

Cell Monitoring using Multi-Frequency Impedance Recordings for Label-free and Time-resolved Cell Response Analysis

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Abstract

Reading the impedance of planar gold-film electrodes that are used as growth substrate for adherent cells reveals changes in electrode coverage or cell morphology. Thus, real time impedance data provide insights in various cell characteristics, such as cell morphology, proliferation, lateral migration or cytotoxicity even over prolonged periods of time. A crucial advantage over standard assays is the 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.

Multi-frequency impedance measurements were used to monitor cell adhesion, cell specific structural changes, proliferation and cytotoxicity. The multi-frequency character of the data allows selecting the most sensitive frequency for a particular application, cell type or detail of the cell response after data acquisition is complete.

Impedance spectra were calculated for HeLa, HEK293 and CHO cells to demonstrate cell-type specific differences under stationary conditions. Data acquisition was performed using the CardioExcyte96 impedance recording system. Cells covering the electrode impede the applied current due to insulating properties of their membranes and the individual tightness of their cell junctions. Cell responses to experimental challenges will induce corresponding impedance changes. Multi-frequency impedance analysis provides a stronger basis for data interpretation as it highlights the changes in specific cell structures.

HeLa, HEK293 and CHO cells show different proliferation curves. Frequency-dependent impedance data reveal individual drifts of the most sensitive frequency, i.e. of the maximal responses indicating cell type specific changes in cell junctions and proliferation dynamics. The expected concentration dependent toxicity was observed for DMSO, Escin and Triton-X by multi-frequency impedance recordings showing decreasing impedance values with exposure time and drifting of the maximal responses to lower frequencies. GPCR-mediated signal transduction was investigated using cells recombinantly expressing the dopamine D₂ receptor. Agonist mode measurements using the endogenous agonist dopamine and antagonist mode measurements using the specific antagonist haloperidol returned potencies that are in line with the literature. Multi-frequency impedance data allow for non-invasive continuous monitoring of cells with the option to zoom in on certain cell aspects or to tailor the readout to individual cell types for improved data interpretation.

