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Label-free monitoring of whole cell vitality

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***Abstract*— The Intelligent Mobile Lab (IMOLA) delivers metabolic and morphological parameters of living cells in a label-free and real time way. It represents a key technology for the development of new cell-based assays. Electrochemical microsensors are used to measure the extracellular acidification (pH), cellular respiration (pO_2), changes in cell number and morphology (electric impedance) in a controlled environment. These parameters are closely linked to the intracellular signaling network of the living cells. They are thus likely to respond sensitively to changes in cellular vitality. A wide spectrum of cell types can be tested with the system, including adherent and suspended cells, continuous cell lines, primary cells or tissue samples. The platform is described in detail and applications in the field's oncology, toxicology and environmental monitoring are shown.**

I. Introduction

Measurement of physiological parameters of cultured cells is routinely used by a number of methods to understand inter-functional relationships at the levels of single cells, organs and organisms [1]. Development and supplement of these methods in order to reach the continuous online and real-time registration of metabolic and morphological cell parameters extend the spectrum of their application, e.g. in toxicokinetics, drug development or chemosensitivity analysis [2,3,4].

II. Materials and Methods

A. Electrochemical platform

The Intelligent Mobile Lab (IMOLA) delivers metabolic and morphological parameters of living cells marker-free, online and in real-time. To do so it uses miniaturized pH-sensors, an amperometric sensor, impedance sensors and a temperature sensor on various kinds of BioChips. The technology was developed in the group of Prof. Bernhard Wolf at the *Heinz Nixdorf – Lehrstuhl für Medizinische Elektronik* of *Technische Universität München* and represents a platform-technology for the development of new cell-based methods and therapies. A detailed description of the whole cell monitoring approach and the related signal processing was given previously [5]. Figure 1 demonstrates the modular construction concept of the system. The analog module contains different electronic assemblies for the operation of the microsensors. The analog module for one BioChip is designed to operate two metal oxide pH-sensors, one amperometric sensor for dissolved oxygen, two impedance sensing structures and one temperature sensor. The fluidic system consists of a controlled pump, a waste container and one or more vessel of fresh cell culture medium.

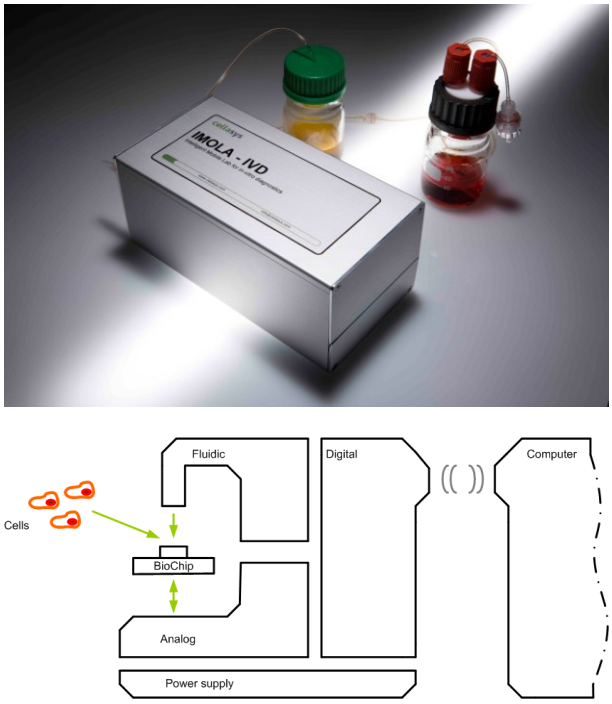

Figure 1. Single IMOLA-System (upper side) with a size of 210 mm x 105 mm x 90 mm and block scheme (lower side) of the system with a sample of living cells, a multiparameter sensor chip, analog module for the micro-sensors' operation, fluidic module for the supply of the cells with a fresh medium and bioactive substances, digital module for the control and data processing, power supply and computer for configuration, data processing and network connection.

The closed fluidic system construction makes the permanent need for a sterile environment unnecessary. Moreover, a simple upgrade allows adjustment of different gas partial pressures within a liquid source vessel. The digital module provides the computer communication, the temporary data storage and the control of the fluidic system and the analog module. Electrical power is supplied either by the integrated accumulator or in a stationary manner by the power supply units. The software module with the name DALiA (Data Acquisition and Link Application) configures the measurement system and controls the experimental run. The pump cycle (stop-and-pump modus), flow velocity and switch of the medium are programmed by DALiA. Furthermore, all the acquired measurement data are

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represented graphically during an experiment. This module is carried out as *Client Application* locally on a computer.

