Mini-Reviews in Medicinal Chemistry, 2007, *7*, 1019-1026 1019

Cell-Based Biosensors in Clinical Chemistry

Spiridon E. Kintzios*

EMBIO/Laboratory of Plant Physiology, Faculty of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

Abstract: Cell-based biosensors represent the next revolution in medical diagnostics, offering a number of significant advantages, such as high speed, portability and low cost. The present review focuses on the most successful technologies used for the detection of ultra-low concentrations of bioactive analytes (such as metabolic markers and pathogens) in clinical samples.

Key Words: Bioelectric recognition, cell-based sensors, cell transfection, hepatitis virus B (HBV), hepatitis virus C (HCV), high-throughput screening systems (HSTSS), membrane-engineering, superoxide anion.

INTRODUCTION

Improvements in the sensitivity of determination of clinically important analytes promise advances in the ability to detect diseases at an earlier stage, when treatment can be most effective. Other advances are in the pipeline in the way of non-invasive and minimally invasive monitoring technologies, which may improve the management of chronic diseases. The estimated worldwide market for diagnostic products is over US$ 30 billion, with an average annual growth rate of 34%. Rapid assay methods and serological techniques present the fastest growing technological sectors, while particular areas of applications expand in the following order: microbiological analyses>immunological tests>clinical chemistry [1]. The momentum of this development is maintained by the presentation of more cost-effective solutions, especially through automation [2].

Blood and urine samples are used to assess a multitude of body processes and disorders. Healthcare economics dictate that diagnostic testing should be performed accurately and in the least amount of time possible [3], i.e. tests should not have to be repeated due to technical problems. Multiphasing screening machines can perform many blood tests quickly and simultaneously using very low sample volumes. As a result of these increasing capabilities, there are more than 250 different blood tests and more than 60 urine tests currently available. Assays are largely based on immuno-enzymatic methods, such as the enzyme-linked immunosorbent assay (ELISA), which has replaced radio-immunoassays as the microbiologists´ favorite method [4]. Among the major advantages of immunoenzymatic systems belongs the vast scope of their applications, ease of operation and, in most cases, a reasonable cost per sample assayed. However, the equipment required for conducting assays by the currently available technology, in particular integrated equipment for automated analysis, is expensive and space-consuming, while there is an additional requirement for trained personnel and laboratory infrastructure [5]. Moreover, the time needed for running a complete analysis varies between a few hours to several days, thus limiting the usefulness of these methods for routine medical diagnostics [6]. Therefore, conventional methods of analysis have considerable disadvantages as far as the issues of practicality, time and, in some respect, cost of each analysis is concerned.

From a commercial point of view, and especially for complex applications, such as the detection of viruses and other pathogens or the determination of short-lived metabolites (such as free radicals), the lack of available rapid and cost-efficient methods is inevitably associated with an inability to meet the ever-increasing demand on diagnostic assays [7-11]. Within the framework of existing technologies, and due to the considerable requirements in labour and capital, it is practically impossible for many private and public diagnostic laboratories to run screening tests on a scale-up level. The ability to detect rapidly, at low cost, very low levels of biological agents in liquids and gases offers highly useful tools to the biomedical society.

When novel medical diagnostic technologies are designed, a number of desired traits must be taken into consideration. First of all, a novel system or analytical principle must be superior to existing methods in terms of high speed, reproducibility, accuracy, selectivity, sensitivity and storability. Furthermore, it is desirable to have a low manufacturing and assay cost, a minimal sample volume and minimal sample preparation time. Medical professionals would also like to be able to monitor at real-time conditions and retrieve as much information as possible during a single assay. Finally, an ideal clinical diagnostic tool should be as compact and portable as possible [12].

