LOW-COST BIOSENSORS: CERAMIC-BASED MULTI-PARAMETRIC SENSORCHIP FOR FUNCTIONAL SCREENING

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Abstract: Biochip technology is considered as a great promise for drug discovery, testing of patients for effective therapies and basic bioscience. The usage of multi-parametric biochips with integrated microsensors for the monitoring of living cells to realize functional assays marks a great advantage in first line identification of the pharmacological properties of an active agent. One limitation in the commercial aspects using multi-parametric biochips are expensive production costs, often caused by the usage of silicon-based technology. Lowering the costs of biochips is the technical challenge, which to be solved for commercial application. To face this challenge, we have developed a ceramic-based multi-parametric biochip, which is equipped with sensors for metabolic and morphologic parameters of living cells or tissue. By fabricating this biochip without silicon technology, it is possible to lower the production costs by about 80%, however providing the same functionality.

Introduction

Functional assays using living cells are commonly used as a primary screen to provide first line results on the pharmacological properties of an active ingredient in pharmacological drug-screening and medical diagnostics. Cellular live is affected by dynamic and complex molecular processes. The regulation of these processes is an essential part of the function of the cell. This includes, that the cells are permanently converting physical and chemical signals of the environment into adequate cellular behavior. This behavior includes the decision about cell division and apoptosis, the activation of different metabolism paths or the production and disposal of proteins. For the monitoring of cellular behavior, typically fluorescence-based methods are used in pharmacological drug-screening. However, these methods are often affected by several disadvantages. Some fundamental cellular parameters are hardly accessible with fluorescent methods, e.g. the study of changes in cell adhesion or cell morphology. Besides, fluorescence assays are endpoint measurements, which means, that the measurement represents only a snapshot recording of the actual cellular state. Due to these disadvantages, microsensor-based assays for metabolical and morphological parameters find their place in various applications [1]. For these parameters there are appropriate electrical and electronic sensors. These microsensors have the advantage that the cells can grow directly on the sensor structures. The measurements are non-invasive and cause no disturbance of the cellular behavior. Due to these facts, microsensors allow real-time multi-parametric monitoring of cellular parameters up to several days. Figure 1 shows an example of a multi-parametric measurement [2].

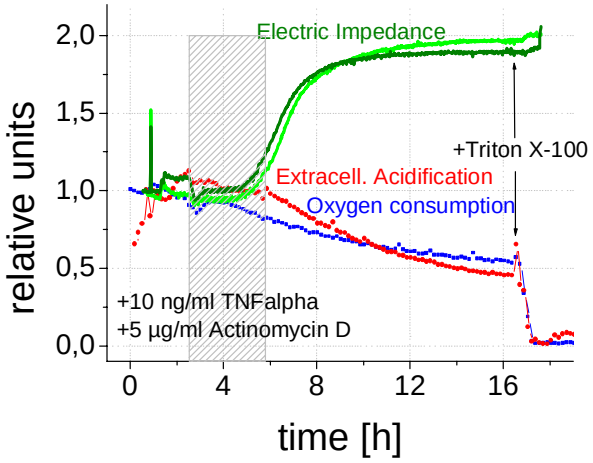

Figure 1: Measurement example: Effects of TNF-alpha and Actinomycin on Hela cells (induction of apoptosis), recorded with microsensors

For the fabrication of the sensorchips often silicon-based technologies are used, e.g. to realize sensors based on field effect transistors (FET) [3]. Due to the relatively large sensorchip area necessary for biochip applications in relation to the integrated FET area, silicon-based technologies cause expensive production costs. As biochips are typically products for one time-usage, the fabrication is not cost effective. One change to face this challenge is to fabricate the biosensor without silicon-based technologies. This requires new sensor technology for some sensor types, e.g. pH-sensors.

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Materials and Methods

Sensors

For the monitoring of the metabolical and morphological parameters of living cells we integrate the following sensors:

pH-sensors:

Acidification of the culture medium is measured by pH-sensors. This acidification provides information on the general cellular metabolism. For planar pH-sensor usually ion sensitive field effect transistors (ISFETs) are used [3]. 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 rutheniumoxyde (RuO_2)-spot allows potentiometric measurements of the pH-value of the culture medium [4]. The sensor is performed as a RuO_2-spot, which is deposited on a platinum electrode by sputtering [5]. Figure 2 shows a microscope image of the RuO_2-spot with the platinum electrode beneath.