Impedance-based assay technology

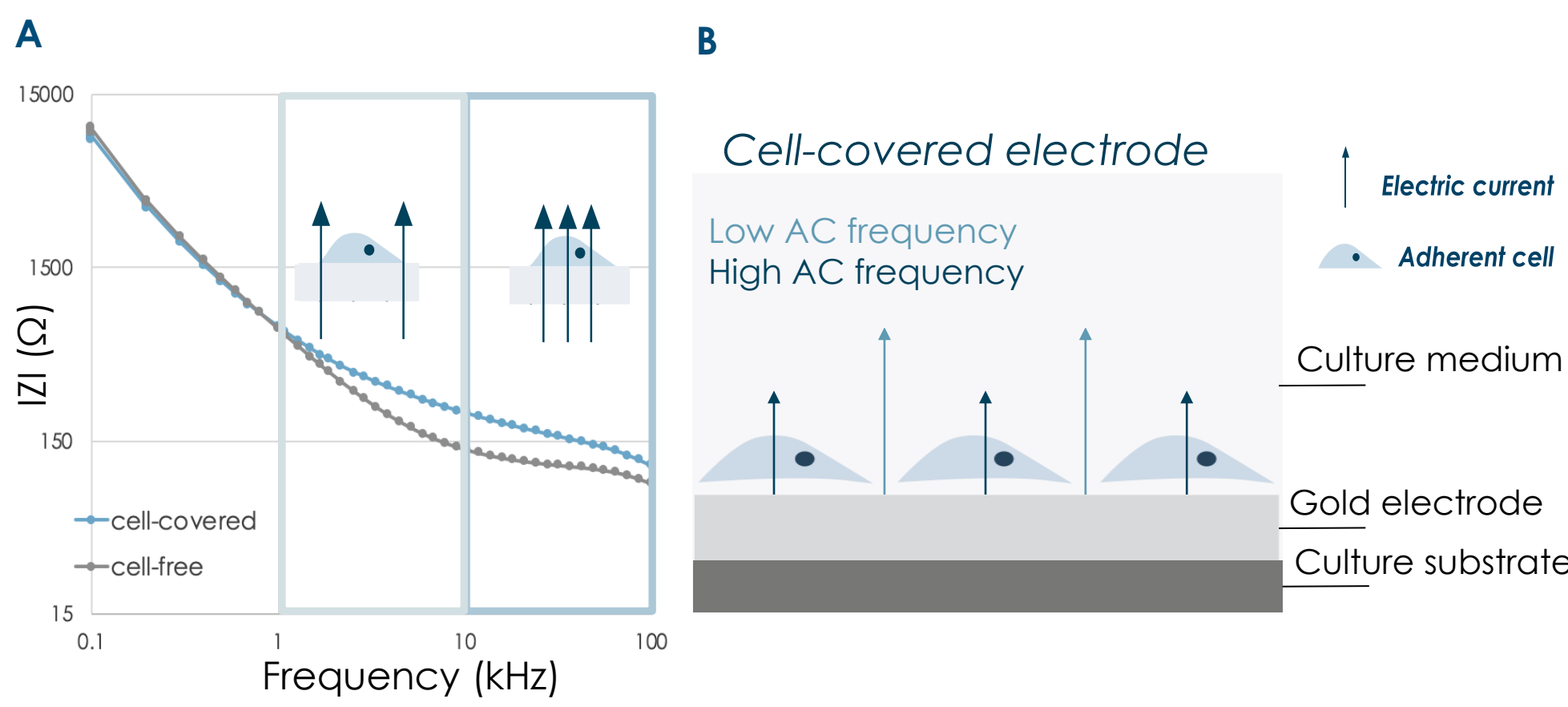


Fig. 2 The frequency dependent impedance of a cell-free and cell-covered electrode in log-log presentation (**A**) reflects the impedance increase due to the cell layer between ~ 1 – 100 kHz. For the experimental setup presented here, the signal is dominated by the paracellular resistance of the cell layer 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 denotes the ratio between the AC voltage applied to a given system and the current through the system that is induced by this applied voltage. In contrast to DC, capacitors have a finite impedance as they become more conductive with increasing AC frequency. Thus, analysis of the frequency dependent impedance reveals the resistive and capacitive properties of the system under test. Therefore, impedance spectra of cells and tissues covering a broad frequency range disclose the passive electric properties of the cell layer.