CELL-BASED SENSORS

A biosensor is a device that detects, transmits and records information regarding a physiological or biochemical change. Biosensors represent the most recent and advanced technology in diagnostics and analytical chemistry. In recent years there has been a rapid increase in the number of diagnostic applications based on biosensors, including live, intact cells and -in some cases- tissues, organs or whole organisms [13-15]. Whole cells provide multipurpose catalysts, particularly

*Address correspondence to this author at EMBIO/Laboratory of Plant Physiology, Faculty of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; E-mail: spiroskintzios@usa.net

1389-5575/07 $50.00+.00 © 2007 Bentham Science Publishers Ltd.

in processes that require the participation of a number of enzymes in sequence; therefore, the utilisation of whole cells as a source of intracellular enzymes is often a better alternative to purified enzymes in various industrial processes. Cell-based biosensors are likely to have improved stability, higher biocatalytic activity, adding low cost in their favour, while their greatest advantage is their ability to provide physiologically relevant data in response to an analyte and to measure the biovailability of the analyte [16].

Research activities in the field of cell-based sensors are rapidly increasing. The number of published reports on cell biosensors increased by almost 70% from 2002 to 2004, representing one quarter of all biosensor-related publications and conference presentations [13]. However, very few of the constructed sensors have been commercialized. The few that have become commercial products are generally used for the detection of a range of substrates and are based on biochemical oxygen demand (BOD) measurement. Most of the biosensors reported use bacterial cells as the sensing element [17-20]. Breakthrough advances in animal cell storage capacity are expected to increase the commercial applicability of cell-based sensors for high throughput pharmaceutical and disease screening. These may result either from improved immobilization substrates, or from the development of functional micro-chamber systems, integrating different microsensor arrays for measuring physical and chemical parameters of cultured cells. In addition, the development of transformed cell lines with an absolute specificity against target compounds, has demonstrated the possibility of constructing biosensors with essentially zero false negative or false positive results.

CELLULAR SENSORS: THE PROS AND THE CONS.

One of the traits that make cell biosensors attractive as a clinical analytical tool is their considerable sensitivity, which is assumed to enable, in some cases, the possibility of detecting just a single target molecule. This theoretical assumption is based on the intricate interaction in nature, which has emerged as a result of relentless trial-and-error testing through countless millennia and allows cells to respond to individual molecules and organisms in rather precise and reproducible ways. This ability, however, comes at a cost, or, rather, a compromise: due to the fact that cells can react in roughly the same manner against an amazingly large number of different molecules, cell sensors can exhibit a very poor selectivity. This is a very common problem in toxicity assays, where cell sensors successfully *detect* the presence of a toxic (or genotoxic) compound, but generally *fail to determine* the exact nature of the toxic analyte [21-23].

In recent years a number of cell transfection methods have been developed for increasing cell specificity, with considerable success. Cell transfection methods are based on the cellular uptake of foreign DNA by, mainly, human B cells, therefore leading to the expression of membrane-bound receptors [24-27]. A quite renowned application is the CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) system developed by MIT Lincoln Lab. CANARY has been used for the detection of *Yersinia pestis* and other pathogens [28]. However, the applicability of cellular transfection is limited by the lack of stability and the frequent, unwanted alteration of cellular phenotype.

An alternative approach to cell transfection is the recently developed technology of membrane-engineering [12, 29]. It is a generic methodology of artificially inserting (usually by electroinsertion) tens of thousands of receptor molecules on the cell surface, thus rendering the cell a very specific responder against analytes binding to the inserted receptors. Receptor molecules can vary from antibodies to enzymes or to polysaccharides. The working assumption of the method is that attachment of the target molecule to its respective receptor causes a change in the cell membrane structure, which is measurable as a change in the cell membrane potential. This has been proven so far in a number of applications, the most prominent being the detection of Hepatitis C virus (HCV) and the determination of very low concentrations of superoxide anion in clinical samples. As demonstrated in Fig. (**1**), detection of HCV with membrane-engineered cells is more sensitive than conventional ELISA assays, but test results derived from either method correlate well to each other.

