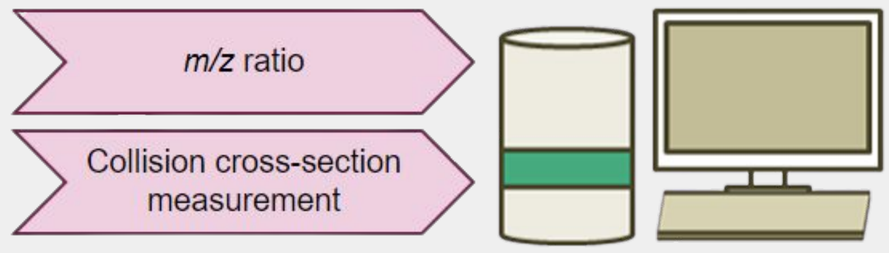
Optimisation of Flow Rate

The balance of inertial and Dean drag forces in the spiral microchannel focusses cells into a stream of ordered single cells.
Different flow rates were tested for cell injection using HepG2 cells, and the flow rate of cells were determined by video recording, and correlated against chromatogram data.

Profiling of Single-Cell Metabolites



Analysis of Single-Cell Metabolites

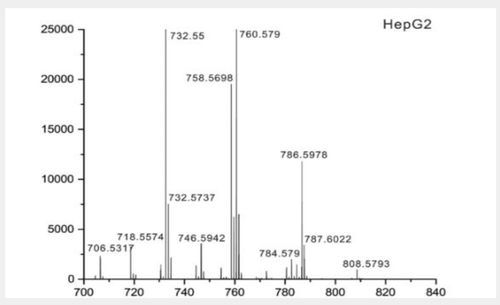


Metabolites were identified by comparing *m/z* and collision cross-section (CCS) measurements against public databases.

RESULTS

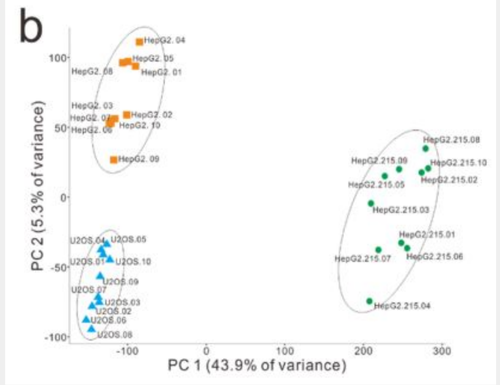
Optimisation of Flow Rate:

At a cell concentration of 6 x 10^2 cells/mL, the maximum flow rate for reliably maintaining separation and hence discriminating cells was 10μL/min.



Profiling of Single-Cell Metabolites
44 possible lipids were identified from three cell lines and cross-matched using CCS measurements. **19 common lipids** were identified for the 3 cell lines, along with **98 slightly mismatched lipid structures.**

Using CCS information to analyse single-cell metabolites
Phosphatidylcholines and **phosphatidylethanolamines** were the main components identified for all cells, with **PC (32:1), PE (P-36:0), and PC (36:2)** being the most prominent lipids.



Discrimination of Cell Types using Principal Component Analysis
Using **principal component analysis,** the three cell lines were differentiated based on the relative abundances of identified lipids between cells

DISCUSSION

Optimal cell concentration and flow rate for injection of queued cells

- The optimal cell concentration and flow rate reduced cell agglomeration, **improving detection sensitivity** using nano-ESI-MS

Profiling of Single-Cell Metabolites
Lipid ions from cancer cells can be **detected reproducibly** using the nanoESI-IMS-TOF MS system and with **greatly improved detection ability**. This is attributed to:

- **Narrow** probe tip (internal diameter: 35 μm) and **low flow** to the nanoESI emitter creating a fine spray of droplets that are more **efficiently desolvated**
- Spray is **more focused** in front of the entrance to the MS so more of the released gas phase ions are transferred into the MS

Using CCS information to analyse single-cell metabolites

- Ion mobility with CCS measurements **improved metabolite detection sensitivity** and confidence of metabolite annotation.

Discrimination of cell types using Principal Component Analysis

- Using PCA, three cancer **cell lines could be differentiated** through the relative abundances of the identified lipids between the cells.

Limitations

- This technique uses cells suspended in 50% methanol solution, resulting in **low concentration of metabolites** compared to live cell suspensions.
- Quantity of metabolites detected can potentially be further improved using a facile live single-cell separation system

CONCLUSION

This novel inertial microfluidic-assisted nano-ESI-MS system can be used to accurately identify cell metabolites and annotate cell structure.

- Reducing cell agglomeration during cell injection in the microfluidic device improved detection sensitivity using nano-ESI-MS.
- Additional CCS measurements using ion mobility further improved the confidence of lipid identification.
- This method was viably applied to screen and differentiate three cancer lines, reflecting the accuracy and sensitivity of this technique.

This method to analyse single cell metabolites has potential applications in the field of metabolomics to analyse single cell metabolites in detail. For example, this can be applied in stem cell research, allowing researchers to identify the stage of differentiation the stem cell is at by examining the contents of each cell.

However, improvements can be made to improve the sensitivity and quantity of metabolite detection for wider biological application.

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