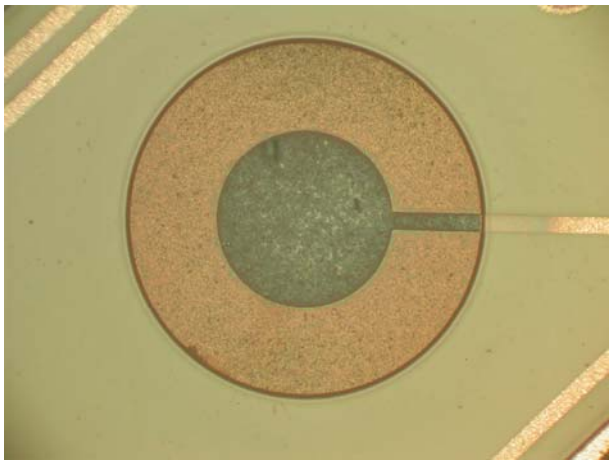

Figure 2: Rutheniumoxyde (RuO_2)-spot which was sputtered on a platinum electrode

Oxygen-sensor:

With the oxygen-sensor it is possible to detect the oxygen consumption of the cells. This allows information on cellular respiration and mitochondrial activity. A planar oxygen sensor, fabricated of a platinum layer by thin film technology is used. The 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:

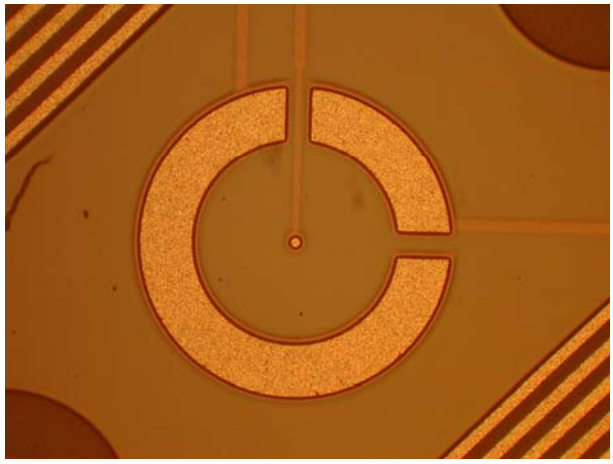

Figure 3: Configuration of the planar amperometric oxygen-sensor with working-, reference- and auxiliary-electrode

Impedance-sensors:

Information on cell adhesion and changes of the morphological properties of the cells is provided by interdigitated electrode structures (IDES) [8]. The structure shape is a finger-capacitor with 50µm structure dimensions as shown in figure 4.

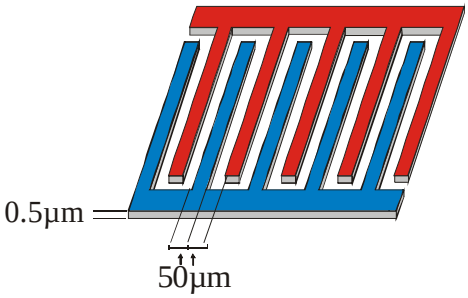

Figure 4: Schematic of an interdigitated electrode structure

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 range, the measured impedance values increased upon cell adherence. Thus, changes in impedance reflect the process of cell spreading, cell adhesion and

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rearrangements of the cytoskeleton linked to cell-cell and cell-matrix junctions [9]. 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 [9].

Temperature-sensor:

For temperature-measurements a Pt1000 platinum resistor is integrated in addition to the metabolical and morphological sensors. With this sensor the temperature of the culture medium can be monitored and facilitate the control of environmental parameters.

Sensorchip

The electrodes for the above described sensor types are fabricated on a ceramic-based substrate by thin film technologies. The RuO_2 for the pH-Sensor is deposited by a sputter-process. Figure 5 shows the layout of the sensorchip. The chip (figure 6) is insolated by a layer of photo resist and is opened on the active sensor surface.

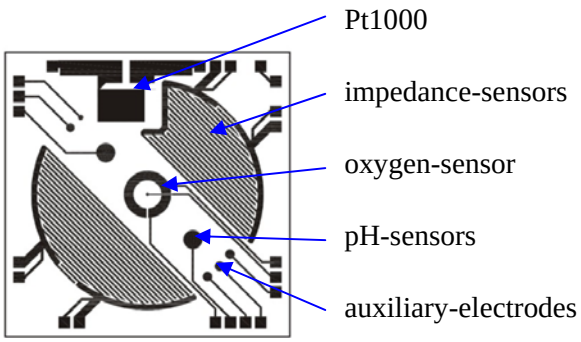

Figure 5: Layout of the ceramic-based sensorchip

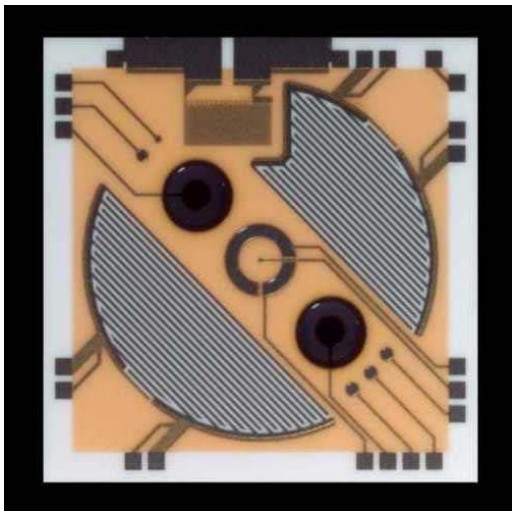

Figure 6: Ceramic-based multi-parametric sensorchip

The sensorchip is fixed on a PLCC68-compatible printed wire board (24 x 24 mm) and is electrically contacted by wire-bonding. To create a culture vessel, the chip is encapsulated by injection molding. The culture vessel is approximately 9.5 mm in diameter. The diameter of the chip area grown with cells is approximately 6 mm. Figure 7 shows the packaged sensorchip. All production processes and all materials which get in contact with cells or culture medium are tested for biocompatiblity according to ISO 10993-5. Prior to use, the chips are sterilized by gamma radiation with approximately 5 kGy.