A wide spectrum of cell types can be tested with the system. The cells are differently classified, e.g. adherent and non-adherent cells, cells in culture, primary cells or tissue samples, or the cells cultured at room/body temperature. Table 1 contains the list of the cells/tissues which have already been tested. The living cells are cultured directly on the BioChips.

TABLE I. LIST OF THE TESTED CELLS/TISSUES

Type	Name	Description
Cells in suspension		
	Yeast	Baker's Yeast
	Escherrichia coli	Bacteria
	Chromera velia	Micro-algae
	Chlorella kessleri	Algae
Monolayer		
	MCF-7	Human breast cancer cell line
	MDA	Human breast adenocarcinoma cell line
	Caco-2	Human epithelial colorectal adenocarcinoma cell line
	HeLa	Human cervical adenocarcinoma cell line
	HepG2	Human hepatocellular liver carcinoma cell line
	PANC-1	Human pancreatic carcinoma cell line
	BxPC3	Human pancreatic adenocarcinoma cell line
	INS-1E	Rat pancreatic β cell line
	HL-1	Mouse cardiac muscle cell line
	L929	Mouse fibroblastic cell line
	3T3	Mouse embryonic fibroblast cell line
	CHO	Chinese hamster ovarian cell line
	Primary cells	Mouse neuron cells
Tissue / 3D		
	Primary cells	Human breast cancer
	Primary cells	Human laryngeal cancer
	Primary cells	Human lung cancer
	Primary cells	Mouse liver
	Primary cells	Sheep pancreas
	Spheroids	Mouse liver microtissue
Special application		
	HepG2 → MCF7	Serial connection of HepG2 (sender) und MCF7 (receiver) cells.
	Primary human hepatocytes	Coating of the BioChip surface with collagen

One BioChip usually contains an amperometric microsensor (e.g. for determination of the dissolved oxygen) and miniaturized sensors for the measurement of pH values and the electric impedance. The integrated temperature sensor records the temperature on the BioChip. The BioChip sensors are made of ceramic, glass or silicon substrates. To start the measurement, the BioChip is placed in the IMOLA and connected to the fluidic system. Figure 2 demonstrates the BioChip-D. It is fabricated with a transparent glass substrate by thin-film technology.

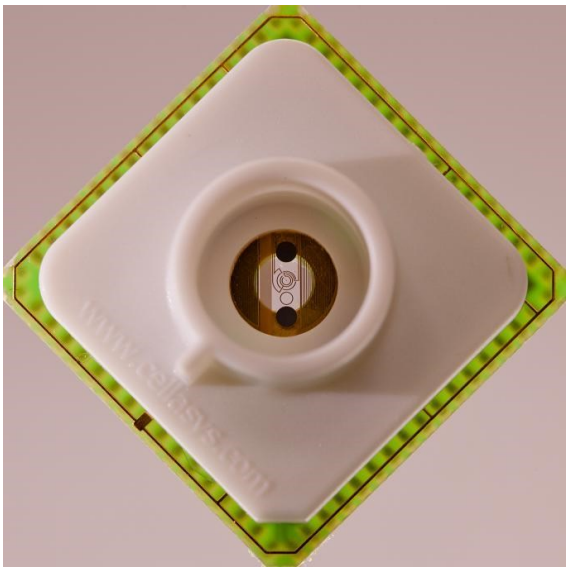

Figure 2. BioChip-D (feed size: 24 mm) with two electric impedance sensors, two pH sensors, one amperometric sensor and one temperature sensor. The PCB was cut open to allow microscopic investigation of the cells.

The following Figure 3 shows the 6xIMOLA-IVD where six parallel measurements in an incubator can be performed.

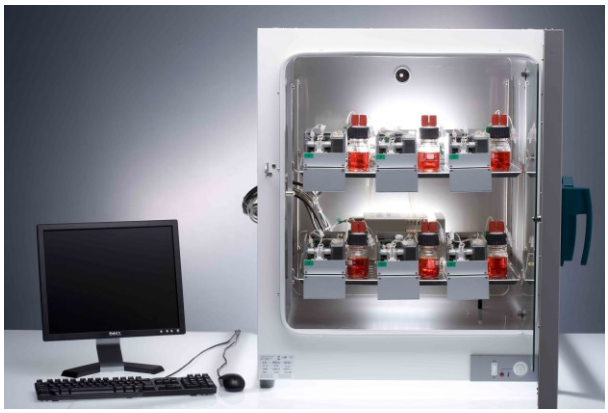

Figure 3. The 6xIMOLA-IVD enables the parallel investigation of six cell culture samples. The systems are preinstalled in an incubator.

