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Unisense microsensors in Cancer Research

Oxygen consumption rate measurements of 4T1 cancer cells using the MicroRespiration System

The application note is based on the research and article by:

Bianchi et al. (2018)

The application note is written by:

Sandfeld et al., Unisense

Introduction

With the Unisense MicroRespiration System it is possible to do real-time measurements of the oxygen consumption of cells. Substrates can be injected while measuring, and the effect of adding e.g. electron transport chain substrates, inhibitors or drugs to the cells can be studied in closed chambers.

Dr. Ravera from the University of Genova has used the Unisense MicroRespiration System to measure the respiration rate of 4T1 cells, a derived murine breast cancer cell line, during the addition of respiratory substrates and inhibitors.

Figure 1: MicroRespiration setup showing a Unisense oxygen microsensor (OX-MR) inserted into a MicroRespiration glass chamber containing the 4T1 cells. The different substrates were injected through the rubber cup with a Hamilton syringe.

MicroRespiration measurements

A Unisense oxygen microsensor (OX-MR) was inserted into a modified MicroRespiration chamber with an injection port (Figure 1). Cells were grown in their standard conditions and detached from the flask or petri-dish by trypsin or a scraper. Normally, 100,000-200,000 cells were used for the experiment (oxygen consumption could be measured even on 10,000 cells). To measure oxygen consumption of the cancer cells, medium containing 137 mM NaCl, 0.7 mM NaH_2PO_4, 5 mM KCl, 25 mM Tris-HCl pH 7.4 and 25 mg/ml ampicillin was used. The cells were permeabilized by the addition of 0.03 mg/ml digitonin for 2 minutes and washed with the respiration medium before the measurements. Slow stirring inside the chamber was maintained and the temperature was kept at 37 °C during the measurements. When the oxygen consumption of the cells stabilized the experiment was started.

To stimulate the pathway composed of Respiratory Complexes I+III+IV, 5 mM pyruvate and 2.5 mM malate were added, while 20 mM succinate was employed to evaluate the respiration through the Complexes II, III and IV pathways. To observe the respiration in coupled condition, 0.1 mM ADP was added after the respiratory substrates.

Conversely, to evaluate the uncoupled oxygen consumption, 5 μM nigericin plus 10 μM valinomycin were added to the cells before the addition of substrate. As respiration inhibitors, 10-50 μM rotenone to inhibit Complex I and 50-100 μM antimycin A to inhibit Complex III were employed. When the respiration was evaluated in the coupled condition, also 10-50 μM oligomycin could be used as an inhibitor of oxygen consumption. No more than 0.05 ml of the substrates were injected into the chamber.

Results and conclusion

Figure 2 shows a typical oxygen consumption curve obtained with the 4T1 cells. It was possible to observe an increment of oxygen consumption after the addition of pyruvate/malate or succinate and an inhibition of respiration after the addition of rotenone and antimycin A. The respiration rate (nmol/h) was calculated from the oxygen concentration measurements in Figure 2 with the SensorTrace Rate software. Using the Unisense MicroRespiration System, the authors could measure the respiration rate of cancer cells and evaluate the effect of adding different substrates and inhibitors to the cells. Besides 4T1 cells, the authors have measured the respiration rate of murine B16 melanoma, CT26 colon carcinoma and L1210 lymphocytic leukemia cells. The researchers have found that treatment with curcumin induces an energetic impairment in the cancer cells by inhibiting ATP-synthase activity and by decreasing ATP generation and oxygen consumption both in vitro and in vivo (Bianchi et al. 2018).

Dr. Ravera and coworkers have also used the Unisense MicroRespiration System to measure the respiration rate of subcellular fractions e.g. rod outer segment disks, isolated myelin vesicles and exosomes (Panfoli et al. 2016).

For further reading please see Bianchi et al. (2018) Curcumin induces a fatal energetic impairment in tumor cells in vitro and in vivo by inhibiting ATP-synthase activity. Carcinogenesis, Vol 39, No. 9, 1141-1150.

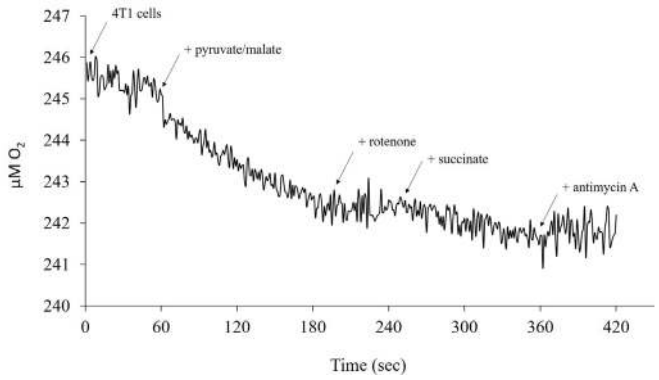

Figure 2: Microsensor measurement of the oxygen consumption from 4T1 cells after the addition of respiratory substrates (pyruvate/malate and succinate) and respiratory inhibitors (rotenone and antimycin A).

Suggested products

- Opto-MR, OX-MR
- MicroRespiration System
- Opto UniAmp, Multi Channel UniAmp
- SensorTrace Rate

Related publications

Ravera et al. 808-nm Photobiomodulation Affects the Viability of a Head and Neck Squamous Carcinoma Cellular Model Acting on Energy Metabolism and Oxidative Stress Production. Biomedicines 2021, 9, 1717.

Villa et al. The Human Fetal and Adult Stem Cell Secretome Can Exert Cardioprotective Paracrine Effects against Cardiotoxicity and Oxidative Stress from Cancer Treatment. Cancers 2021, 13, 3729.

Ravera et al. Concentration-dependent metabolic effects of metformin in healthy and Fanconi anemia lymphoblast cells. J Cell Physiol. 2018; 233: 1736–1751.

Marini et al. Discovery of a novel glucose metabolism in cancer: The role of endoplasmic reticulum beyond glycolysis and pentose phosphate shunt. Scientific Reports 2016, 6:25092.

Marini et al. Divergent targets of glycolysis and oxidative phosphorylation result in additive effects of metformin and starvation in colon and breast cancer. Scientific Reports 2016, 6:19569.

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