The physiological oxygen partial pressure (so-called physioxia) in the tissues of living organisms is much lower than its atmospheric values (normoxia). For example, for various human organs, it ranges from a few to a dozen or so percent, and in a tumor it may drop even below 1% (hypoxia)! Therefore, conducting scientific experiments in *in vitro* models, where oxygen pressure is near atmospheric does not reflect the physiological state and may be the source of many artifacts. Even short exposure of cells or organs to sub-optimal oxygen conditions can significantly affect the expression of genes regulating important cellular processes, and thus the cells response. This, in turn, may have a profound impact on the result of the experiment and its interpretations. This translates into our knowledge and understanding of many biological processes, including those which are disturbed in pathological states, i.e. cancer, cardiological or inflammatory diseases. Therefore, it is necessary to ensure appropriate conditions for the handling of biological material (including those obtained from patients) throughout the experimental procedure.

Therefore, the team of Professor Claudine Kieda from the Laboratory of Molecular Oncology and Innovative Therapies (LOMTI) of the Military Medical Institute (WIM) in Warsaw were funded by Ministry of Science and Higher Education with a grant of 1.5 million PLN establish a system for the preparation and cultivation of cells in vitro under controlled oxygen conditions.

The X3 system from Biospherix was purchased, which enables to carry out in vitro experiments under controlled O2, CO2, temperature and humidity conditions. All intravital procedures, i.e. preparation of cells / organoids, culture, and microscopic observations, are carried out without leaving the system. This significantly limits the disturbance of cell functions during the course of the experiment. At the same time, the systems allows to put (through a special airlock) materials to and from the system, to use other equipment present in the Laboratory; ie centrifuges, flow cytometer and others. The system allows simultaneous cell culture in 3 different oxygen pressure values, and independent workstations allow simultaneous operation of 2 researchers, without the risk of cell or tissue exposure to atmospheric air. The system is also equipped with an inverted fluorescence microscope (AxioObserver7, Zeiss) allowing observation of cultured cells. Thanks to the control of the pO2 conditions and the temperature of the microscope chamber, it is possible to perform observations in real time (including time-lapse films) at the set oxygen level. This allows to assess the effect of oxygen pressure on cell morphology and movement, protein expression and intercellular interactions. An additional measurement system of O2 dissolved in fluids (OxyLite, Oxford Optronics) enables the monitoring of changes in oxygen levels in culture medium due to cell metabolism.

The purchased equipment is an integrated system that allows to perform profound research on the effects of different oxygen pressure on cells and tissues in vitro. It allows to measure many indicators in vitro and to conduct complicated research protocols (thanks to independent control of settings in incubators and their large capacity). The system will be the basic tool for experiments planned in the research program of the Laboratory of Molecular Oncology and Innovative Therapies in WIM. The system is located in the Laboratory of Alternative Models of Preclinical Laboratory of Molecular Oncology and Innovative Therapies.

Similar systems are found in a few scientific units in Poland: Technology Transfer Center (CePT) of the Medical University of Warsaw and the Institute of Experimental and Clinical Medicine of the Polish Academy of Sciences in Warsaw and at the Jagiellonian University in

Kraków and the Pomeranian Medical University in Szczecin. Most of them are designed for clinical trials, which hampers scientific cooperation.

That is not the case for the system in LOMTI / WIM- all researchers interested in conducting experiments using the described equipment are encouraged to contact and cooperate!

Team of LOMTI