A second point in favor of cellular sensors, in particular when the detection of pathogens is needed, lies in their inherent similarity with cell culture, namely the dual character of a detection system that is also a host of the pathogen under detection. The culture of cells for detecting and even determining the presence of a pathogen is considered as the golden standard of the diagnostic community. However, several weeks may be required before a clearly defined symptom is observed in a test culture [30-32]. On the contrary, cell sensors required significantly less time (from a few moments to a day) in order to produce a reliable result. This is very important, because the ability to detect a single type of molecule is not necessarily associated with the ability to evaluate the virulence of a pathogen. For example, viral strains expressing novel antigens may escape detection by the most advanced immunoanalytical systems. Nevertheless, they will be detected by an appropriate cell biosensor, provided that the cells used in the analytical system are susceptible (or responding otherwise) to the virus under detection.

A critical issue limiting the broad application of cellular sensors is their unsatisfactory storability. This can be extended by providing cells with an adequately controlled culture environment, which in turn requires controlled nutrient medium flow and, in the case of animal cell sensors, a CO_2-enriched culture atmosphere and a constant temperature of 37ºC [33]. These conditions can be satisfied by means of sophisticated equipment (such as pharmacological cell screens, which are discussed next), but are certainly hard to meet with portable, low-cost biosensor units. Currently, advanced cell sensors are based on cell immobilization rather than cell culture.

THE TRANSDUCTION OF THE SIGNAL: AVAILABLE OPTIONS

Biosensor responses representing cellular signaling events are usually employing some sort of optical detection technology. This is mainly due to the advent of cellular engineering to include optical or luminescent reporting elements in cells [34]. Another approach is based on cross-linking antigens with membrane receptors, which results in a detectable increase in the concentration of cytosolic Ca^{2+} [35]. An ex-

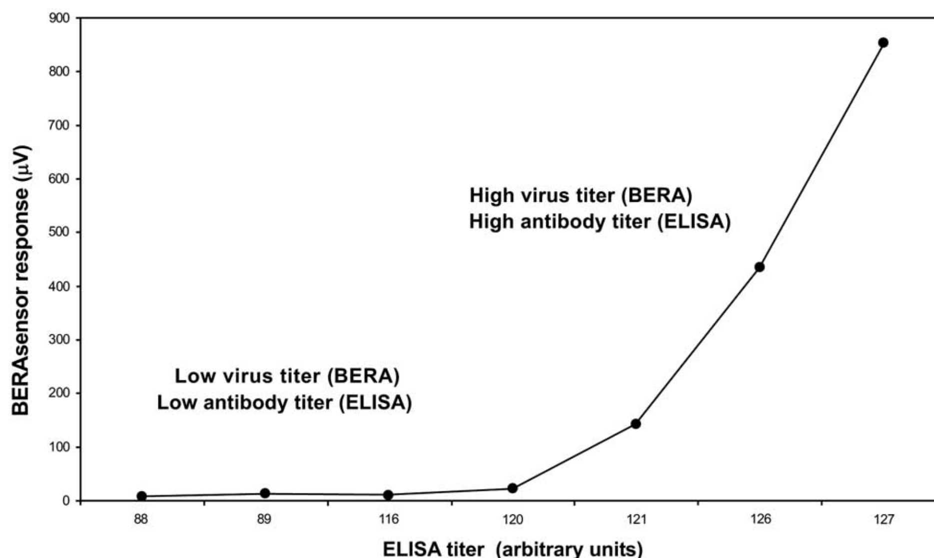

Fig. (1). Correlation between the response of the BERA-HCV sensor and the corresponding antibody titer as determined by conventional ELISA assay (Abbott Laboratories´microparticle-based chemoluminescent assay for the detection of HCV core antigen). Note that the correlation between the two assays is high at high virus concentrations but is practically zero at virus concentrations below the detection limit of the immunoenzymic assay. Results were received after analyzing a total of 50 HCV(+) blood samples collected from patients of the Hippokrateion General Hospital (Athens, Greece) (source: author's own research, unpublished results).

ample is presented in Fig. (**2**), whereas the depletion of calcium ions from cells bearing virus-specific antibodies was observed after treatment with either Hepatitis virus B (HBV) or HCV-positive samples [12, 36]. The same approach has been used for the detection of low concentrations of oxidized glutathione in blood samples, using membrane-engineered cells bearing glutathione reductase on their membranes (Fig. (**3**)) [37]. The instrumentation used in order to detect visible, fluorescent, or luminescent signals from cells or tissues, includes microscopes, fiber optics, CCD cameras and other optical equipment [13].

