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Review Article

A short review on cell-based biosensing: challenges and breakthroughs in biomedical analysis

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Abstract

Current cell-based biosensors have progressed substantially from mere alternatives to molecular bioreceptors into enabling tools for interfacing molecular machineries and gene circuits with microelectronics and for developing groundbreaking sensing and theragnostic platforms. The recent literature concerning whole-cell biosensors is reviewed with an emphasis on mammalian cells, and the challenges and breakthroughs brought along in biomedical analyses through novel biosensing concepts and the synthetic biology toolbox. These recent innovations allow development of cell-based biosensing platforms having tailored performances and capable to reach the levels of sensitivity, dynamic range, and stability suitable for high analytic/medical relevance. They also pave the way for the construction of flexible biosensing platforms with utility across biological research and clinical applications. The work is intended to stimulate interest in generation of cell-based biosensors and improve their acceptance and exploitation.

Keywords: biosensing, electro-optical assays, synthetic biology, cell dynamics, cell physiology, theranostics

Introduction

Although whole-cell-based biosensors are not as sensitive to environmental changes as molecular-based ones, cell-based sensing platforms are uniquely capable of providing functional information related to sample toxicity or pharmacology[1] *via* the cell physiology assessment, thus becoming a significant enabling resource for biological research and the pharmaceutical industry[2]. As a result, their applicative potentials become paramount in environmental and biomedical analyses and their developments are constantly in the spotlight. Significant progress is related to new analytical methods and sensing configurations, as well as an improved biological relevance, in terms of the type of cell culture, organ mimics, or whole organism interconnection. Cells not only yield quantitative response to specific stimuli, but also help in quantitatively analyzing bioeffects of complex samples. Most importantly, they enable drug-ligand interactions analysis, monitoring the effect of bio-available/active agents, and assessment of basic cellular functions, ageing, disease pathogenesis and pathology progress. To this effect, biosensing platforms involve living cells coupled to specific transducers and analytic systems to quantify cell specific signals. Choosing between different transduction mechanisms is often dependent on the types of cells utilized and

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the functional approach envisaged for platform development. As a result, the flexibility in determining the sensing strategy, the relatively simple and cost-effective fabrication and potential to integrate the tremendous advances in the field of synthetic biology, microfluidics and system engineering synergistically contributed to the progress in the field. However, challenges related to impractical cell biosensor regeneration[3] (required for the development of multianalyte biosensors and their reuse), are often reported among persistent issues against realization of the full potential of cell-based biosensors. One of them is limited standardization associated with reduced storage life (*i.e.*, intrinsic reduced cell viability with time) and with heterogeneity in cell populations, high interferences (*i.e.*, limited specificity of cellular responses) and high costs of accessory equipment. The list also includes the limited ability to detect low concentration of bioactive compounds, the reduced sensitivity and specificity and long-time responses[4], access to one-shot (end-point) information rather than to time-course dynamical data, and limited access to multiplexed/multiparametric assays[5].

Despite the challenges, with the emergence of engineered cell reporters and fast cell-based assay with modulation of cellular reactivity (*via* synthetic gene circuits[6–7]), cell-based biosensors have evolved from mere tools for detecting specific analytes into multiparametric devices for real time monitoring and assessment[1,8] and actual theranostic tools (***Fig. 1***). Theranostics, an emerging field of medicine, merges drugs and/or techniques into unique combinations to simultaneously or sequentially diagnose and treat medical conditions. In this respect, the cell-based theranostic platforms offer unprecedented diagnosis and treatment options and unique personalization avenues[7].

This mini review is set to complement the recent reviews[1,8] with concerning on cellular platforms. It presents, from a unique perspective lying at the intersection of different scientific disciplines, the exciting progress in the field of (mammalian) cell-based biosensing platforms merging new detection concepts and engineered cells. It aims to reflect the potential of cell-based biosensing platforms as enablers of breakthroughs in biomedical analyses. For specific aspects of microbial derived biosensors, one can address reviews[9] on prokaryotic-cell-based biosensors and the methods to tune their response, along with literature within.

Significant results in the last years are briefly reviewed along two main converging directions: i) new sensing concepts of physiological responses of cells incorporated in biosensing platforms and ii) cellular modification enabling tunable assays for medical diagnostic applications.