Six IMOLAs are installed in an incubator as a parallel arrangement. The system's architecture allows one to perform the marker-free, continuous, parallel and real-time measurements. The experiments are run in a programmed stop-and-pump cycle. Thereby, the BioChip measures the cellular oxygen consumption, the extracellular acidification and morphological changes of the cells during the stop-phase. During the pump-phase, cell culture medium is transported to the BioChips to supply the cells with fresh nutrients and to allow the sensors' recalibration. In the same way, the test substances are given to the cells in order to elicit the specific responses. The measurement cycle can be perpetuated over several days.

III. Results

A. Oncology

The tumor interstitium is characterized by a micro-environment that differs from corresponding normal tissues. For example, the tumor tissue phenotype has a reduced extracellular pH, a low oxygen concentration and a poor nutrient supply to the cells [4]. The reasons for these phenomena include a patho-metabolic syndrome e.g. changes in glucose consumption, acidification rate and mitochondrial activity [6]. Identification and classification of these characteristics lead to the development of new methods for diagnosis, prognosis and therapy in oncology. Chemosensitivity is among other things a responsiveness of tumor cells to cytostatic drugs in the context of cancer therapy. Chemoresistance of tumor cells is usually mediated by genetically determined impairments of intracellular signal transduction pathways. It was shown that the acidified microenvironment of tumor cells protects them from induced cell death and supports cell proliferation [7]. Moreover, it is known that the hypoxic conditions in the microenvironment assist the clonal expansion of tumor cells [6]. For the above reasons, cancer researchers pay their particular attention to the role of core metabolism in recent years. In this context the system was used for the measurement of metabolic parameters in a study where the chemosensitivity of MCF-7 cells to the cytostatic drug cisplatin was determined. For this purpose the tumor cell line MCF-7 was cultured on the BioChip and treated with different concentrations of that drug. MCF-7 cell metabolic activity was continuously measured over a period of 3 days and in real time.

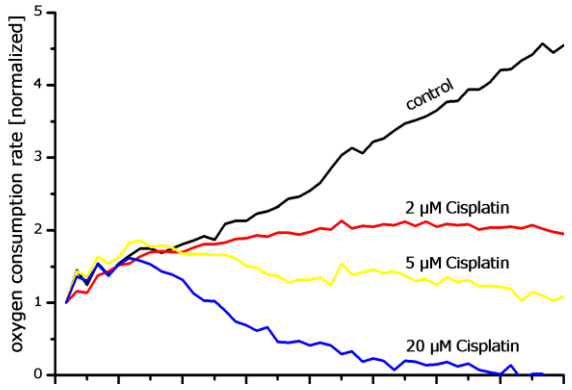

Figure 4. Oxygen consumption rates of MCF-7 cells under the influence of different concentrations of cisplatin.

A fresh cell culture medium with 5µM and 10 µM of cisplatin was connected to the fluidic system after 18-24 hours of measurements. The change of the cell metabolic activity before and after the addition of different cisplatin concentrations could be observed. Figure 4 shows the extracellular acidification rates of three MCF-7 cultures before and after the treatment with cisplatin, measured in parallel. The control measurement performed without the addition of cisplatin demonstrates the continuous slight increase of the acidification rate until the end of the measurement. MCF-7 cultures treated with 5µM and 10µM of cisplatin showed a significant dose dependent reduction of the extracellular acidification rate. For method validation the results were compared with the outcome of the parallel performed WST-test and cell counting assay (CASY) [8].

B. Toxicology

Toxicokinetics describes the temporal development of intake, distribution, metabolism, storage and elimination of foreign substances in an organism. It was shown that the effect of a substance at the cellular level provides feedback to the reaction of an organism. For example, the electrochemical Cytosensor Microphysiometer (CM) – commercialized by Molecular Devices, USA – measures the extracellular acidification rate of living cells. The CM was subsequently accepted as an alternative method to animal use in "Draize Eye Irritation Test" performed on rabbits [9]. Our study of concentration dependent effect of toxins showed that 5 µM of mercury(II)chloride ($HgCl_2$) hardly affects the 3T3 fibroblasts. A significant reduction of the extracellular acidification was, however, measured after the addition of 20µM $HgCl_2$. This cellular effect seems to be partly reversible because toxin removal leads to the restoration of the acidifying activity of the cells almost up to the untreated level (Figure 5).