Cell proliferation and cell toxicity experiments

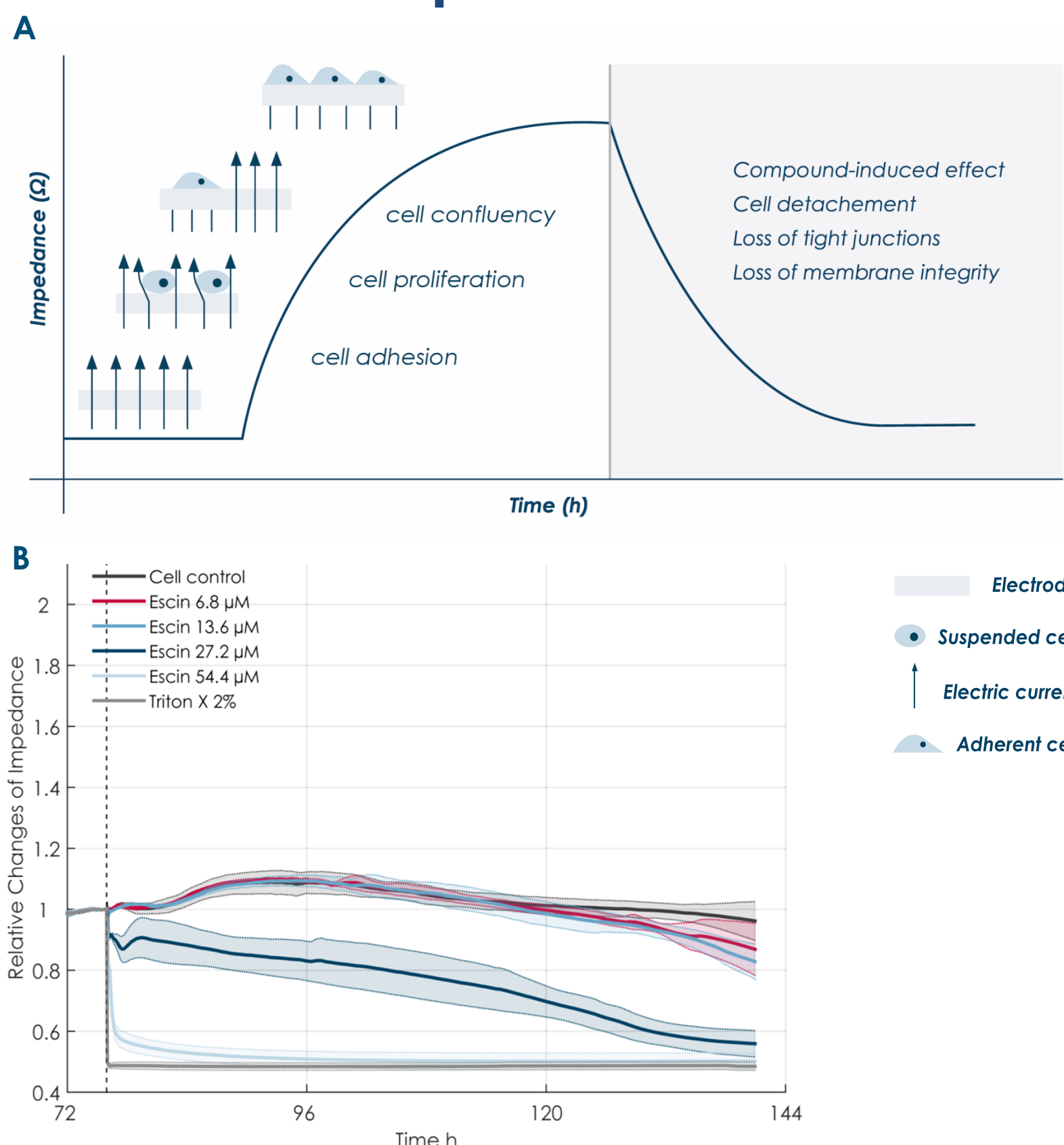


Fig. 3 A Time-dependent impedance measurements at a single fixed frequency (e.g. 10 kHz) reveal the time course of cell adhesion, proliferation or cell death. Such cell type-specific profiles of various cell phenotypes are e.g. relevant in cancer research. **B** Typical cell death experiment. Effect 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 last data point prior to addition of chemicals or control treatments and the time point of compound addition.

Multi-parametric cell monitoring with CardioExcyte 96



Nanon's cell monitoring technology is widening its application area. 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.