Advances in sensing technologies

Electrical cell-substrate impedance sensing (ECIS)[10], and light addressable potentiometric sensor[11], together with fluorescent imaging are among the favored transduction/detection methods[1] exploited in the development of many of the mammalian cell biosensors.

In particular, electrochemical impedance sensing (EIS) platforms have gained an undisputed front place in the development of whole-cell biosensors due to their label-free monitoring capability of cell-substrate interaction, assessment of attachment, spreading, motility (including micromotion), growth and pro-

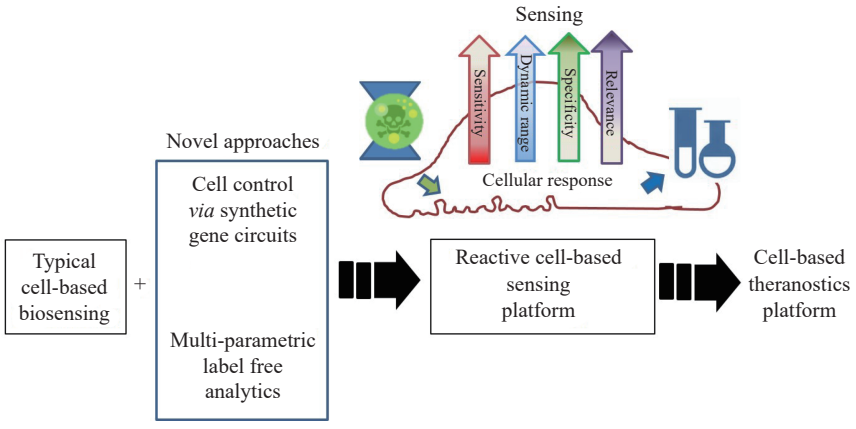

Fig. 1 **Fusion of multiparametric label free analytics with innovative cellular control *via* synthetic gene circuits (enabling modulation of intrinsic cell signaling cascades and establishment of engineered cell reporters and actuators) makes possible the development of new cell-based platforms with enhanced biosensing and theranostic capabilities.**

liferation[12–16], as well as cellular state[17] (*i.e.*, the functionality of cell-to-cell connections, the metabolic and electrophysiological status), all sensitive indicators of cellular physiopathology and response to external stimuli. Furthermore, several spatially resolved impedance measurement techniques have been developed and applied for the investigation of localized sample properties in complex heterogeneous structures/mixtures, to cell and particle research, including electrical impedance tomography, scanning electrochemical microscopy, and microelectrode arrays[18].

As such, bioelectrical impedance cell culture platforms have shown a broad utility in biomedical applications and cancer research[19–20]. ECIS or different bioelectronics recognition assays (*e.g.*, BioElectric Recognition Assay, BERA) were demonstrated to enable assessment of subtle cellular responses to chemical, physical, and biological stimuli[16]. Cell culture systems integrated with electrodes are routinely providing new insights into disease pathogenesis and physiology. Cardiomyocyte-integrated microelectrode array technology, that allows for the measure of spontaneous activity in excitable cells *via* recording the extracellular local field potentials *in vitro*, undergoes standardization for the specific assessment of drug-induced cardiac toxicity.

As recently[21–22] demonstrated, *via* bio-analytical platforms that combine HT-29 cell cultures on gold film electrode arrays with multi-frequency impedance measurements and hypoxic conditions, electrical cell-based biosensors provide the ability to assess bioeffects of hypoxia and gain insight into dynamic changes of cellular processes after exposure to low oxygen environments and carbonic anhydrase IX inhibition. Given the exquisite role of carbonic anhydrase as a clinically relevant target for novel cancer therapeutics specific for hypoxic tumors in relation to haematological assays and from a biosensing perspective, the wide applicative impact of this type of platform is well supported.

Notably, the bioelectrical impedance cell culture platforms are fully characterized and the findings are confirmed with those of optical microscopy and electrochemical (including pH) assays[23], which are also among the preferred techniques for whole cell platform development.

No wonder that *in situ* combination of analytic tools provides a step forward in cell-based biosensing platforms bestowing flexibility and higher sensitivity, dynamic range, stability and analytic/medical relevance. As demonstrated by Bodnarenko *et al*[24], implementation of an electrochemical push-pull probe, combining a microfluidic system with a micro-electrode, is an effective tool for locally altering the microenvironment of few adherent living cells and achieving sensing functionalities not achievable using the individual techniques separately. By working in two different perturbation modes, namely electro-chemical (*i.e.*, electrochemical generation of a chemical effector compound) and microfluidic, full control over the chemical composition of the extra-cellular space of cell monolayers was demonstrated to equip cell-based biosensing platform.

