24-WELL-MICROPLATE WITH SENSORS FOR METABOLIC, MORPHOLOGIC AND ELECTROPHYSIOLOGIC PARAMETERS OF LIVING CELLS OR TISSUE

J. Ressler, V. Lob, M. Brischwein, H. Grothe and B. Wolf

Technical University of Munich, Heinz Nixdorf-Chair for Medical Electronics,
Arcisstr. 21, D-80290 Munich, Germany

ressler@tum.de

Abstract: High-throughput-technology is a central platform for functional cellular screening and has been widely applied in biomedical and pharmaceutical diagnostics. Functional assays using living cells are commonly used as a primary screening to provide first results on the pharmacological properties of an active agent. Compared to endpoint-measurements, the use of optical and electrical microsensors allows real-time monitoring of cellular parameters for extended measurement periods. Based on the experience with multiparametric sensorchips for the monitoring of living cells, we have combined the microplate-format with appropriate sensorchips for measurements of metabolical, morphological and electrophysiological parameters of living cells. Depending on the intended application, the microplate can be equipped with the required sensor types. Therefore, we provide a range of appropriate sensorchips.

Introduction

In pharmacologic drug-screening and medical diagnostics, a trend towards the utilization of functional assays using living cells can be observed. Cellular live is determined by dynamic and networked molecular processes. The regulation of these processes is fundamental for the entire organism. Biological cells are permanently converting physical and chemical signals of the environment into adequate cellular behaviour. This behaviour includes the decision about cell division and apoptosis, the activation of different metabolism paths or the production and disposal of proteins. For pharmacological drug-screening, typically fluorescence-based methods are used. However, these methods are often affected by several disadvantages. On the one hand, fluorescence assays are endpoint measurements, which means, that the measurement represents only a snapshot recording of the actual cellular state. On the other hand, some important cellular parameters are hardly accessible with fluorescent methods, e.g. the analyse of changes in cell adhesion or cell morphology.

Due to these handicaps, microsensor-based assays for metabolical, morphological and electrophysiological parameters find their place in various applications [1]. For these parameters we allocate optical read-out sensors and electrical sensors, which are appropriate arranged on the sensorchip, depending on the intended application. In case of electrical microsensors, the cells can grow directly on the sensor structures. The measurements are non-invasive and cause no disturbance of cellular behaviour. Due to these facts, the sensors allow real-time multiparametric monitoring of cellular parameters up to several days [2]. Figure 1 shows an example of a multiparametric measurement.

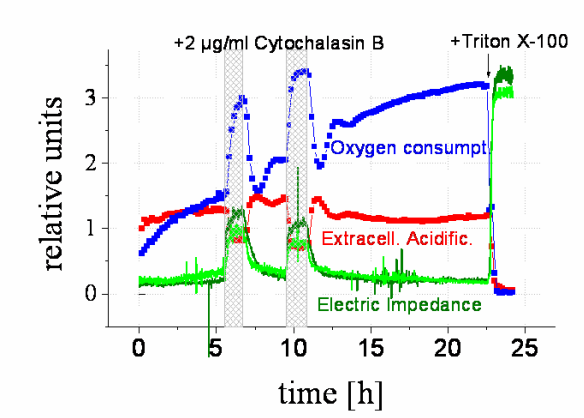

Figure 1: Effect of Cytochalasin B on LS 174 cells (colon carcinome), recorded with microsensors

Materials and Methods

Sensors

For the monitoring of the metabolical, morphological and electrophysiological parameters of living cells the following sensors are available:

Oxygen - sensors:

With the oxygen-sensor it is possible to detect the oxygen consumption of cells. This allows obtaining information on cellular respiration and mitochondrial activity. The sensorchips can be equipped with two types of oxygen-sensors.

(1) An optically read-out sensor, based on a fluorescent dye, which is fixed on the sensorchip surface [3]. The oxygen concentration of the environment of the sensor is affecting the fluorescence properties of the dye [4], which can be detected by an

ISSN: 1727-1983

optical read-out system. Figure 2 shows sensor-spots which will be fixed on the sensorchip.

