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Bioimpedance microelectronics in a 24-microwell plate with metabolic-sensors for testing chemosensitivity of tumor cells and tissues

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Abstract. Living cells react to external influences such as pharmacological agents in an intricate manner due to their complex internal signal processing. Cell reactions are an impact on vitality, cell-cell or cell-matrix interaction and morphological changes. A number of published techniques on impedance spectroscopy (IS) of adherent cells with planar electrodes address these changes. However, IS can merely serve as an indicator of cellular events rather than provide detailed information on a specific cell process. Thus our approach is a 24-microwell sensor-plate with impedance-electrodes in parallel to pH- and O_2-sensors, capable of being integrated into a fully automated screening system. For the purpose of IS, high precision impedance-electronics have been developed based on integrated circuits and validated against a Solartron 1260 impedance analyzer. IS data is correlated to the metabolic-sensors and additionally compared with cell images shot by an inverse optical microscope which is also part of the screening system. Proof of principle is demonstrated by experimental growth monitoring of a MCF-7 culture and cellular response to chemotherapeutics. Furthermore, the potential to monitor living tissue probes is presented for the first time.

1. Introduction to IS for label-free cellular monitoring in cell culture plates

In standard assays, the cellular response is determined by end-point methods usually involving labeling and optical methods. However, cell labeling is expensive, it is a possible source of artifacts, and long-term monitoring is hard to realize. Thus, the kinetics of an effect remains unclear in many cases and recovery effects may completely escape detection. For these reasons impedance spectroscopy (IS) appears to be a suitable method for monitoring cultured cells label-free and in real-time. A significant scientific interest arose when a first report on IS in cell culture appeared in 1984 [1]. This led to commercialization of products which are now in some extent automated screening systems.

A brief review of three systems is provided by [2]: ECIS [3], RT-CES [4] and CDS [5] are purely impedance based technologies utilizing different electrode arrangements, materials and measurement strategies. Each of these systems shares basic principles in IS [6]: In a parallel approach, a plausible physical model has been constructed and correlated with the measured impedance data. When based on a profound theory, mathematical models or electrical equivalent circuits can be parameterized. When modeling cultured cells on electrodes, one has to account for electrode impedance in series with the impedance contribution of the cellular layer. A lot is known on both domains, but one or the other is often neglected. Although modeling is thought to be descriptive, combining these impedance fractions increases complexity enormously. Since these systems suffer from data indexation, limited bandwidth or even predefined frequencies, they remain unique and studies are not easily comparable.

The concept of an outright novel screening system called “intelligent microwell-plate reader” (IMR), in which an IS module is embedded, has previously been published elsewhere [7,8]. It has been clearly shown that micrometabolic sensor technology and image documentation provide compounded insight into distinct aspects of cellular life and cellular responses, which can be correlated with data

about cell morphological changes obtained by impedance measurements. The development of miniaturized impedance electronics to address planar electrodes in microwell-plates is essential, since there is no measurement apparatus available, which is sufficiently packaged to integrate all system components in a small unit, meets the needs for non-invasive cellular measurements and provides complete impedance information over a large frequency range.

2. Materials and Methods

Core of the IMR (figure 1) is a sensor-equipped microwell-plate (MWP). Together with the IMR system components, this plate is placed into a standard cell culture incubator. Integrated microelectronics measure the complex cellular impedance with planar interdigitated electrodes (IDES, figure 2). This information is correlated with data on cell metabolic rates retrieved with optochemical pH- and pO_2-sensor spots. These are read out by a moveable sled-mounted process-microscope in parallel to image capturing. Cell culture media (and drug solutions) are regularly replaced by an automated pipetting-robot. A central control unit ensures long-term experiments up to days and weeks.

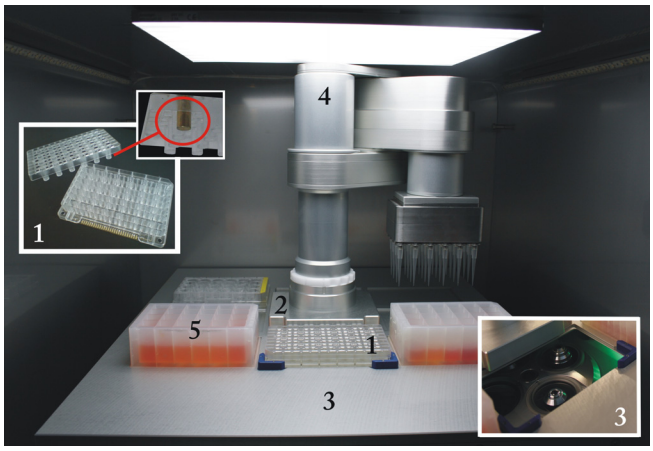

Figure 1. Intelligent Microwell-plate Reader (IMR): Microwell-plate with cover plate (1), impedance electronics (2), inverse microscope (3), pipetting robot (4) and cell-culture medium reservoirs (5).