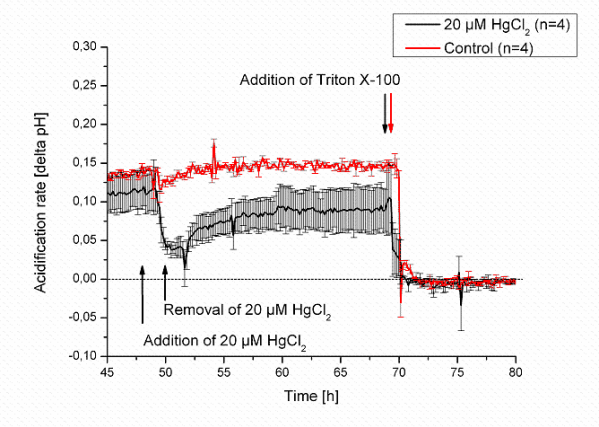

Figure 5. Extracellular acidification rate of 3T3 mouse fibroblasts after addition and remove of mercury chloride.

During the experiment it was shown that the toxin had no measurable effect on cellular respiration. However, an irreversible increase of the electric impedance was observed. After the end of the experiment, the non-ionic detergent Triton X-100 was added to all the samples including the control, leading to a complete destruction of cell membranes and cells death as a positive control [10]. This study demonstrates that the method is suitable for providing information about interactions between cells and active substances. One of the major advantages of this system is the possibility to perform marker-free long-term investigations, excluding any foreign influences on cells, and allowing the observation of long-term effects after chronic exposure to toxins. Moreover, it was shown that the technology is capable of differentiating metabolic and morphological cellular effects with respect to different toxin concentrations.

This is an essential feature for any technology attempting to replace animal experiments currently used for determining the toxicity of new chemicals.

C. Environmental Monitoring

Experiments with the test system and *Chlorella kessleri* described the influence of the xenobiotic Metamitron on the photosynthetic activity of green algae. As shown in figure 6, the oxygen production by the algae significantly decreases after the addition of 1 mg/L metamitron. This effect was also reversible and a restoration of the photosynthetic activity took place after removal of the substance [11].

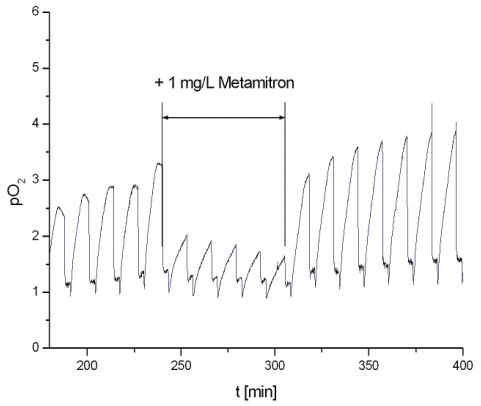

Figure. 6. Photosynthetic activity of the green algae after addition and removal of Metamitron.

Results of these experiments show that the high sensitive test system is suitable for various applications in limnology.

IV. Summary

Numerous successful studies and experiments demonstrated the possibilities of the technology – the marker-free continuous acquisition of the cellular vitality. In cancer medicine the possibility to monitor the tissues prior to, during and after an ex-vivo modeled therapy in real-time is a clear benefit. On the basis of a validation in prospective and interventional clinical trials, cancer patients may benefit from enhanced, personalized information about the effectiveness of different options for medication. Moreover, the selected anticancer drug may be optimally dosed to achieve the highest possible efficacy and avoid the systemic toxic reactions.

In the field of toxicology the marker-free principle of measurement has an important advantage because the cellular effects of a test substance do not undergo any interference with the labeling chemicals. In addition, the fluidic-system allows for long-term investigations, which permit an identification of long-term cellular or tissue effects. The technology makes it possible to determine and to classify a degree of the toxic impact of chemical compounds on an organism.

In environmental research, the system is able to evaluate the effects of pollutants on the relevant metabolic pathways using photo-synthetically active organisms as signal transducers, for example by measurement of photosynthesis. As the IMOLA is also suitable for mobile applications, pollution analyses in the areas of environmental research (fresh waters) and public services (municipal water supply, mineral springs) may be carried out locally.

Remark

This article is an updated version of an article which appeared in German language as "*Elektrochemische Technologieplattform zum Monitoring lebender Zellen*" in "B. Wolf (Editor) *Bioelektronische Diagnose- und Therapiesysteme* – m³: microelectronic meets medicine, Shaker Verlag, 2012, 31-50, ISBN: 978-3-8440-0831-9"

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