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Modulation of Cellular Reactivity for Enhanced Cell-Based Biosensing

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Abstract

Cell-based sensing platforms provide functional information on cellular effects of bioactive or toxic compounds in a sample. Current challenges concern the rather extended length of the assays as well as their limited reproducibility and sensitivity. We present a biosensing method capable of appraising, on a short time scale and with exquisite sensitivity, the occurrence and the magnitude of cellular alterations induced by low levels of a bioactive/toxic compound. Our method is based on integrating optogenetic control of non-electrogenic human cells, modified to express light sensitive protein channels, into a non-invasive electro-optical analytical platform enabling quantitative assessment of the stimulus dependent, dynamical cellular response. Our system exploits the interplay between optogenetic stimulation and time lapse fast impedance assays in boosting the platform sensitivity when exposing cells to a model exogenous stimulus, under both static and flow conditions. The proposed optogenetically modulated cell-based sensing platform is suitable for in field applications and provides a new paradigm for impedance-based sensing.

Graphical Abstract

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Despite their limited sensitivity and specificity to environmental composition/changes when compared to molecular-based biosensors, current whole-cell-based biosensing systems are uniquely capable of providing functional information on the impact of a sample on cell physiology including related toxicity or pharmacology effects.¹ As a result, cell-based sensing has been evolving into a significant enabling technology for biological research, environment assessment, and the pharmaceutical industry.² In particular, whole-cell biosensors based on electrical assays^{3–9} enabled a variety of important biomedical, environmental, and security related applications.

An electrical cell-level assay particularly relevant to our work is electrical impedance spectroscopy (EIS) which relies on reading the current induced by applying an alternating electric field of low amplitude and variable frequency to a set of microelectrodes to measure the electric properties of a biological sample placed between the electrodes. EIS has gained an undisputed front place in the development of whole-cell biosensors due to its capability for label-free monitoring of cell-substrate interactions, attachment, spreading, motility (including micro-motion), growth, and proliferation,⁴ as well as of the status of intercellular junctions.⁵

Among EIS techniques, time lapse electrical cell substrate impedance spectroscopy (ECIS) has proven its ability of assessing subtle cellular responses to chemical, physical, and biological stimuli.⁶ However, the direct applicability of EIS platforms for biosensing is affected by possible confounding effects, dependence on cell type,⁷ the large time scale of analysis (~days), and the equivalent circuit⁸ dependent relevance of multiparametric electrical analyses. Also, EIS platforms do not typically cover the short time scale of physiologic response (~ μ s) typical in micro(multi)electrode array (MEA) approaches for assessing the activity of excitable cells (e.g., neurons and cardiomyocytes) or the effects of drugs via recording of extracellular local field potentials in vitro.⁹ MEA systems face the particular challenges of recording artifacts, interelectrodes inconsistencies, and the alteration of pH due to faradaic reactions especially during long stimulation periods required for drug screening¹⁰ on top of the biological challenges associated with the use of excitable cells (e.g., poorly adherent, requiring special cultivation conditions, and having genotype, phenotype, and time dependent channel repertoire¹⁰).

Reporter cell lines (microbial¹¹ or mammalian¹²), modified cells capable of generating a measurable reporter response (e.g., fluorescence, luminescence) when specific signal transduction cascades are activated by the presence of bioactive stimuli,¹³ have shown

potential to enlarge the set of cellular characteristics and processes accessible through biosensing via optical platforms.

In spite of important advances in cellular-based sensing platforms, the state of the art of the field faces serious challenges: the limited ability to detect a low concentration of bioactive compounds; the reduced sensitivity and specificity, as well as long-time responses;¹⁴ access to one-shot (end-point) information rather than to time-course dynamical data. In addition, rapid characterization of compounds for activity or toxicity hazard evaluation following acute, chronic, or developmental exposures should ideally also consider multiplexed/multi-parametric assays,¹⁵ not currently available.