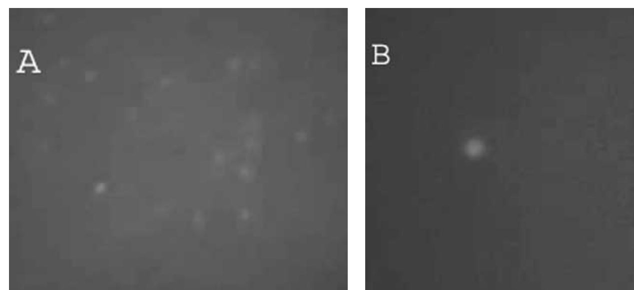

Fig. (2). Changes (expressed as differences in fluorescence intensity) in the intracellular Ca^{2+} concentration in membrane-engineered Vero cells bearing HCV-specific antibodies before (A) and after (B) incubation with a Hepatitis virus C (HCV)-positive sample. Virus attachment to cells caused a rapid decrease of $[Ca^{2+}]_{cyt}$, which could be quantitatively measured by means of fluorescence spectrometry and correlated with viral titre in the sample (source: author's own research, unpublished results).

Using a different approach, electrically active cells can be interfaced with microelectrodes which allow the capture of extracellular spikes or impedance changes associated with cellular or tissue response. An example is the Bioelectric Recognition Assay (BERA) which has been originally developed for the detection of human viruses (HBV, HCV, herpes viruses) and veterinary disease agents (foot and mouth disease virus, prions, blue tongue virus) on the basis of their specific interaction with appropriately immobilized, mammalian cells and the measurement of the change of the electric potential that is caused by the aforementioned interaction [38-40]. Recently, the method has been used for the routine detection of tumour-specific antigens in clinical samples [41].

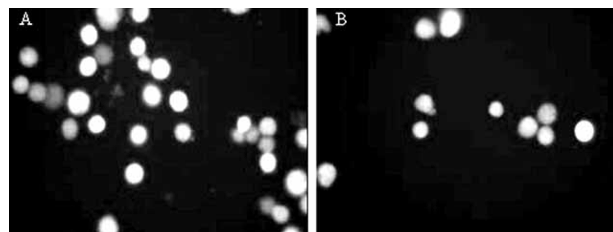

Fig. (3). Reduction of the intracellular Ca^{2+} concentration before (A) and after (B) treatment of membrane-engineered Vero cells bearing glutathione reductase (GR) with 5mM oxidized glutathione (GSSG). Bars represent 70 μm. Changes in cellular fluorescence intensity were futher used for the quantitative determination of GSSG in standard solutions and unknown (modified from [37]).

Fluorescence-based systems are less practical than electrophysiological ones, since they are not suitable for quantitative determinations. They are also far more expensive than electrode-based techniques [42]. Fluorescent dyes may also have considerable side-effects on cellular physiology, such as uncoupling of respiratory inhibition and membrane permeabilization [43, 44] and may interfere with the assayed cell parameter [45].

SYNTHETIC TISSUES FOR PHARMACOLOGICAL RESEARCH

In vivo pharmacological studies using animals provide information on complex intercellular and organ-to-organ effects not readily accessible from *in vitro* tests. However, *in vivo* studies have many and significant disadvantages: they are expensive, labor intensive and time consuming, while ethical concerns have decreased their popularity in recent years. *In vivo* assays are essential because of the complex nature of vascular responses to test reagents, responses that no *in vitro* model can fully achieve [46].