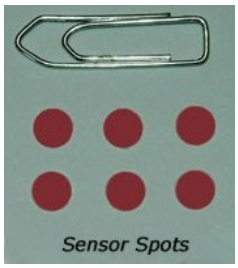

Figure 2: Optically read-out sensorspots for oxygen [5]

(2) A planar oxygen sensor, fabricated of a platinum layer by thin film technology. This sensor consists of three electrodes (working-, reference- and auxiliary-electrode) and realizes an amperometric measurement method. A constant potential is applied between reference- and working- electrode. Additionally, an auxiliary-electrode is used to keep the reference-electrode without current [6]. The resulting current essentially depends on the concentration of the oxygen in the culture medium. The relatice surface areas of working-, reference- and auxiliary-electrodes are about 1 : 1000 : 3000. The relatively small surface of the working-electrode minimizes oxygen self-consumption of the sensor itself and ensures that all measured changes in the electrochemical current are due to changes in the oxygen concentration caused by the cells [7]. The main electrochemical reaction taking place at the electrodes and causing the measured current:

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

Figure 3 shows the three-electrode configuration of the used planar oxygen-sensor:

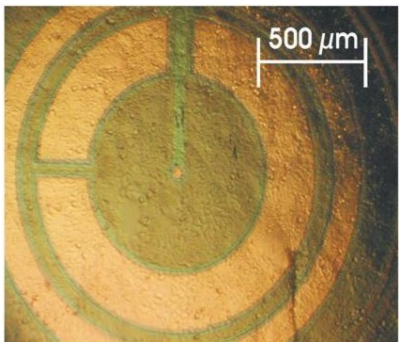

Figure 3: Configuration of the planar amperometric oxygen-sensor with working-, reference- and auxiliary-electrode

pH - sensors:

Acidification of the culture medium is measured with pH-sensors. This acidification provides information about global metabolic rates. There are two sensor types available:

(1) An optically read-out sensor, with a similar operating mode as the optical oxygen-sensor [8]. However, the fluorescent properties of the dye are influenced by the pH-value of the environmental culture medium [9]. Both, the optical oxygen- and optical pH-sensors are provided by PreSens - Precision Sensing GmbH, Regensburg, Germany. Figure 4 shows sensorspots which will be fixed on the sensorchip.

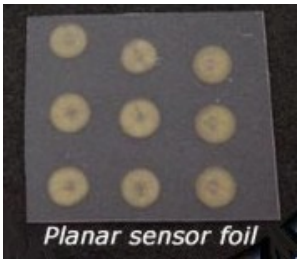

Figure 4: Optically read-out sensorspots for pH [5]

(2) A microelectric pH-sensor implemented as a rutheniumoxyde(RuO_2)-spot, which is deposited on the surface of the sensorchip. For planar pH-sensor usually ion sensitive field effect transistors (ISFETs) are used [10]. To avoid the cost expensive silicon technology, we have integrated a metaloxide-based pH-sensor. Together with a reference-electrode which is integrated in the fluidic-system, a RuO_2-spot allows potentiometric measurements of the pH-value of the culture medium [11]. The sensor is performed as a RuO_2-spot, which is deposited on a platinum electrode by sputtering [12].

Impedance - sensors:

Information on cell adhesion and changes of the morphological properties of the cells is provided by interdigitated electrode structures (IDES) [13]. The structure shape is a finger-capacitor with 50µm structure dimensions as shown in figure 5.

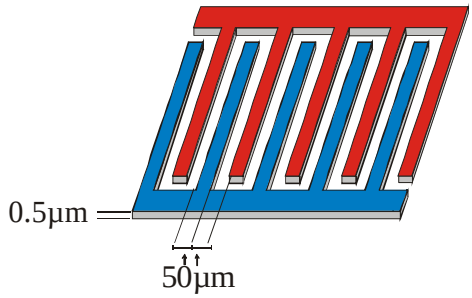

Figure 5: Schematic of an interdigitated electrode structure

It is contacted with two seperate paths each for current and voltage in order to eliminate path resistances. Like the planar amperometric oxygen-sensor, it is fabricated from a platinum layer by thin film technology methods. A low-amplitude alternating current in a frequency range of about 10 - 20 kHz is applied to the electrode structure. Due to the insulating properties of the cell membranes in this frequency

ISSN: 1727-1983

range, the measured impedance values increased upon cell adherence. Thus, changes in impedance reflect the process of cell spreading, cell adhesion and rearrangements of the cytoskeleton linked to cell-cell and cell-matrix junctions [14]. An equivalent circuit with a resistance and a capacitance in parallel (C_{par}, R_{par}) is selected for the specification of the complex impedance. These parameters are used for the description of results [14].

Micro-electrode-arrays:

For the monitoring of electrophysiological parameters of electrically active cells, a micro-electrode-array is used. It consists of 64 electrodes arranged in an 8x8 array. The array is fabricated from a platinum layer with a gold spot for each of the 64 sensor-electrodes. With this micro-electrode-array electrical activity generated in networks of cultured neuron or muscle cells can be detected [15,16].