Accordingly, the next section is dedicated to perspectives offered by combined technologies for cell-based sensing.

New perspectives offered by combined technologies for cell-based sensing

The important challenges for the adoption of new technologies for cell-based sensing reside in their ease-of-use and seamless integration into existing workflows. The electrochemical and optical methods are the most commonly used in transducing the signals for both microbial and mammalian biosensors and their combination with other techniques has been often attempted. For instance, the capability to combine impedance assays (*e.g.*, EIS) with other techniques to gain new understanding of dynamic cellular processes was demonstrated by us as early as 2012[25]: an all electrochemical system (combining EIS and cytochrome c-based amperometric biosensor) allowing the simultaneous and real-time monitoring of both cell adherence and superoxide release into the extracellular space was developed and enabled real-time multiparametric characterization of renal cell behavior when exposed to calcium oxalate, a calculus-forming salt. It was discovered that calcium oxalate crystals decrease cell adherence and at the same time induce oxidative stress by an overproduction of superoxide. Subconfluent cells, without fully developed tight junctions, appear to be more vulnerable than confluent cells with tight junctions, indicating the important protective role of these junctions[25] also in *in vitro* assays. As a side note, dielectric formalism of characterizing cells[26] and their connection[27–28] with EIS and tests on tissue samples[17] highlighted gap junction connectivity as a "universal" collective sensing mechanism, affected cells loosing cell-cell contacts.

A combination of EIS and surface plasmon resonance (SPR) was demonstrated[29] as a suitable label-free sensing platform for evaluating effect on

living cells of the amyloid fibrils that are involved in Alzheimer's disease. In this multiparametric label-free assay it was proposed a novel quantitative analysis of the SPR dip combined with advanced EIS as a tool for dynamic cell assessment. It revealed a biphasic cellular response upon $A\beta_{42}$ exposure corresponding to changes in cell-substrate adherence, cell-cell tightening and cytoskeletal remodelling, and provided insight into dynamics of cell-cell junction[30]. The shape of the cell adhesion kinetic curves assessed by a waveguide grating biosensor concept (Epic BenchTop) has been reported[31] as a relevant assay to differentiate between cytostatic and cytotoxic effects. This optical biosensor employs evanescent waves and their sensitivity to changes in local refractive index in the vicinity of the sensor surface for detecting redistribution of cellular contents. This is done *via* recording changes in incident angle (that primarily reflects the stimulation-triggered dynamic mass redistribution perpendicular to the sensor surface) and the shape of the resonant peaks (associated to stimulation-modulated inhomogeneous surface redistribution of cellular contents). In contrast, the combined EIS-SPR platform provided the first demonstration of multiparametric, enhanced resolution on cell-surface and cell-cell interactions modulated by membrane related protein apparatus, applicable to all adherent cells and other amyloidic compounds such as lysozyme[32]. The proposed platform also highlighted modified junctional protein expression and functional alteration of barrier properties as result of cellular impact of a bioactive substance, thus of a strong biomedical[33–35] and even biomaterial analysis relevance[36].

Advances in cellular sensors

Fast cell-based assay with modulation of cellular reactivity

However, the applicability of standard electrical impedance or SPR platforms for biosensing is affected by possible confounding effects[37], evolutions depending on cell type and the large time scale of analysis (~ days). To meet the real world requirements in terms of sensitivity, dynamic range, stability and applicability, an innovative sensing concept was recently proposed[38–39]. It 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) to achieve the controlled perturbation of the membrane potential of a model human embryonic (non-excitable) cell line. Fast time lapse EIS is used to monitor and quantitatively assess cell responses following both light stimulation (alone, as reference dynamics) and light stimulation combined with exposure to the analyte of interest.

The approach[38–39] assimilates electrical and optical sensing platforms through the use of optogenetics, a powerful technique[40] which allows control of cellular activity with high spatial and temporal precision[41]. Optogenetics offers control of cell signaling, cell migration[42], and deep insights into biological systems (metabolism and electrical activity) *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 level[43].