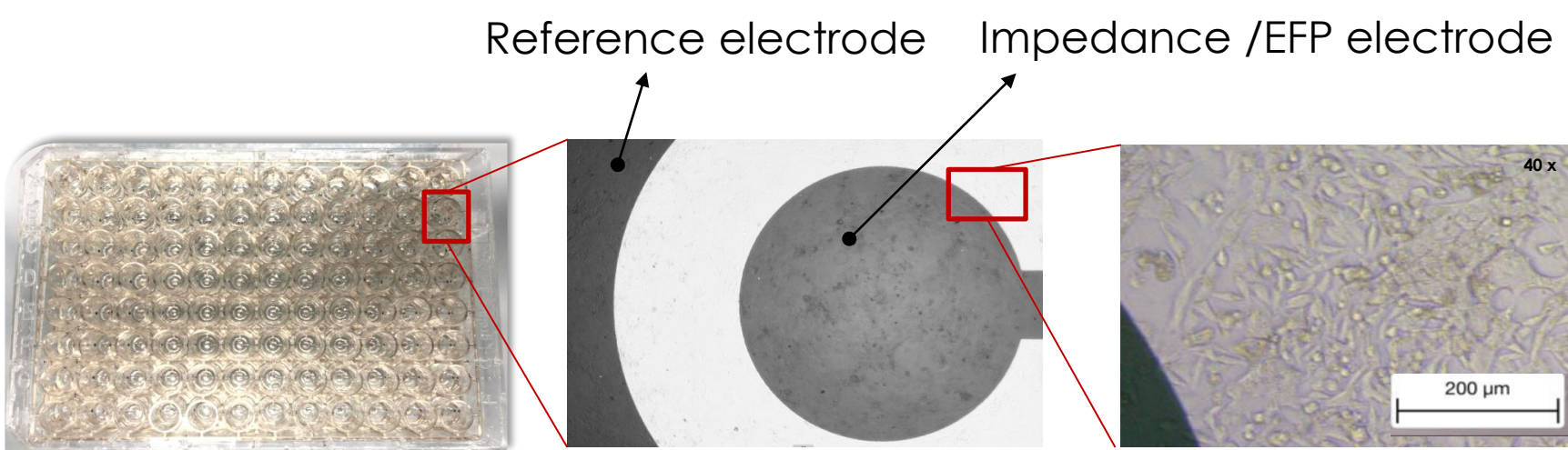


Fig. 1 Multiple functional assays can be combined in a single plate. Transparent NSP-96 sensor plates are compatible with standard plate readers and automated instrumentation, thereby giving you freedom to combine your assays in one plate.