In order to address these challenges, we propose a novel biosensing method¹⁶ capable of assessing cellular alterations induced by low levels of bioactive/toxic compounds over short time scales and with exquisite sensitivity. The proposed sensing concept involves enhancement of cellular reactivity to analytes by applying additional stimulation, e.g., by lighting either per se, or accompanied by mechanical one (involving microfluidics). In this study, controlled perturbation of the membrane potential of a model human embryonic (non-excitable) cell line is achieved by lighting to modulate cell reactivity and obtain enhanced cell-based biosensing. Fast time lapse EIS is used to monitor and quantitatively assess cell responses following both lighting (alone, as reference dynamics) and lighting combined with exposure to the analyte of interest.

Our approach assimilates electrical and optical sensing platforms through the use of optogenetics, a powerful technique¹⁷ which allows control of cellular activity with high spatial and temporal precision,¹⁸ via specific light sensitive proteins (i.e., opsins). Expressed in mammalian cells, these proteins undergo light induced conformational changes and trigger disturbances of membrane permeability at cellular and subcellular levels. As a result, optogenetics offers control of cell signaling¹⁹ and of cell migration^{19–23} and deep insights into biological systems (metabolism and electrical activity).²⁴ Although the progress in optogenetics paralleled the one in whole-cell biosensors based on reporter genes, mostly developed in bacterial cells, its application in cell-based biosensing has not been discussed until the present work.

More precisely, we integrate optogenetic control of non-electrogenic human cells, stably modified to express ChR₂ light sensitive protein channel, into a non-invasive electro-optical analytical platform enabling evaluation of the effects induced by bioactive/cytotoxic compounds on reference cellular dynamics. By pacing the membrane potential, V_m , (i.e., by selective depolarization using light), we gain rapid access to an inner reference dynamics and a convenient way to enhance cellular reactivity, irrespective of the nature of the targeted bioactive analyte. This effective cell sensitization due to optogenetic stimulation is well supported: restoration of light induced ionic unbalance requires active cell processing (with cell energetics as well as cell signaling components), and there is an established impact of the plasma membrane potential on cell cycle progression cell survival, proliferation, and differentiation^{25,26} as well as on nanoscale reorganization of membrane lipids and receptor proteins.²⁷ We rely on time lapse fast impedance measurements to analyze cellular dynamics of optogenetically modified cellular sensing platform (subsequent to modulation of ionic

fluxes and cell membrane electrical parameters typical for bioactive compounds and optogenetic control), achieve increased response sensitivity, and address *in field*, wider applicability. Emphasis is placed on parametrization of the whole dynamics (as opposed to selected time points assays) in relation to specific actuation conditions to illuminate the mechanisms behind boosted cellular sensitivity toward establishment of light activated/controlled cellular biosensors of wide basic and applied research relevance. Optogenetics per se is not needed to detect the responses to any target bioactive substance. This is a leap forward from existing cellular sensors that function under the restriction of specific cellular intrinsic gene expression processes or a signal transduction cascade (e.g., using reporter proteins,²⁸ optoswitches,¹⁷ or simply dyes^{29,20}) for generating a measurable response when exposed to bioactive stimuli¹³ and are thus inherently slow and specifically developed for a particular target.

Our study innovatively demonstrates the virtues of optogenetically modulated cellular dynamics to reveal even low concentrations of bioactive/toxic analytes under short exposure time and, when combined with time lapse fast impedance assays, provide an effective biosensing tool. As a proof of concept, we test our approach to rapidly and sensitively detect a reference toxicant (CdCl₂) with emphasis on assessment of a low concentration (10 μ M) which challenges the capabilities of current cellular sensors. However, we expect that the same approach can be similarly used to address a large variety of bioactive analytes.

We believe that the integration of cell biology, electrical impedance spectroscopy, and optogenetics provides an important opportunity for the development of a next generation of rapid and high-sensitivity electrical cellular sensors.

EXPERIMENTAL SECTION

Biosensing Platform.

The novel cell based biosensing platform (according to Scheme 1) comprises the following: (1) optogenetically modified non-excitabile cells; (2) an illumination module connected to a (3) flow through cell culture chamber and (4) an impedance analyzer to appraise cell dynamics upon stimulation. On the basis of quantitative appraisal of cellular dynamics via time lapse fast EIS at selected frequencies,³⁰ the study demonstrates the capability of controlled illumination and tailored microfluidic parameters to reproducibly boost cellular reactivity of optogenetically modified cells when exposed to bioactive/toxic analytes.