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 in these recent reports[38–39]. Cell-based biosensing is achieved *via* optogenetic control of nonelectrogenic human cells, stably modified to express ChR2 light sensitive protein channel, and integrated into a noninvasive electro-optical analytical platform. The platform is unique in enabling analyte detection without requiring active transcription and translation of a reporter protein and in providing a rapid access to an inner reference dynamics and a convenient way to enhance cellular reactivity, irrespective of the nature of the targeted bioactive compound. This is gained by pacing the membrane potential, V_m, by selective depolarization using light[43]. V_m results from actively maintained balance of ions across the cell membrane, conformational changes of channel proteins interfering with various molecules (*e.g.*, viral proteins-ion channels emerged as therapeutic targets for viral infections[44] while direct modulation of V_m related to viroporins[45] is well established) as well as changes of the lipid composition of the cell membrane in response to variation in the proximate cellular environment. As such, effective cell sensitization due to optogenetic stimulation and V_m modulation 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 V_m potential on cell cycle progression, cell survival, proliferation, differentiation[46] as well as on nanoscale reorganization of membrane lipids and receptor proteins. As a side note, it was reported that hyperpolarization of the engineered cell membrane occurs as a result of the interaction of engineered cells

(antibody-bearing on the cell membrane) with the specific antigens[47].

The approach[38–39] innovatively demonstrates the virtues of optogenetically modulated cellular dynamics to reveal even low concentrations of bioactive/toxic analytes under short exposure time. It relies on time lapse, fast impedance measurements and optogenetically modified cellular sensing platforms. In the presence of bio-active compounds, capable to alter ionic fluxes and cell membrane electrical parameters, the cellular dynamics typical for mild optogenetic control are specifically modified enabling detection. The platform is the ideal starting point to accommodate other engineered cells, and achieves increased response sensitivity towards wider basic and applied research relevance.

As demonstrated by rapidly and sensitively detecting a reference toxicant ($CdCl_2$) in the concentration range (approximately 10 μmol/L) which challenges the capabilities of current cellular sensors, the approach can be used to address a large variety of bioactive analytes. This applicability is indirectly supported by other related approaches, for instance a cell-based biosensor for the direct detection of the SARS-CoV-2 S1 spike protein antigen[47] with changes in cellular bioelectric properties measured by means of BERA. The biosensor is based on membrane-engineered mammalian cells bearing the human chimeric spike S1 antibody. The attachment of the protein to the membrane-bound antibodies resulted in a selective change in the cellular bioelectric properties.

Existing cellular sensors function under the restriction of a specific cellular intrinsic gene expression processes or signal transduction cascade (*e.g.*, using reporter proteins[48], opto-switches[40] or simply dyes[49–50]) to generate a measurable response when exposed to bioactive stimuli[51–52] and are thus inherently slow and specifically developed for a particular target.

Cell-based assays with modulation of cellular reactivity[38–39] provide a leap forward and are well suited to integrate a wide variety of engineered cell reporters that are enabled by recent advances in synthetic biology.

Engineered cell reporters

Mammalian cells are inherently capable of sensing extracellular environmental signals and activating complex biological functions on demand. Engineering mammalian-cell-based devices that monitor and therapeutically modulate human physiology is a promising and emerging frontier in clinical synthetic biology. Advances in synthetic biology have made it possible to engineer cells to sense the presence of custom biological molecules or design new sensing strategies. Synthetic biology enables designing synthetic gene circuits consisting of interconnected gene switches to programme time-dependent and context-dependent target gene activities in living cells[6], paving the way for engineering new cellular functionalities. As such, cells become powerful additions[53] to the field of "theranostics". (The term "theranostics" was coined to explain developments in science to establish more specific and individualized therapies for various pathologies, and to bring about a union of diagnostic and therapeutic applications into a single agent thus leading to a promising therapeutic paradigm involving diagnosis, drug delivery and monitoring of treatment response. It is traditionally associated with multifunctional nanomaterials that combine therapeutic and diagnostic functions[54] in a single nanostructured complex.)

Engineering new cellular functionalities

The capacity to engineer new cellular functionalities is limitless. The development of reporter cell lines (microbial[55] or mammalian[56]) designed to provide a simple, rapid and reliable method to monitor the activation of intracellular signaling pathways induced by extracellular stimuli has matured into a wide array of (mostly optical) biosensors. Thus, by harnessing the power of natural receptors (animal or bacterial[57]) to sense various molecules and carefully rewiring their downstream signaling, one can program mammalian cells to sense a wide range of extracellular cues and provide various output functions in response[58] and turn them into theranostic agents.