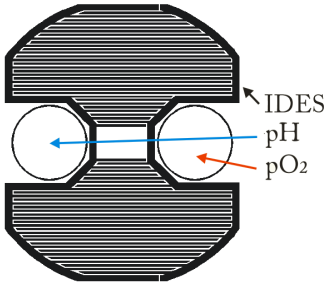

Figure 2. Interdigitated electrode structure with optochemical sensor spots and window for microscopy.

2.1. Microwell-plate

The MWP is a composed of a 24-well polymer corpus and a glass bottom plate with 24 IDES and optochemical sensor spots. Each cell culture well has a diameter of 7 mm. IDES are fabricated by thin film structuring of Pt, both electrode width and distance are 50 µm. Two side chambers connected to the culture well at the bottom serve as access points to the pipetting robot. Equilibration of hydrostatic pressure differences thereby result by medium flowing through the culture well. A lid with inserts to each well confines the assay volume to 23 µl. For the first time, Au-plated inserts are utilized for vertical impedance measurements against bottom IDES in cell culture wells (figure 1).

2.2. *Microelectronics*

Novel high precision IS microelectronics have been developed on the basis of the AD5933 impedance converter IC from Analog Devices. This impedance analyzer in a chip derives complex impedance by a DFT-algorithm from a sinusoidal stimulus signal. Measurement accuracy of 0.5% could be attested from 5 kHz to 100 kHz for high impedances greater than 1 kΩ. To overcome bandwidth limitations, to be able to measure small impedances and to avoid interference with other electrical sensors, several modifications have been implemented in our design (figure 3). These include flexible clocking, galvanic decoupling and the development of an analog frontend. Because a true two-electrode measurement is performed on IDES, stimulus voltage and resulting current are regulated well below

26 mV or 4 mA/cm^2, respectively, in order to monitor a quasi-linear system [9]. As a result, impedance spectra of 24 wells from 500 Hz to 100 kHz can be measured within 40 s.

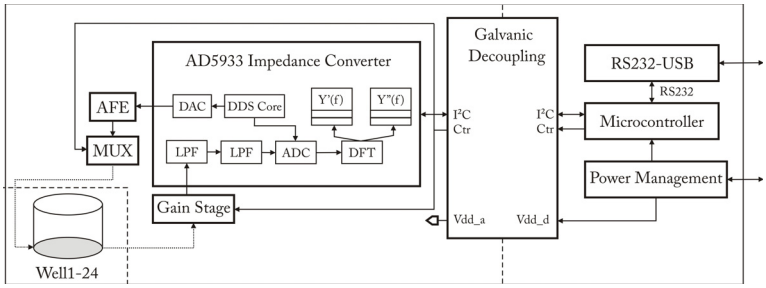

Figure 3. AD5933 bioimpedance electronics peripheral block diagram

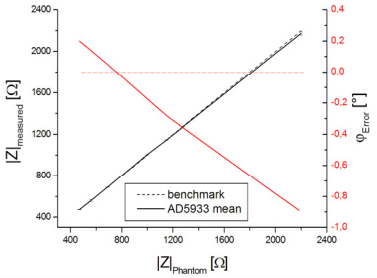

Figure 4. System accuracy (10kHz)

As a gold-standard the frequency-response analyzer Solartron 1260, which yields 0.1% accuracy within a frequency range from 10 µHz up to 32 MHz, was chosen. System accuracy was determined to be less than 2% for either magnitude or phase with the use of R-C impedance phantoms as compared with the reference (figure 4). Dummy circuits were therefore assembled based on experimental results to match true cell impedance in the frequency bandwidth of interest. Working bandwidth of 50 µm Pt-IDES was found to be from 100 Hz to 10 kHz which overlaps with the operation range of the analyzers. Moreover, β-dispersion of biological tissue is expected in the same region [10].

2.3. Cell measurements

MCF-7 cells (human breast cancer cells) were inoculated into the central chamber of the MWP with 500 µl Dulbecco's Modified Eagle Medium containing 5% fetal calf serum. Since a medium with reduced pH-buffer capacity was used for enhanced pH-sensitivity, the cell culture medium was exchanged in 10-20min cycles. Impedance data was interpreted in magnitude $|Z(\omega)|$ and phase $\varphi(\omega)$. For simple detection of events, change in impedance $\Delta|Z(\omega)|=[(|Z(\omega)|-|Z_0(\omega)|)]/|Z_0(\omega)|$ was the normalized cell impedance, with Z_0 being the impedance of pure medium. As experimental compounds, cytochalasin B and cisplatin were added with the pipetting robot from a separate medium reservoir. Cytochalasin B is an alkaloid drug known to inhibit glucose import into the cell (thereby altering the cell metabolic profile) and to act on actin filaments in the cellular cytoskeleton. Cisplatin is a cytostatic drug used for cancer chemotherapies. Addition of the non-ionic detergent Triton X-100 yields baseline values of sensor date validates reversibility and correctness of the signals.