Controlled illumination (optogenetic stimulation and recovery in the dark) is used to determine a sequence of subliminal (of reduced amplitude and cellular impact) V_m excursions from the resting state values to more positive ones, and back to (hyper)polarized states, due to cellular self-recovery. Time lapse EIS is used to analyze both the reference signal (due to lighting) and the one involving cell dual stimulation (lighting and analyte exposure).

Applications performed in field or involving automatic operation require flow conditions. While fluidics per se can also provide reference/controlled stimulation (besides light pulses), relatively high flow rates should be avoided since they may affect the electrical properties of

the sample via flow dependent cell-surface attachment and electrode coverage changes, hence masking the ones induced by mere analyte exposure. The parameters of the interlinked elements under stress conditions (be it optical, mechanical-flow, or chemical stimulation) have been carefully considered for maximizing the effect of the targeted stimulus. To determine the experimental constraints to augment cellular response to a toxic analyte by controlled cell stimulation, we assessed the effect of flow as an additional means besides lighting to enhance the sensitivity of the cellular platform in particular for low concentration range and provide fast response times in configurations amenable to automatic operation and in the field application.

Therefore, cell platform testing is considered both in static condition and with continuous flow, with or without a prestimulation step (i.e., with analyte applied concomitant with or subsequent to a standard illumination) to reveal the concentration dependence of cellular response to a model cytotoxic analyte (e.g., CdCl_2) under optimized conditions relevant for each configuration.

Aware of the inherent complexity of cell-toxicant interaction, reflected at the cellular level as both rapid (direct membrane effect, activation of a signaling transduction cascade) or slow (interaction with DNA repair mechanism, generation of reactive oxygen species, modification of cell proliferation, differentiation, and induction of apoptosis) processes, we propose comparative analysis of the dynamics recorded for individual concentrations with or without prestimulation as indicative of multiple interaction levels or dynamics.

The addressed questions concern the following: (1) quantitative evaluation of analyte induced changes of reference dynamics (black curve, Scheme 1) to reflect, on a shorter time scale and with exquisite sensitivity, the presence of low levels of a bioactive/toxic compound; (2) dynamics parametrization to reveal target specific cellular responses; (3) an optimal stimulation/sensing protocol; (4) effectiveness of the assay in fluidic conditions.

Cell Line Generation and Maintenance.

Optogenetically modified stable cell lines were generated on the basis of HEK293-Flp-In System (Life Technologies, Invitrogen) using the FRT genomic targeting and the pcDNA3.1/hChR₂ (H134R)-EYFP (a gift from Karl Deisseroth, Addgene plasmid no. 20940³¹) plasmid by means of site specific recombination.³² The optogenetic control cells (FLPN, parent HEK293) were cultured in Dulbecco's modified Eagle's medium (DMEM high glucose) with 10% fetal bovine serum (FBS) and penicillin-streptomycin (100 IU/mL–0.1 mg/mL), while for “O” cells the medium was supplemented with hygromycin (100 $\mu\text{g}/\text{mL}$). Cells were grown in a humidified atmosphere/cell culture incubator (MCO-20AIC, Sanyo, Japan) with 5% CO_2 at 37 °C until fully covering surface electrodes in a cell monolayer. The quality of electrode coverage (i.e., monolayer development) is tested prior to experiment using impedance spectroscopy (10^2 – 10^5 Hz frequency range) and optical microscopy inspection.

All culture media and supplements were purchased from Invitrogen. CdCl_2 , chosen as the model toxic analyte, was purchased from Sigma-Aldrich. Fresh CdCl_2 solutions were prepared prior to experiments from stocks in deionized (Millipore) water.

Light Stimulation.

Achieved using a computer controlled home-built module presenting a 470 nm Rebel LED (Luxeon, Quadica Developments Inc., Canada) which delivers over the individual flow culture chambers, with selected repetition rates, light pulses with fluence of 1.3 mW/mm^2 and duration ranging from 0.2 to 3 s. A succession of individual pulses of chosen duration and intervals between the light pulses (LPs) synchronized with the impedance assay forms the core of the cell sensitization protocol. A specific module was implemented to control and integrate the illumination protocol with EIS measurements.

