InsightCell™: Real-Time Metabolite Monitoring

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InsightCell enables appropriate nutrient, substrate and drug delivery to living cells located within the sensitive area of the NMR spectrometer. Real-time monitoring of metabolite content under physiological conditions provides an innovative basis for non-invasive observation of 3D cell culture systems.

Introduction

InsightCell permits the study of dynamic metabolic processes upon specific metabolic stress situations, test for drug treatments, substrate, toxicological or inhibitor challenge and allows for real-time pathway analysis. In this application it is aimed to characterize metabolite content in human fibroblasts suffering different mitochondrial diseases. Specific adaptations of metabolic fingerprints or biomarkers during interventions are expected to improve physiological insight and possibly hold diagnostic potential.

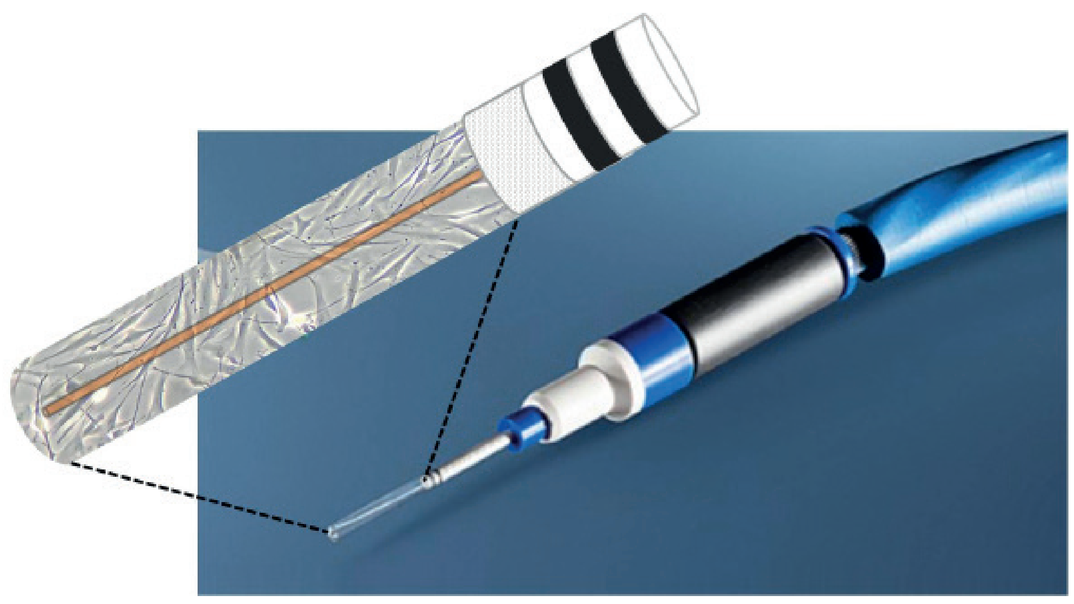

Fig. 1: InsightCell flow unit containing human fibroblasts imbedded in collagen based matrix.

Technical Details

Cells are cultured in standard conditions in incubators, then imbedded in matrigel and transferred into the InsightCell tool (figure 1). The culture medium is pumped through the NMR spectrometer and is taken up in fraction collector after outflow for further metabolomical measurements, pH estimation (externally and via histidine resonances) and oxygen control (T_1-measurements and external liquid gas analysis). Cell viability is determined afterwards by flow cytometry.

