MICROFLUIDIC NMR FOR *IN SITU* CULTURE AND METABOLOMIC ANALYSIS OF HEPATOCELLULAR CARCINOMA CELL LINES

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ABSTRACT

We demonstrate the use of microfluidic NMR for *in situ* culture and continuous quantitative analysis of metabolism in hepatocellular carcinoma (HCC) cell lines. The ability to observe intracellular responses in a time-resolved manner provides a more detailed view of a biological system and how it reacts to stimuli. This has significant potential in the pharmaceutical field for drug development, particularly toxicity testing.

KEYWORDS: Hepatocellular carcinoma (HCC), Microfluidic NMR (µNMR), Metabolism

INTRODUCTION

Microfluidic systems provide a precisely controlled culture environment that can be manipulated to mimic physiological or therapeutic conditions. Utilising the non-invasive nature of NMR, metabolic data from live systems can be ascertained by detecting multiple metabolites simultaneously, at low concentrations and without harming the cells. The combination of microfluidics and NMR creates a powerful, non-destructive and non-invasive technique for *in situ* quantification of metabolism in biological samples under tightly regulated conditions. Previous work in our group showed that metabolic information can be obtained from as few as 1500 cells, using the MCF-7 cell line[1]. However, in that work the culture was done externally to the NMR system, and the culture chip had to be repeatedly removed from the incubator and placed in the NMR spectrometer manually. Apart from being inefficient, this leads to repeated disruption of the growth conditions, and limits the time resolution to 4-8 hours.

The present work addresses these problems by enabling continuous microfluidic culture inside of the NMR spectrometer over a 24h period, thus producing quasi-continuous NMR data. We apply this new modality to compare the metabolism of different HCC cell lines.

Changes to cancer cell metabolism are well described in their role in survival and sustaining tumorigenesis[2]. Rapid metabolism by cancer cells creates a harsh, immunosuppressive tumour microenvironment (TME) in solid tumours such as HCC[3]. This allows tumour growth to continue, unchecked by immunosurveillance that would otherwise prevent it. Understanding cancer cell metabolism in the TME, its response to drugs, and the effect on other cell types will inform the understanding of such tumour growth and how best to treat it.

A metabolic analysis technique able to quantify the metabolic capacity of cancer cells or tumour-infiltrating immune cells could inform the development of new therapies. Current metabolomic technologies require in the order of one million cells and are often destructive, end-point analyses, however our technology requires only 1,500 - 2,000 cells per device and preserves the cells for future tests. This reduction in scale will be invaluable for optimising the use of small samples such as those typically extracted from patient biopsies of diseased tissue.

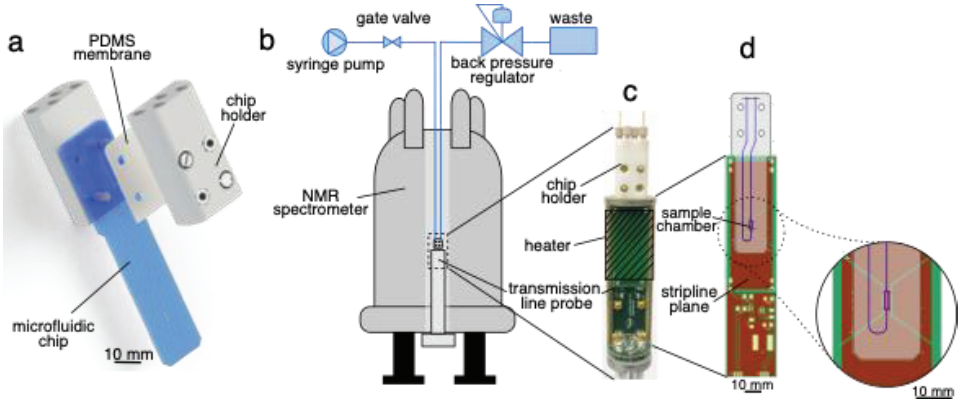

Figure 1: Experimental set up. A) Microfluidic device in a 3D printed chip holder. B) Devices are filled by syringe pump and 1.2Bar back pressure is applied. C) Enlarged view of the transmission line probe structure. D) Internal view of the probe.

EXPERIMENTAL

We have created a flexible microfluidic platform fabricated from a transparent plastic, compatible for cell growth within an NMR spectrometer. The microfluidic device sits inside a transmission line NMR probe designed by our group, with a heating system to keep the cells at 37°C while in the probe, allowing continuous observation. This technology can measure mM concentrations, at µl volumes, within a few minutes - sufficient to observe changes in primary energy metabolism, such as glucose, lactic acid and amino acids.

RESULTS AND DISCUSSION

We have demonstrated the growth of HCC cell lines in our microfluidic devices and attained a detailed, time-resolved picture of cellular processes, with data points every 17 minutes for 24 hours. Clear changes in glucose and lactic acid indicate glycolytic rate, with apparent differences between cell lines. This data has highlighted the different metabolic profiles of cell lines from different stages of advancement of the disease.

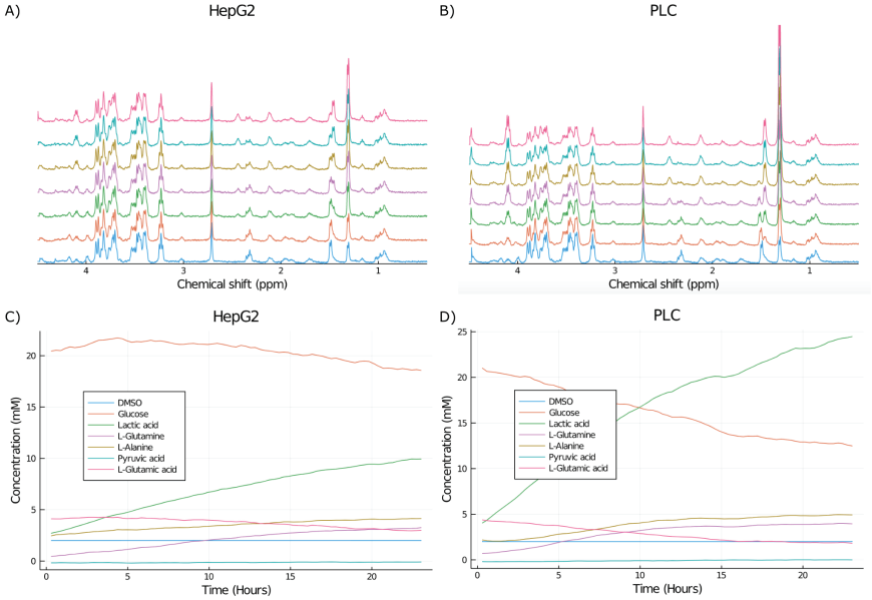

Figure 2: In situ microfluidic NMR analysis of two hepatocellular carcinoma cell lines, HepG2 and PLC. A-B) waterfall plots of spectra over 24 hours and C-D) concentration changes of six chosen metabolites.

CONCLUSION

HCC cell lines can be successfully cultured and simultaneously analysed using µNMR. This method can be used to observe changes in extracellular glucose and lactic acid in real time, indicating differences in glycolytic rate between cell lines. Interesting changes in a range of extracellular amino acids can also be observed.

ACKNOWLEDGEMENTS

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