Electrical Impedance Spectroscopy Measurements.

The cells were cultured at a concentration of 4.5×10^5 cells/mL in flow through culture chambers equipped with sets of electrodes, a circular, $250 \mu\text{m}$ diameter working electrode and a co-planar, larger counter electrode, for ECIS assays and the experimental protocols were performed under static and flow conditions. Both the commercial (8W10E, flow 1F8 $\times$ 10E) and the custom designed ones³⁰ were tested providing similar evolutions. A peristaltic pump Watson Marlow (8 line) was used to achieve controlled flow ($16 \mu\text{L/min}$).

A custom designed fast impedance analyzer was used for recording the time series of impedance data (amplitude and phase) from the individual channels or the cell culture platform, at single frequencies. The 4294A precision impedance analyzer (Agilent, Japan), operated in burst mode via a custom developed LabView interface was used to validate the custom developed impedance analyzer data and characterize via impedance spectroscopy in the 100 Hz to 100 kHz frequency range the quality of cell cultures. To characterize cellular dynamics during illumination protocols, we performed fast, time lapse impedance assays at 1 kHz. This frequency was proven optimal for characterization of cell-cell junctions formation³³ and morphological changes together with cellular micromotion,³⁴ and thus was selected for the development of the sensing concept. Impedance values, modulus of the complex impedance at the selected frequency $Z^*(\text{fr}, t) = |Z(\text{fr}, t)|e^{i\phi(\text{fr}, t)}$ were normalized using the formula $|Z(\text{fr}, t)|_{\text{nor}} = |Z(\text{fr}, t)| - |Z(\text{fr}, 0)|$ with t_0 corresponding to the initiation of the LPs protocol. Data analysis was achieved using the LabView interface and OriginPro 8.5 (OriginLab, USA).

Statistical Analysis.

All results are expressed as mean $\pm$ SD of indicated independent experiments and are represented as normalized values relative to a value corresponding to a fixed time point (e.g., onset of illumination or initiation of baseline recording).

Impedance Data on an Optogenetically Modified Cellular Platform.

Fast impedance assay performed at 1 kHz reveals dynamics of impedance data on an optogenetically modified cellular platform upon stimulation. Preliminary tests (data not shown) were performed to set the parameters of light stimulation that allows measurable impedance responses and cell recovery after light stimulation and under fluidic actuation. The 1 kHz frequency was selected for bioanalysis being proven adequate (support data not shown) for the sensitive assay of cellular changes subsequent to modulation of ionic fluxes and cell membrane electrical parameters (including membrane potential³⁵) typical for

optogenetic control. Illumination protocols (10 LP of 1 and 3 s duration, 300 s apart, followed by 15 min of recovery in the dark) are considered optimal to achieve the aimed subthreshold cell stimulation and the characteristic reference dynamics (comprising the distinct evolutions during illumination and in postillumination, recovery phases). After each individual pulse, there are specific dynamics corresponding to recovery processes of cellular ionic, membrane potential equilibria perturbed by illumination. As a result, the specific light stimulation determines specific dynamics of ChR₂ modified cells, both during illumination and in postillumination, recovery phases, that are statistically different from the control cells—CTRL (FLPN, parent HEK293 line without light sensitivity)—and are well correlated to the magnitude (duration) of the optical stimulus.

This dependency on the magnitude of the stimulus and on cell reactivity and capacity to regain equilibrium after perturbation as powerful hinges for (1) quantitative evaluation of analyte induced changes of the reference dynamics to reflect, on a shorter time scale and with exquisite sensitivity, the presence of low levels of a bioactive/toxic compound; (2) the target specific cellular responses and its concentration dependency; (3) the effect of multiple stressors (i.e., the assay in fluidic conditions), as addressed in Results and Discussion.