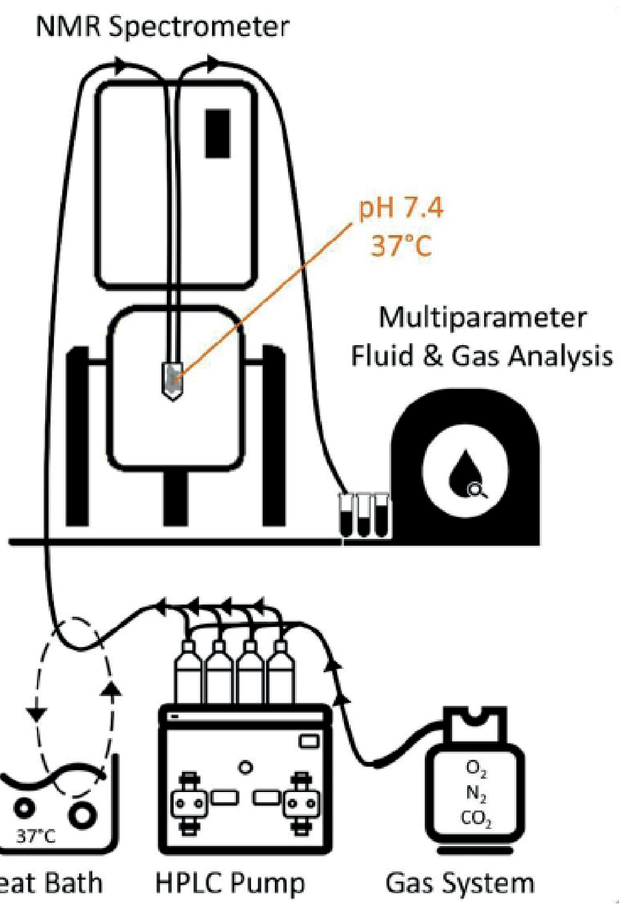

Fig. 2: Bioreactor inside a Bruker AVANCE II 500 MHz NMR spectrometer with HPLC pump, water bath for temperature control of the perfused oxygenated medium.

As first proof of concept, human fibroblast metabolite content was monitored in real-time during intervention. Time evolution of lactate and glucose before and after rotenone (mitochondrial complex I inhibitor) and 2-dehydroxy-D-glucose addition was determined in real-time (Figs. 3,4).

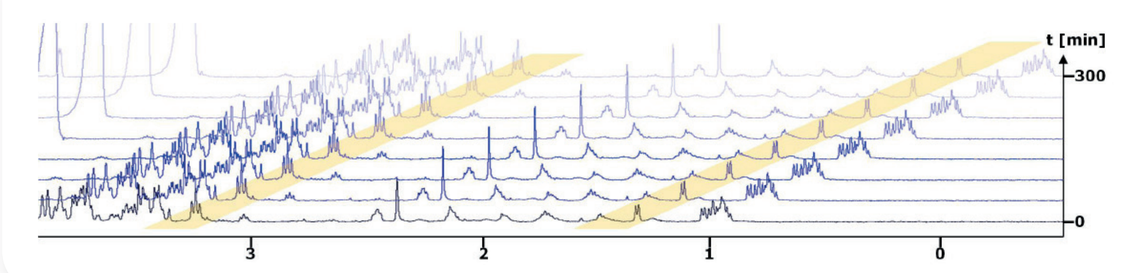

Fig. 3: Overlay of NMR spectra vs time (5 h) showing the ^{1}H 1D aliphatic region. Over 30 metabolites were identified.

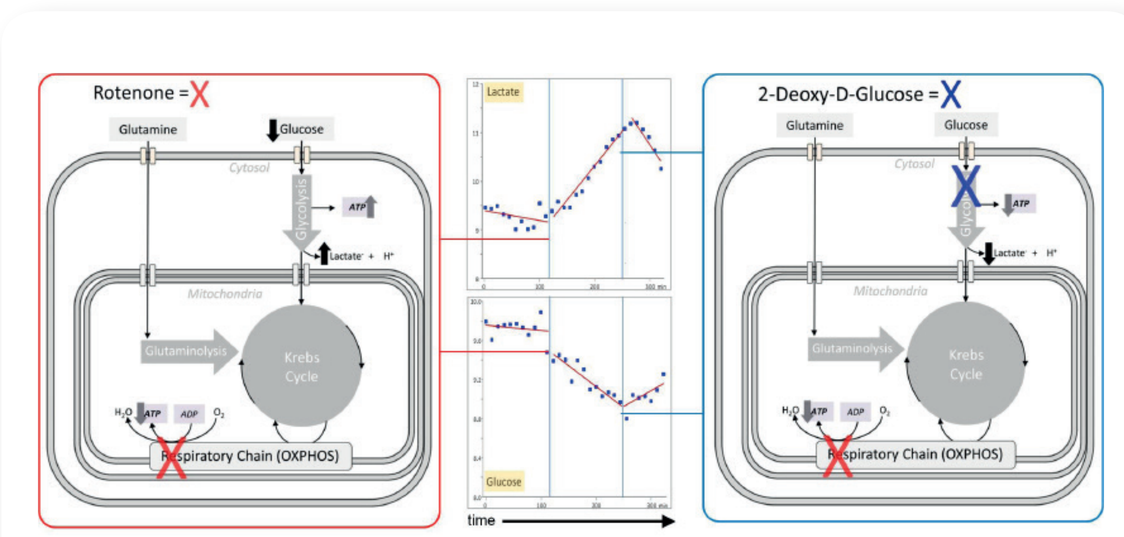

Fig. 4: Enzymatic pathway inhibited by Rotenone (left) and 2-Deoxy-D-Glucose (right). Lactate and Glucose kinetic profiles (middle) showing clear and reversible regulation of the glycolytic pathway.

All data courtesy of Prof. P. Vermathen, PD Dr. J. M. Nuoffer, D. Hertig, University of Bern