High-content multi-frequency impedance cell monitoring for label-free and time-resolved cell toxicity analysis of various cell types

Elena Dragicevic[1], Krisztina Juhasz[1,2], Michael Skiba[3], Joachim Wegener[3], Marta Lemme[1], Ulrich Thomas[1], Max Engelstädter[1], Michael George[2], Sonja Stoelzle-Feix[1], Niels Fertig[2]

1 Nanion Technologies, Munich, Germany; 2 Institute for Nanoelectronics, TUM, Germany; 3 Institute of Analytical Chemistry, Chemo- and Biosensors, Faculty of Chemistry and Pharmacy, University of Regensburg, Regensburg, Germany

• contact : elena@nanion.de •

Introduction

Reading the impedance of planar gold-film electrodes that are used as growth substrate for adherent cells reveals changes in cell morphology. Thus, real time impedance data provide insights in various cell phenotypes such as cytotoxicity even over prolonged periods of time. A crucial advantage over standard assays is the non-invasive continuous cell monitoring. Advanced information content is obtained by using multi-frequency impedance readouts as they allow zooming in on changes in membrane topography, cell-cell or cell-matrix junctions deconvoluting the complex whole cell response, for instance, to G-protein-coupled receptor (GPCRs) activation. The multi-frequency character of the data allows selecting the most sensitive frequency for a particular application or cell type after data acquisition is complete.

Multi-parametric cell monitoring with CardioExcyte 96

Next to electrophysiology, contractility and viability recordings now the deconvolution of complex whole cell responses is possible with the new software tool **SpectraControl** running on **CardioExcyte 96**. In addition to high quality consumables and controlled temperature and environment, the system comes with an automated liquid handling system for cell seeding, compound applications and medium exchange.

Fig. 1 Multiple functional assays can be combined in a single plate. Transparent NSP-96 sensor plates are compatible with standard plate readers and automated instrumentation, thereby giving you freedom to combine your assays in one plate.

Impedance-based assay technology

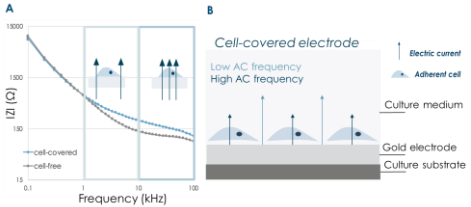


Fig. 2 The frequency dependent behavior of a cell-free and cell-covered electrode presented in a so-called Bode diagram (**A**) reflects the impedance increase due to the cell layer between ~ 1 – 100 kHz. For the experimental setup presented here, the signal is dominated by the paracellular cell layer resistance between about 1 – 10 kHz (range indicated by light blue frame) and is increasingly influenced by capacitive currents across the cell membranes between 10 – 100 kHz (range indicated by darker blue frame). **B** Schematic side view of a cell-covered electrode.

Impedance describes the electrical resistance in response to an AC signal. It takes into account that capacitors become more conductive with increasing AC frequency. Thus, impedance spectroscopy that uses AC signals with individually modulated frequencies within a spectrum enables a more diversified study of the passive electric properties of the cell layer.

Cell proliferation and cell toxicity experiments

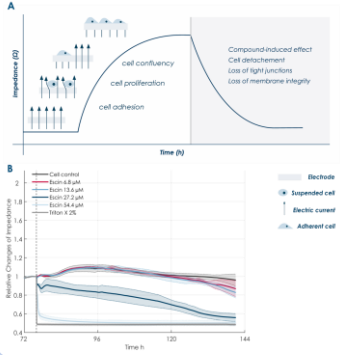


Fig. 3 A Base impedance measurements at a single fixed frequency (e.g 10 kHz) reveals cell adherence, proliferation or cell death. Hereby identified cell-specific profiles of different growth and cell behavior pattern are e.g. relevant in cancer research. **B** Exemplary cell death experiment. Effect of Escin on the impedance signal of CHO cells in concentrations of 6.8 µM, 13.6 µM, 27.2 µM and 54.4 µM (n = 4). Effect of Triton X-100 (2 %) is also shown. Data are normalized to the respective compound reference without cells and the compound addition time point.