Sensorchips

The sensor types described above can be integrated on glass-based sensorchips and will be arranged depending on the intended application. Figure 6 shows three possible arrangements. However, every custom arrangement is possible. Due to the transparency of the sensorchips, the microplate can also be employed for all kinds of microscopic applications.

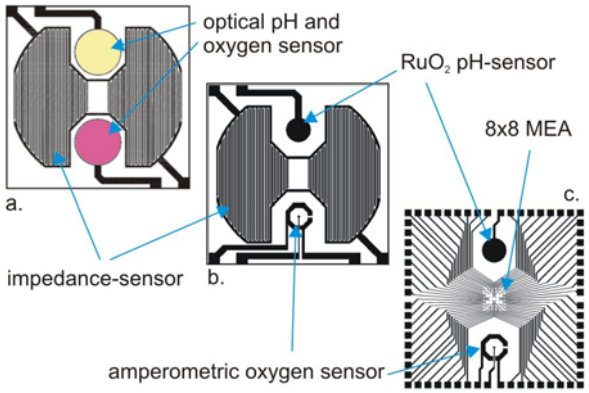

Figure 6: Three possible sensor-arrangements on glass-based sensorchips: a. with optical sensors; b. with electric sensors; c. Neurochip with metabolic sensors

Microplate

The sensorchips are fixed beneath a 24-well-microplate and electrically connected to an adequate contact-frame as shown in Figure 7. The microplate can be custom selectable equipped with different sensorchips, allowing different assays on one plate. As the format of this plate complies with other commercial microplates, it can be used with all customary liquid handling systems and is easily integrated into industrial process lines.

Figure 7: Mounting with sensorchips and electrically contacting of the 24-well-microplate

Discussion

Together with an appropriate analyzing system, which provide the the complete measurement electronic equipment for sensor control and data aquisition for all described sensor-types and an adequate liquid handling system, this microplate enables a wide range of possible applications. The beginning of each experiment includes seeding of cell suspensions directly in each well (about 2 x 10^5 cells per well) and incubation for 48 h in a humidified atmosphere for adherent growing cell cultures. After incubation, the microplate is inserted in the analyzing system. Figure 8 shows an example of an analyzing system, which has been developed together with the microplate.

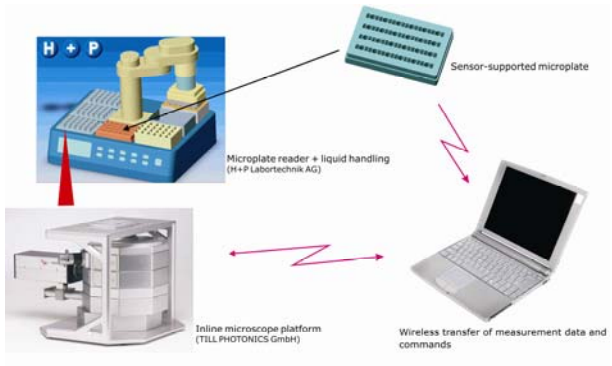

Figure 8: Screening system for living cells supported by microsensors and microscope platform

On-line and thereby dnamic monitoring of the effects of active agents on living cells is possible with multi-parametric sensor system based on technologies described above. Sensor-based cellular assays are non-invasive and do not not need any fluorescence labels. Measurements of morphological properties of cell cultures are possible. To obtain information about cellular metabolism, a liquid handling system is operated in an interval mode and the kinetics of primary data of pH- and oxygen-sensors are monitored. The

ISSN: 1727-1983

measurement of absolute values of pH and oxygen is not pursued. Only relative changes in the course of an experiment are analyzed and all possible interpretations are referenced to a baseline which is typically defined by the fresh culture medium properties at the end of one flow-cycle.

Conclusions

Due to the transparency of glass-based sensorchips, the microplate can also be employed for all kinds of fluorescence applications such as live cell microscopy. The combination of multiple sensor data and data from microscope imaging frequently allows conclusions about the mechanism of action of added drugs. As the format of this plate complies with other commercial microplates, it can be used with all customary liquid handling systems and is easily integrated into industrial process lines. For this reason there are many possible fields of application in biomedical or pharmaceutical areas like drug screening, chemosensitive testing, environmental engineering and basic research.