RESULTS AND DISCUSSION

Experiments reveal that cell response in EIS assays is highly dependent on the nature, timing, and interplay among different stimulation modalities: optical, mechanical-flow and chemical (i.e., cytotoxic analyte), and evaluating their influences is a prerequisite for establishing the optimal biosensing protocol and the platform's performances. An important finding of our investigations is that the reactivity of cells to a bioactive compound is boosted in a (dynamic) flow environment under optimal illumination conditions (for achieving reproducible behavior in a flow system), and one can achieve unrivalled biosensing capabilities (sensitivity and analysis time) especially for a low-concentration range when using controlled stimulation. Hence, our results are discussed by emphasizing three critical elements:

- a. the effect of mechanical stimulation (flow) on performances of the cellular platform under optical stimulation;
- b. the effect of timing of stimulation, i.e., analyte exposure performed simultaneously or subsequent to illumination, on the reactivity of cells within the platform to a bioactive analyte;
- c. the biosensing relevance, i.e., dependence of cellular response, on the concentration of a model analyte (CdCl₂).

Effect of Flow on Cellular Response To Control Illuminations.

To set the optimal light pulse duration in conjunction with the combined cellular effect of shear stress (flow) and illumination, the characteristic cell responses to control illumination protocols for sequences of 10 LP with durations of 1 and 3 s, respectively, were evaluated under both static (no flow) and flow conditions. Under static conditions, EIS assays show a monotonous dependency of cell response on light intensity stimulus; the larger the stimulus

is, the more pronounced is the effect in the dynamics recorded in impedance data (inset Figure 1). Under flow, however, the larger stimulus (i.e., LPs of 3 s) triggers a dynamics not reaching a quasi-stationary level by the end of the 90 min assay, in stark contrast with the shorter LPs—and subsequently milder stimulation case (Figure 1). For this stimulation the dynamics in flow conditions is comparable with the one in the static case that, however, presents a larger deviation versus control.

According to the results presented in Figure 1, to relate the (combined) effect of both illumination and flow/no flow conditions to a quasi-stationary level, similar to the one presented by the control, the stimulation conditions to assess platform sensitivity to a chemical stressor under flow are set to light pulses with duration of 1 s.

Effect of Timing of Stimulation on the Reactivity of Cells within the Platform.

Since the effect of shear stress (flow) and illumination dependent cellular changes will be modulated in the presence of a chemical stressor, we evaluated the characteristic cell responses to control illumination protocols in the presence of a model bioactive compound at a concentration where direct cellular effects are not recorded in typical EIS assays.

To assess the effect of timing of stimulation on the reactivity of cells within the platform, a chemical stressor (25 μM CdCl_2) was added together with or after stimulation with the illumination protocol, optimized to boost sensitivity under flow: 10 LP of 1 s duration, 300 s interval between pulses. According to Figure 2 the effect of a model analyte (25 μM CdCl_2), applied simultaneously with or following light stimulation, is indeed augmented by the optogenetic stimulation and flow conditions as hypothesized.

This result confirms the synergic effect of flow conditions and optogenetic (lighting) control on increasing the cellular reactivity to a noxious compound, revealing reproducible effects of a cytotoxic compound at a subthreshold concentration; it also reveals a large enhancement of cell reactivity by (pre)stimulation with light pulses, not reported so far and only accessible in flow conditions. Whereas in our setup, this is advantageous, the results on flow-through chips (such as IBIDI 1 F8 $\times$ 10E with 0.36 mm height) can be proven irregular due to large shear stress.

The sensitivity of the platform to optogenetic (light) stimulation under flow for a panel of concentrations of the analyte is further assessed.

Concentration Dependency for Cytotoxic Effect.

On the basis of literature reports,¹³ cadmium induced (time and dose dependent) cellular effects are only notable at large time scales (a minimum of 12–18 h corresponding to 6 h exposure and 6–12 h postexposure required to develop the fluorescent indicator¹³), and for concentrations above 30 μM . Notably, this biosensing platformx already decreased the classical assay time (24–36 h) with the use of optical sensor cell line probing effects using fluorescence, classical ECIS, and end point measurements via MTT.