A recent review[53] highlights key innovative approaches to engineering new cellular functionalities towards construction of theranostic (sensing and therapy) cells *via* the development of an outstanding range of sensor systems for detecting various extracellular environmental cues that can be rewired to custom outputs. Simply put, the downstream signaling from natural and synthetic receptors that bind to various biological and chemical molecules are connected, either by using the natural downstream signaling of the receptor or by fusing some effector modules to trigger target transgene expression. Their ectopical expression determines cells to respond to target binding, and their ligand specificity bestows cells with sensitivity to extracellular analytes (*e.g.*, disease markers, microbial and viral components), and even ability to secrete therapeutic proteins when necessary. These so-called theranostic cell-based devices have the ability to sense a disease state or the presence of extracellular microbial components and

reverse the pathology *via* a feedback mechanism and by precisely controlling the expression of a specific output.

While there is a vast array of specific recognition elements (antibodies[47], aptamers[59]) that can be specifically expressed in cells, the later are also equipped with an exquisite display of receptors and ion channels wired to intricate intracellular transduction mechanisms. *Via* toolkits[60] for composing customizable genetic programs in mammalian cells it is thus possible to expand the nature of external stimuli sensed by the engineered cells from the biochemical into the physical and even the mechanical realms. From this perspective it is worth noting that the model cell line used in whole-cell sensing platform based on light modulated cellular dynamics (*i.e.*, physical stimulus) carried a larger repertoire of ionic channels enabling membrane potential homeostasis[38] and fluorescent reporters (yellow fluorescent proteins). Sensitivity to mechanical stimuli and surface properties is well known and often wrought in biosensing concepts[61], control of stem cell differentiation[62–64] and for empowering engineered nanomaterials as adjuvants to potentiate the adaptive immune responses to antigens[65–66]. Interestingly, by conjugating an ion channel receptor to a functional protein and a functionalized (anti-GFP) nanobody it is also possible to engineer mammalian cells sensitive to radio waves[67]. Even some unconventional sensor development strategies that harness the biophysical movement of rationally designed chimeric proteins for engineering non-immune cells have also been reported.

In terms of applicability of optogenetic theranostic cell modification, the emerging field of optogenetic medicine[68–69] covers synthetic therapeutic solutions precision-guided by light. The span could be as wide as from semiautomatic glucose homeostasis in diabetic mice with smartphone-controlled optogenetically engineered cells[70] to visual prostheses[71] and beyond, given the ever expanding access to modular extracellular sensor architectures. Among the possible applications enabled through access to modular extracellular sensor architectures for engineering mammalian-cell-based devices[72] are: detection of biologically active signaling molecules[73], fabrication of complex and smart cellular constructs[74], future anti-infective strategies (including antiviral[75]), *in vivo* glucose homeostasis[76] and cancer fighting.

Concerning this last field, cell-based therapies have emerged as a promising treatment modality for diseases such as cancer and autoimmunity. Yet, due to high risks for severe toxicity and inflammatory side effects, their effective administration, on a routine basis, is challenging. Addressing this issue, refined temporal and spatial control over engineered therapeutic cells[77] was demonstrated based on exogenously imposed specific regulation through the use of small molecules to gate cellular functions[78].

Conclusions

This review summarizes key innovations in the latest years toward the development of cell-based biosensors for various applications in biomedical analyses. Overcoming current challenges concerning the length of the assay, analytical methods, reproducibility, and cell sources, bioengineered cell based platforms have been evolving to sense various stimuli, allow innovative cell-based biosensors with tailored performances. Advances in (hyphenated) sensing technologies proved essential to support this progress towards generating platforms capable to fulfil the sensitivity, dynamic range, stability requirements. Modulation of cellular reactivity and engineering cell reporters with new cellular functionalities are two of the enabling concepts that pave the way for the establishment of robust, reliable, and flexible biosensors with broad utility and analytic/medical relevance.

While future trends can only be speculated and the importance of cell-based biosensing platforms for viral detection as required by the recent COVID-19 pandemics is second to none, it is exciting to witness and contribute to the progress of cellular platforms from the canonical view of living cells as mere alternatives to molecular bioreceptors, and cost effective, ethical replacements to animal tests into enabling tools for interfacing molecular machineries and gene circuits with microelectronics and development of advanced sensing and even theranostic platforms that enable mammalian cells to work as "doctors" in the body[53].

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