Acknowledgments

The authors would like to thank the current staff of the Heinz Nixdorf-Chair for Medical Electronics involved in this work. This work is supported by the Bayerische Forschungsstiftung under reference no. 572/03, in part by the Stiftung Industrieforschung under contract no. S641 and by technical cooperation with Heraeus Sensor Technology GmbH, Kleinostheim (Germany), H+P Labortechnik AG, Oberschleißheim (Germany), PreSens GmbH, Regensburg (Germany), Microcoat GmbH, Bernried (Germany) and Rawe Electronic GmbH, Weiler i. Allg. (Germany).

References

[1] BRISCHWEIN M., MOTRESCU E. R., OTTO A.M., CABALA E., GROTHE H., WOLF B. (2003): 'Functional cellular assays with multiparametric silicon sensor chips', *LAB ON A CHIP*, **3**, pp. 234-240

[2] OTTO A.M., BRISCHWEIN M., NIENDORF A., HENNING T., MOTRESCU E., WOLF B. (2003): 'Microphysiological testing for chemosenitivity of living tumor cells with multiparametric microsensor chips', *Cancer Detection and Prevention*, **27**, pp. 291-296

[3] JOHN G.T., KLIMANT I., WITTMANN C., HEINZLE E. (2003): 'Integrated optical sensing of dissolved oxygen in microtitre plates: A novel tool for microbial cultivation', *Biotechnology and Bioengineering*, **81**, pp. 829-836

[4] DESHPANDE R.R., HEINZLE E. (2004): 'On-line oxygen uptake rate and culture viability measurement of animal cell culture using microplates with integrated oxygen sensors', *Biotechnology Letters*, **26**, pp. 763-767

[5] PreSens GmbH, Internet site address: http://www.presens.de/

[6] BARD A.J., FAULKNER L.R. (1969): 'Electrochemical Methods, Fundamentals and Applications', 2 ed., John Wiley & Sons, Inc.

[7] WIEST J., BRISCHWEIN M., RESSLER J., OTTO A.M., GROTHE H., WOLF B. (2005): 'Cellular assays with multiparametric bioelectronic sensor chips', *Chimia*, **5**, pp. 243-246

[8] JOHN G.T., GOELLING D., KLIMANT I., SCHNEIDER H., HEINZLE E. (2003): 'pH-sensing 96-well microtitre plate for the characterization of acid production by dairy starter cultures', *Journal of Dairy Research*, **70**, pp. 327-333

[9] JOHN G.T., HEINZLE E. (2001): 'Quantitative screening method for hydrolases in microplates using pH indicators: Determination of kinetic parameters by dynamic pH monitoring', *Biotechnology and Bioengineering*, **72**, no. 6, pp. 820-627

[10] LEHMANN M., BAUMANN W., BRISCHWEIN M., EHRET R., KRAUS M., SCHWINDE A., BITZENHOFER M., FREUND I., WOLF B. (2000): 'Non-invasive measurement of cell membrane associated proton gradients by ion-sensitive field effect transistor arrays for microphysiological and bioelectronical applications', *Biosensors and Bioelectronics*, **15**, pp. 117-124

[11] FOG A., BUCK R.P. (1984): 'Electronic semiconducting oxides as pH sensors', *Sensors and Actuators*, **5**, pp. 137-146

[12] KREIDER K.G., TARLOV M.J., CLINE J.P. (1995): 'Sputtered thin-film pH electrodes of platinum, palladium, ruthenium and iridium oxides', *Sensors and Actuators B*, **28**, pp. 167-172

[13] EHRET R., BAUMANN W., BRISCHWEIN M., SCHWINDE A., STEGBAUER K., WOLF B. (1997): 'Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures', *Biosensors and Bioelectronics*, **12**, pp. 29-41

[14] EHRET R., BAUMANN W., BRISCHWEIN M., SCHWINDE A., WOLF B. (1998): 'On-line control of cellular adhesion with impedance measurements using interdigitated electrode structures', *Medical*

ISSN: 1727-1983

& Biological Engineering & Computing, **36**, pp. 365-370

[15] GROSS G.W, WEN W.Y., LIN J.W. (1985): 'Transparent indium-tin oxide electrode patterns for extracellular, multisite recording in neuronal cultures', *Journal of Neuroscience Methods*, **15**, pp. 243-252

[16] KEEFER E.W., GRAMOWSKI A., STENGER G.A., PANCRAZIO J.J., GROSS G.W. (2001): 'Characterization of acute neurotoxic effects of trimethylolpropane phosphate via neuronal network biosensors', *Biosensors and Bioelectronics*, **16**, pp. 513-525

ISSN: 1727-1983