Indeed, when using impedance monitoring of cells exposed to CdCl_2 without cell prestimulation (i.e., without illumination), there is an obvious limit of quantitation, the 10–

50 μM concentrations (Figure 3) providing dynamics not statistically different from the ones of the mere control (black). When applying illumination prior to CdCl_2 exposure, a significant increase of cell dynamics due to CdCl_2 effect is revealed. The minimal concentration used, i.e., 10 μM , leads to plateau values exceeding more than twice the one corresponding to 50 μM , i.e., a 5 times higher concentration applied to nonstimulated cells.

Given the boosted sensitivity of our sensing concept, the evaluation of cellular platform response for different concentrations of CdCl_2 (10, 25, 50, and 100 μM)—applied simultaneous with or after an optical stimulus (Figure 4)—indicates in 1–2 h of exposure quantitative changes of cellular impedance corresponding to concentration dependent CdCl_2 bioactivity effects even for the concentration challenging the capabilities of current cellular sensors.

It is worth noting that a statistically relevant change is visible even for the 10 μM (orange curve) concentration when exposure to target analyte is realized postillumination and in flow. Although the total duration of the assay is doubled, this is an interesting option for photoreactive analytes (that upon absorption of photons undergo molecular changes or generate reactive oxygen species) expanding the range of analytical relevance. For shorter assays, as highlighted in Figure 4B, CdCl_2 applied simultaneously with the optical and flow stimuli reproducibly alters the characteristic (reference) dynamics in a concentration dependent manner also with evident biosensing potential: marked differences of dynamic response are recorded even for reportedly low, e.g., 10 or 25 μM CdCl_2 concentration and 1 h duration of exposure when compared to the static case.

The optimized biosensing platform based on optogenetically modified cells reveals a concentration dependent change both of the reference dynamics during LPs and of the plateau values after recovery and indicate exquisite sensitivity in the low-concentration range.

The calibration curves derived on the basis of curve parametrization (plateau values, initial slopes, and inflection points) demonstrate exquisite sensitivity of the assay. Such calibration curves are presented in Figure 5A, corresponding to the dependency of the plateau value with the concentration, and reveal the highest sensitivity when the analyte is applied, in flux, after illumination—red as compared to the case of simultaneous application with the illumination; black, in flux; or green, no flux.

The overall detection time is below 2.5 h; yet, since, in flow conditions, the plateau values are not always reached, the calibration curve based on the slope values (Figure 5, inset) is a preferable option, significantly reducing the analysis time to ~30 min! As highlighted in

Figure 5B, a modified Hill function with offset (S): $\Delta Z = S + (E - S) \frac{x^n}{k^n + x^n}$, where $k =$

Michaelis constant and $n =$ cooperative sites, can be used to fit the impedance evolutions (the fit parameters are given in Table 1; note, however, that since we make no assumption on the nature of the interaction process, in our approach the parameters have only empirical relevance). Further parametrization of the evolutions is possible with subsequent use of

principal component analysis,³⁷ and the two parameters of the measured impedance (i.e., both modulus and phase or resistance and capacitance components).

Cellular dynamics upon analyte exposure is modulated by bioeffect magnitude as well as by the timing and sequence of illumination

As a widespread toxic pollutant of occupational and environmental concern, concentration dependent cadmium at the cellular level has a range effects: genomic instability, interaction with DNA repair mechanism, generation of reactive oxygen species, modification of cell proliferation, differentiation, and induction of apoptosis, processes that have different time scales and concentration dependent occurrence.

It is worth noting that under flow and optogenetic stimulation, the cellular platform reveals (Figure 6) a biphasic evolution when exposed to 50 μM concentration of CdCl_2 in both experimental protocols: when applied simultaneously or postillumination (arrow in Figure 6), with slightly faster dynamics (higher slopes) in the latter case. Although assessment of different types of cell death or cell stress and evaluation of different types of biological responses as well as how they would compare exceeds the scope of the present work, the biphasic evolution recorded when exposing the platform to 50 μM concentration of CdCl_2 , (a concentration high enough to affect cell response due to the different cellular effects and scales mentioned above) can be related to the different cadmium effects at the cellular level, with dynamics modulated by stimulus magnitude, further confirming that the proposed assay is able to provide detailed access to various types of analyte induced cellular dynamics.