Frequency spectra reveal the *sweet spots:* *"Where do I find the maximum resolution of my effect?"*

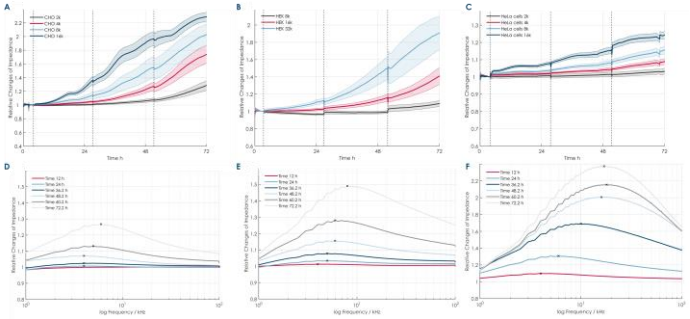
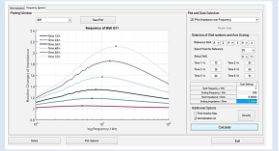


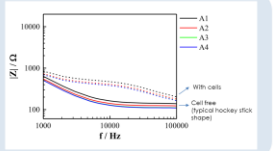
Fig. 4 *"Which frequency can spot the highest impedance change?"* Averaged relative changes of the impedance signal with associated standard deviation due to cell proliferation of CHO (**A**), HEK (**B**) and HeLa (**C**) cells with initially seeded cell densities of 8k, 16k and 32k / well over a time period of 72 h (n=12 each). Data is normalized to medium reference without cells and to the time point of cell addition to wells. Frequency spectra at time points 12 h, 24 h, 36 h, 48 h, 60 h and 72 h for CHO cells with initial cell densities of 2k (**D**), 4k (**E**) and 16k / well (**F**). Data is normalized to medium reference without cells and cell addition time point. *Sweet spots*, maximum of amplitude change, is marked with "x".

Barrier function – GPCR agonists relate to changes in barrier properties

The CardioExcyte 96 software provides a SpectraControl tool to record and analyze impedance spectra.

Changes in cell shape and cell number can be investigated. The method is suitable for monitoring cell adhesion, cell specific structural changes, proliferation and toxic effects of compounds.





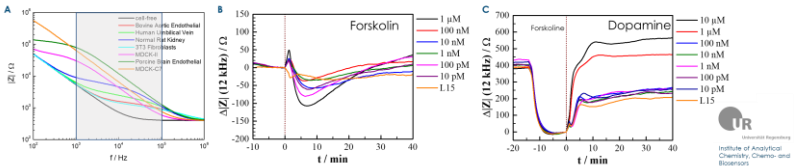


Fig. 5 Frequency-dependent impedance recordings are the basis for many applications of impedance technology in cell analysis. **A** shows simulated impedance spectra illustrating cell-type specific dielectric properties of listed cell lines for a 0.6mm recording electrode. Additionally, we were able to monitor GPCR activation in various cell types by applying specific agonist in dose-response studies. **B,C** Here, we were able to monitor GPCR-mediated signal transduction by applying the endogenous agonist (dopamine) in dose-response studies or receptor-independent agents that are used for pathway deconvolution (forskolin) (L15 indicates control).

Conclusions

Electric cell-substrate impedance sensing with frequencies ranging from 0.1 kHz – 100 kHz are successfully integrated in Nanion´s cell monitoring technology (CardioExcyte 96/FLEXcyte 96).

Continuous and multi-parametric cell monitoring has an advantage over time- and cost- intensive individual endpoint assays.

Nanion´s experimental setup reveals the kinetics of cell behavior and allows an in-depth mechanistic understanding in real-time.

Multiple research areas benefit from impedance recordings at a frequency spectrum.