3. Results and Discussion

Experiments were performed to monitor cellular growth on electrodes (figure 5) whilst verifying the proliferation process with microscopy (figure 6). After attaining a confluent cell layer, cytochalasin B was applied for three hours and withdrawn thereafter to investigate the impact on cell morphology and potential for recovery. In a second assay, cells were treated with cisplatin to compare the effect on cell morphology with the effect on cell metabolism (figure 7). To examine the responses of cells within their native 3D tissue architecture, pieces of explanted sheep pancreas were analysed within the system (figure 8).

The results show the successful integration of IS microelectronics for non-invasive cell culture monitoring and its benefit compared to established laboratory methods. Parallel live-cell imaging is a valuable tool for the validation of sensor data interpretation. As a next step, it is envisaged to use sensor data for image acquisition. Furthermore the attempt of applying vertical IS in culture-wells appears promising for monitoring cultured tissue probes. In conjunction with metabolic monitoring, a more complete picture of cellular physiology and its responses to experimental treatment

is obtained. A particular focus of application is the use of the system for improving and individualising cancer chemotherapies by on-chip cell culturing systems.

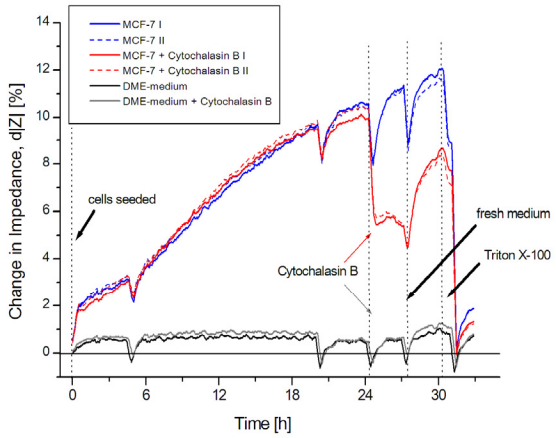

Figure 5. Parallel assays of MCF-7 cells ($8x10^4$ per well) monitoring proliferation and morphological changes after addition and removal of 6µM cytochalasin B.

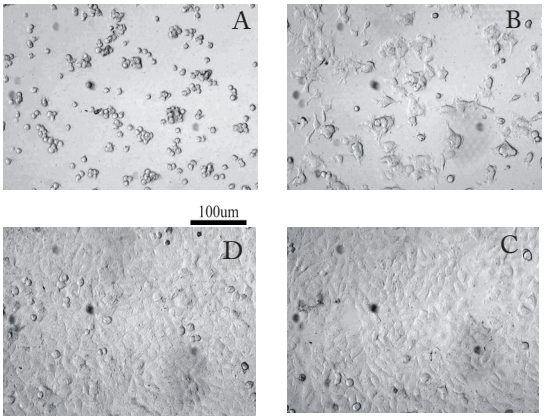

Figure 6. Automated picture documentation: Cells after seeding (0h, A) and spreading (5h, B), confluent cell layer (20h, C) and cells after agent addition (27.5h, D).

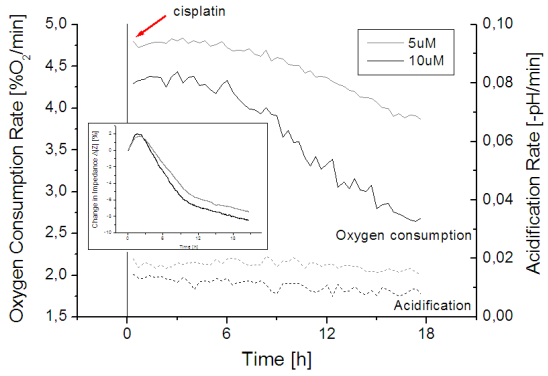

Figure 7. Metabolic reaction in comparison to the change in impedance during long-term treatments of MCF-7 assays with 5µM and 10µM cisplatin.

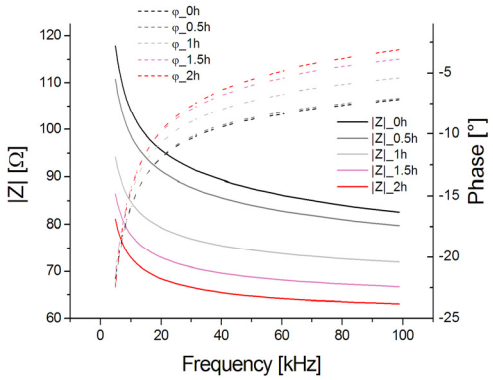

Figure 8. Triton X-100-induced cell destruction of sheep pancreas tissue in a microwell-plate: Impedance spectrum vertically recorded against gold-plated lid.

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