CONCLUSIONS

Typical cell-based biosensors ascertain activation of a downstream reporter, cell death, or lack of proliferation as defining events in evaluation of bioactive (inhibitory, toxic, or stimulatory) compound presence (i.e., half-maximal effective concentrations, EC_{50} , or median lethal concentration) upon long time incubations. In contrast, the current work is directed to establishing a sensitive method to quantify specific cellular responses (aside cell death) operant in assessing the presence of substances with bioactive potential, at a minimal effective concentration as established in long-term exposure tests (e.g., see ref 13; cadmium induced time and dose dependent cellular effects were only notable at large time scales—12–18 h, corresponding to 6 h exposure, and 6–12 h postexposure required to develop the fluorescent indicator—and for concentrations above 30 μM), within experimental conditions and time durations suitable for point of need applications.

We demonstrate a novel biosensing method¹⁶ in which selective control of the membrane potential of a model human embryonic (non-excitabile) cell line is achieved as a way to modulate cell reactivity and achieve enhanced cell-based biosensing, i.e., capability of assessing cellular alterations induced by low levels of bioactive/toxic compounds over short time scales and with exquisite sensitivity, a substantial gain in terms of sensitivity and information gained as compared to other impedance technologies and current cell-based biosensors.

The key elements of our approach are related to three main pillars: (a) cell biology, (b) optogenetics, and (c) electro-analytics, providing improved cell response to sample/analyte exposure, speed, sensitivity, and a level of detail not achievable with current biochemical or optical methods.

- a. According to cell biology knowledge, cellular systems have the capacity to preserve their dynamic stability after perturbation, by regulating key variables (including cell volume, electrolyte concentrations, pH, membrane potential, and concentrations of intracellular ions, and reactive oxygen species (ROS)) within physiologically relevant ranges, and this capacity plays the central role in shaping the response of cells to external perturbation, be it toxic or stimulatory.
- b. Optogenetics enables versatile control of cellular functions with light sensitive proteins; so, by using light for modulating cells' dynamic stability, we developed robust non-excitable cells with tailored light responsiveness via stable expression of ChR₂ channels and introduced them into an electro-analytic assay with integrated controlled microfluidics and optical stimulation.
- c. With electro-analytics, we show that time lapse fast impedance assay is capable of revealing with exquisite sensitivity the minute changes of the cellular state triggered by exposure to bioactive compound/stimuli.

Optogenetics control of the membrane potential of a non-excitable cell provides a convenient way to enhance cellular reactivity, irrespective of the nature of the targeted bioactive analyte. Moreover, CdCl₂ has high analytic relevance (i.e., is a model compound for a wide range of hazards and threats associated with environment, pharmaceuticals, and biosecurity); thus a wider applicability and impact of the proposed biosensing platform can be foreseen. When applied to this model bioactive (toxic) compound, the optimized biosensing platform was shown to be more sensitive to low levels of CdCl₂ when the cells are preactivated using a soft light stimulus (10 LP of 1 s each, with 5 min between them), in a microfluidics setup. The synergistic effect of both flow and illumination was demonstrated. The flow system allows further integration of chemical separation techniques (e.g., capillary electrophoresis³⁶) for improved discrimination power. With a highly efficient chemical separation, the cellular response can be attributed to an individually fractionated component, if needed.

Taken together, the results highlight a novel real-time, label-free, highly sensitive cell-based biosensing platform that (a) enables quantitative assessment of cell dynamics, (b) provides increased cell reactivity and enhanced cellular responses to even minute exogenous stimuli (optical, chemical or mechanical), and (c) demonstrates capacity to discriminate between different analyte concentrations, especially in the low range, at time scales suitable for point of need tests.

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Figure 1.

Effect of flow: (A) reference impedance evolutions for cells exposed to illumination protocols under flow (sequences of 10 LP of 1 (black) and 3 s (red) durations; inset, evolutions of the platform under static conditions as a function of the magnitude of the light simulation); (B) reference impedance evolutions for cells exposed to illumination protocol (sequence of 10 LP of 1 s duration) in static (blue) and flow conditions (black). As control, impedance dynamics of optogenetically modified cells subjected to flow without illumination. The data represent the mean evolutions of four separate experiments; shadow areas are corresponding to $\pm$ SD.