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

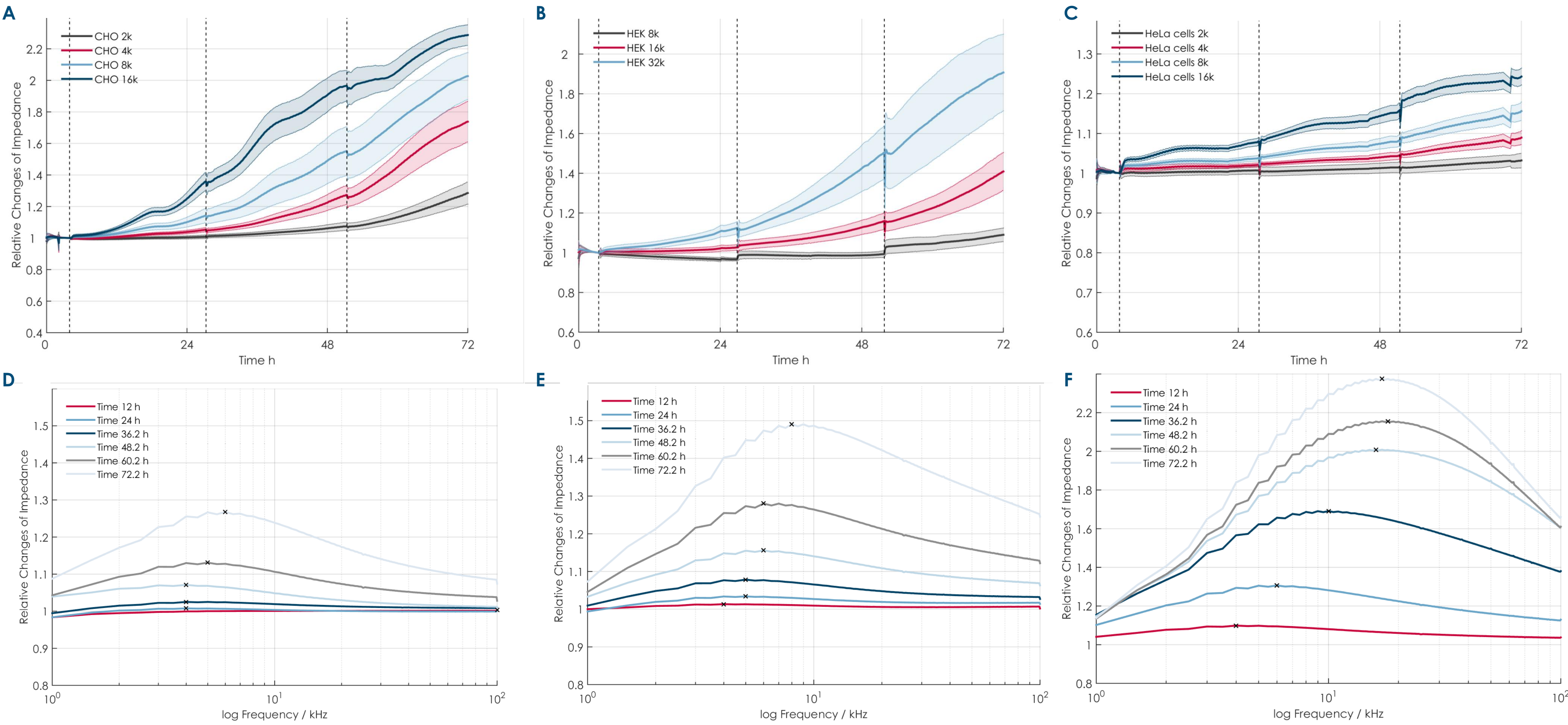


Fig. 4 "Which frequency reports on the highest impedance change?" Averaged relative changes of the impedance signal with associated standard deviation due to cell proliferation of CHO (**A**), HEK (**B**) and HeLa (**C**) cells with initially seeded cell densities of 8k, 16k and 32k / well over a time 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".

Stimulation of GPCRs is mirrored in time-dependent impedance profiles

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.