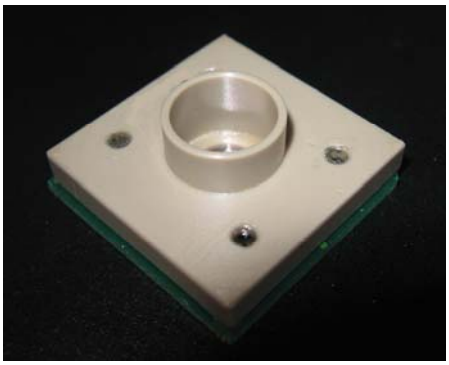

Figure 7: Encapsulated sensorchip module with culture vessel

Measurement methods

Together with appropriate analyzing systems [1,2,7] which provide the the complete measurement electronic equipment for sensor control and data aquisition for all described sensor-types and an adequate fluidic perfusion system, the sensorchip enables a wide range of possible applications. The beginning of each experiment includes seeding of a cell suspension directly on the chip surface (about 1.5 x 10^5 cells) and incubation for 48 h in a humidified atmosphere for adherent growing cell cultures. After contacting the chip on a PLCC-Socket, a fluid insert is connected enclosing a cell culture microchamber with a volume of about 6 µl and providing a regular exchange of cell culture medium and drug addition. The fluidic system is designed to avoid large pressure changes with possible adverse effects on the cells. Figure 8 shows an example of an analyzing system, which have been developed together with the sensor chip.

Figure 8: Intelligent Mobile Lab (IMOLA) for the monitoring of living cells

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The appropriate measurement method for the monitoring of cell respiration and the acification of culture medium is marked by a pump operating in stop and flow mode.This periodic operation of the fluid perfusion system (typically 7 min flow-off followed by 3 min flow-on with a rate of 100 µl/min) shape a periodic oscillation of pH- and oxygen-sensor signals. During the flow-off intervals, the cells are affecting the surrounding culture medium directly by extrusion of acidic metabolic products and by consuming dissolved oxygen. To evaluate the pH-sensor raw data, the slope of the graph (voltage vs. time) during the flow-off interval is calculated by linear regression. The resulting value is expressed as 'dU/dt'. The raw data of the planar amperometric oxygen-sensor (current vs. time) is calculated in a similar way, besides that only the first three minutes of the flow-off interval is considered and the resulting slope is corrected by the sensitivity of the sensor. The resulting value is expressed as 'dI/dt'. A measure of sensitivity is obtained from the sensor signal during the flow-on intervals, when fresh culture medium saturated with oxygen flowed into the microchamber. Thus, every flow cycle resulted in exactly one value of dU/dt and dI/dt. Here, absolute values of pH and oxygen concentration is not of interest. Therefore, the calculation of cellular metabolic rates is based on the determination of relative changes during short time intervals, where long term signal drift is negligible [7]. Figure 9 shows the principle of the sensor data analysis considering as an example of pH raw data.

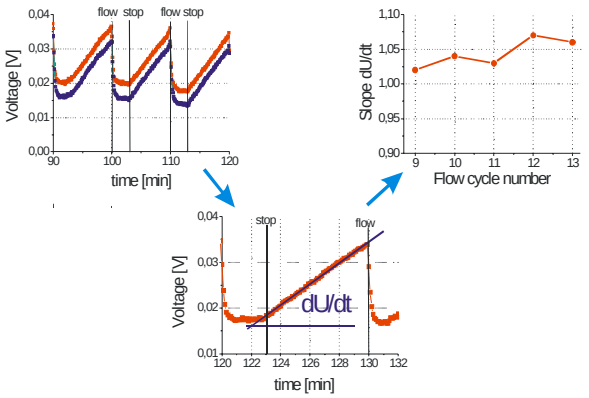

Figure 9: Analysis of sensor signal raw data of the pH-sensor: absolute voltage data results in relative values of acidification

In order to obtain baseline signals (sensor readings without viable cells), a adequate ingredient (e.g. the non-ionic detergent Triton X-100) is added at the end of each experiment [2].

Discussion

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 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. From an economic point of view, the use of low-price technologies such as chips based on ceramic-substrates is superior to silicon-based technologies.

Conclusions

The ceramic-based multi-parametric sensorchip described above can deliver information on metabolical and morphological parameters of living cells. For this reason there are many possible fields of application in biomedical and pharmaceutical areas, environmental engineering and basic research.

By fabricating this sensorchip without silicon technology, it is possible to lower the production costs by about 80%, however providing the same functionality and performance as silicon-based technologies. This approach combines the advantages of biochip technology with cost effective usage for commercial applications.

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