Figure 2.

Effect of stimulation (illumination and flow) on modulating cellular response to a low concentration of a toxic compound ($25 \mu\text{M CdCl}_2$) when applied with the illumination protocol (CdCl_2 -I, initial) or after the illumination protocol (red; CdCl_2 -F, final). As control, CdCl_2 is applied in flux on optogenetically modified cells but without illumination and without flow, respectively (gray). The data represent the mean evolutions of four separate experiments, and the shadow areas are corresponding to $\pm\text{SD}$.

Figure 3. Comparison between CdCl_2 induced effects without light stimulation (controls: $0 \mu\text{M}$, black; $10 \mu\text{M}$, orange; $25 \mu\text{M}$, green; $50 \mu\text{M}$, red) and for cells prestimulated by light pulses (+LP), exposed to the lowest CdCl_2 concentration, $10 \mu\text{M}$ (orange trace).

Figure 4.

(A) Concentration dependent dynamics of the cell-based biosensing platform with the analyte applied in flux after light stimulation (10 μM , orange trace; 25 μM , green trace; 50 μM , red trace; 100 μM , blue trace); comparison between concomitant and sequential (indicated with arrow) exposures (10 μM , orange trace; 25 μM , green trace); exposure to target analyte postillumination and in flow revealing dynamics and measurable responses even for the lowest tested concentration (10 μM CdCl_2). (B) Comparison between flow and static conditions for concomitant exposure to the analyte (10 μM , orange trace; 25 μM , green trace; 50 μM , red trace; 100 μM , blue trace) and illumination. The shaded areas represent $\pm\text{SD}$ for a minimum of $n = 4$ experiments.

Figure 5.

Quantitative biosensing. (A) Calibration curve based on modulus data for 10, 25, 50, and 100 μM CdCl₂ concentrations for stimulated cells (with LP applied simultaneously or prior to CdCl₂ exposure) under static and flow conditions. Inset: calibration curves under flow and prestimulation conditions based on plateau values (red) and slope values (blue). (B) Concentration dependent dynamics of the cell-based biosensing platform with the toxic analyte applied in flux after light stimulation (10 μM , orange trace; 25 μM , green trace; 50 μM , red trace; 100 μM , blue trace). Black lines correspond to time domains used for quantitation—fit with a Hill function.

Figure 6.

Characteristic evolutions of impedance data of cell-based platform exposed to LPs (black), simultaneously exposed to LP and 50 μM CdCl_2 (blue curve), or 50 μM CdCl_2 applied postillumination (red curve). LP of 1 s duration in flow conditions. The data represent the mean evolutions of three separate experiments. The biphasic behavior revealed for the 50 μM CdCl_2 concentration is indicated (dash lines).

Scheme 1. Biosensing Concept^a

^aOptogenetically modified cells are grown on ECIS type flow culture wares, stimulated using an optimized illumination protocol and evaluated using fast time lapse impedance assay to reveal the presence of bioactive compounds. The characteristic response to control light stimulus (black curve) undergoes significant, specific changes of the dynamics, both during the light pulses and in the recovery phase, under combined stimulation (e.g., mechanical, chemical stressors, and illumination, red curve; for analyte concentrations too small to induce measurable changes in control experiments, blue line).

Table 1.

Examples of the Fit Procedure Applied for the Initial 20 min after Exposure to the Analyte (Fit Values for Figure 5B)

	100 μM	std error	50 μM	std error	25 μM	std error	10 μM	std error
reduced χ^2	3.057		6.95		0.8508		4.9848	
adj R^2	0.9996		0.9978		0.9995		0.9949	
START	-19.153	0.0638	-21.66	0.05601	-24.97	0.02105	-27.88	0.0755
END	319.22	0.07313	143.21	0.0917	114.729	0.06439	125.248	0.53342
k	3.05	0.00104	4.369	0.0024	5.80	0.00264	7.788	0.03737
n	1.69168	0.00109	3.0165	0.00492	2.2956	0.00193	1.4257	0.00498