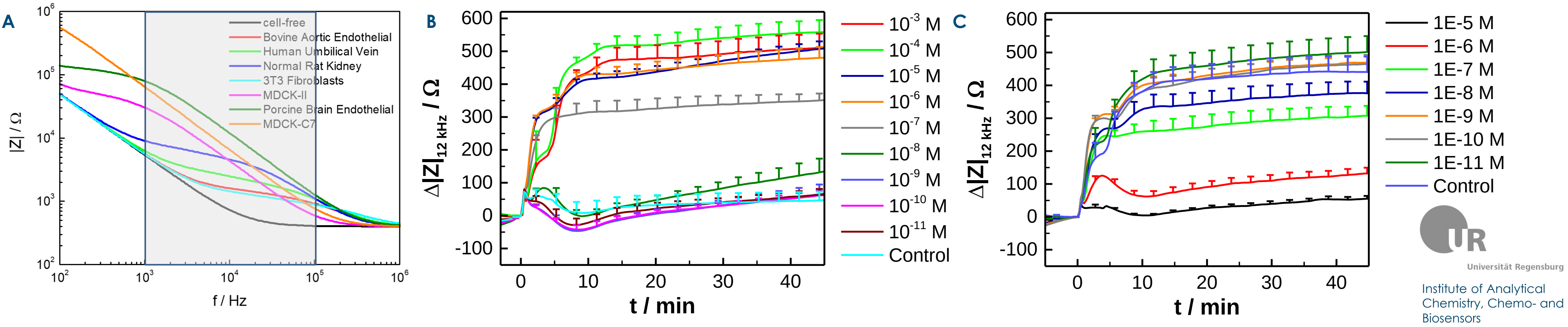
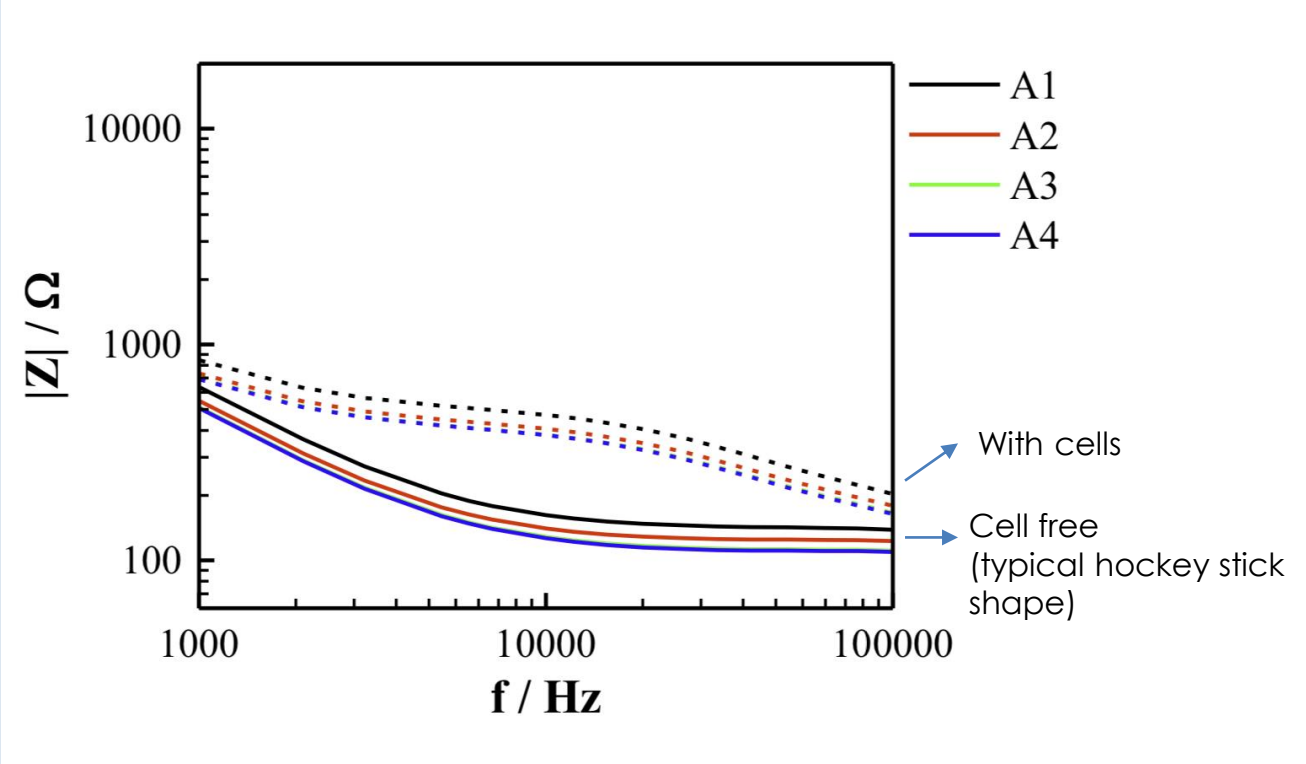
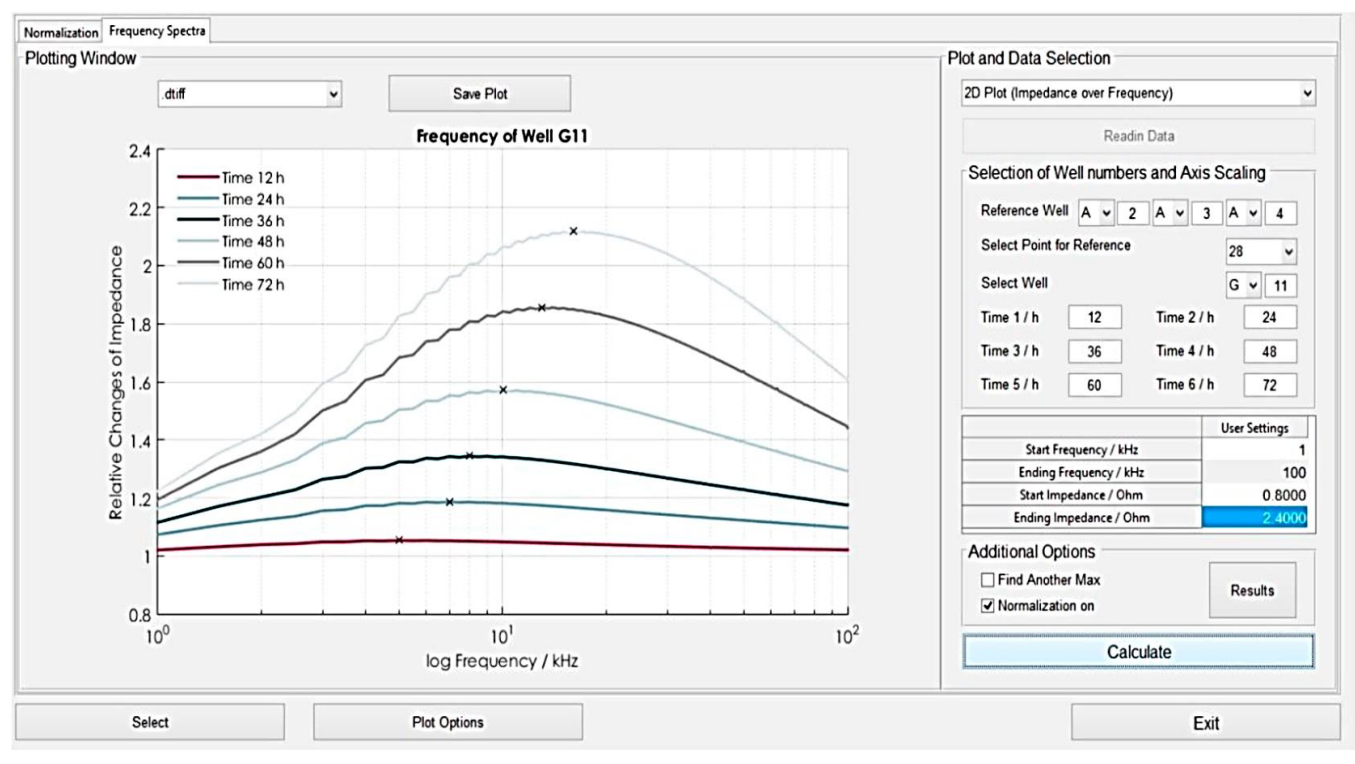


Fig. 5 Frequency-dependent impedance recordings are the basis for many applications of impedance technology in cell analysis. **A** shows simulated impedance spectra illustrating cell-type specific dielectric properties of the listed cell lines for a 0.6mm recording electrode. Additionally, we were able to monitor specific GPCR (here dopamine D2 receptor) activation by applying the endogenous agonist dopamine in agonist (**B**) and antagonist mode (**C**) using haloperidol as specific antagonist. Concentrations in **B** refer to dopamine concentrations in absence of antagonist. Concentrations in **C** denote concentrations of haloperidol pre-incubation prior to addition of 10 μM dopamine at time zero. Agonist mode measurements returned a pEC₅₀ of (7.3 ± 0.1) for dopamine whereas the antagonist mode measurements returned a pIC₅₀ of (6.8 ± 0.2) for haloperidol.

Conclusions

- Electric cell-substrate impedance sensing with frequencies ranging from 0.1 kHz – 100 kHz are successfully integrated in Nanon'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.
- Nanon'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 multiple frequencies.