In vitro cell-based assays are valuable, can be carried out expeditiously, and lend themselves to quantification, but they are more difficult and time-consuming to perform, and therefore limit the number of tests that can run at any one time. Furthermore, they are often of limited predictive value because they do not represent accurately the complex environment to which a drug candidate is subjected within a living organism. Consequently, *in vitro* tests are best viewed as providing initial information that is subject to confirmation by *in vivo* assays.

Common *in vitro* assays refer to cell proliferation (usually determined by colony formation assays, flow cytometry and use of selective stains for DNA and RNA determination) and cell viability, while a number of assays that correlate with either functional or reproductive cell capabilities have been developed. These usually employ criteria such as the assessment of survival and growth in tissue culture, functional assays, metabolite incorporation and membrane integrity [47, 48]. Different methods do not necessarily correlate well to each other, while the usefulness of conventional assays is often limited by technical and practical considerations.

In recent years, various biosensor designs have emerged that are oriented towards facilitating pharmacological screening based on cell proliferation and/or viability assays. The progress in three-dimensional microfabrication technology has opened new possibilities for miniaturising epithelial cell culture and analysis devices. Miniaturisation offers high potential for both fundamental research and clinical diagnostics. Most, if not all, of these systems are based on assaying parameters of a cell suspension culture [49-51]. The activation of cellular receptors usually causes transient or sustained increases in acidification rate, i.e. the excretion of lactic and carbonic acids formed during the energy metabolism. The archetypal silicon microphysiometer invented by Owicki and Parce [52] was developed as a biosensor-based instrument that detects changes in the physiological state of cultured living cells by monitoring the rate at which the cells excrete acidic products of metabolism. Furthermore, other researchers opted for the measurement of surface pH [53] or electrical properties [54].

Mammalian cells can be immobilized in polymers, such as collagen matrixes, which represent the material that is closest to the natural tissue environment. In this way it is possible to construct functional 3-D cell-based biosensor platforms [55-57]. A particular group of immobilized cell systems are spheroid cultures on polymer-coated culture surfaces [58] or in a porous substratum [59, 60, 61]. Spheroids are three-dimensional organoid cultures, in particular hepatocyte cultures; isolated primary cells in this culture type spontaneously form spherical multicellular aggregates and maintain their morphological and functional characteristics *in vitro* [62, 63]. By combining microfabrication and microcontact printing techniques, it is possible to pack primary hepatocytes in dense spheroid microarrays, maintaining liver-specific phenotypes, such as liver-enriched transcriptional factors, albumin secretion, urea cycle enzymes, and intercellular adhesion molecules. In spite of the usefulness of the spheroid culture technique, several obstacles hinder their widespread use for biological application, such as the inability to immobilize spheroids at a defined location and cell necrosis occurring within the core of large and coalesced spheroids because of oxygen depletion [64, 65].

A popular system is the HμREL®, originally developed at Cornell University. HμRELs are patented microfluidic circuits that can reveal interactions among multiple tissue types and one or more pharmacologic compounds. The system is currently available in a commercial version [66]. The physical features of a HμREL® embody parametric values derived from a physiologically-based pharmacokinetic model. The geometry and fluidics of the device are fashioned to stimulate the values for drug residence time, circulatory transit time, organ cell density, tissue size, shear stress, and certain other physiological parameters found in the living animal, so as to mimic the fluid-mediated interactions of the organ systems represented in the microfluidic circuit [67]. A HμREL® microfluidic circuit comprises an arrangement of separate but fluidically interconnected “organ” or “tissue” compartments. Each compartment contains a culture of living cells drawn from, or engineered to mimic the primary functions of, the respective organ or tissue of a living animal (Fig. (**4**)) [68-69]. Typically, four organ compartments are included in each device: a “liver” compartment to represent the organ primarily responsible for xenobiotic metabolism, a “lung” compartment to represent a target tissue, a “fat” compartment to provide a site for bioaccumulation of hydrophobic compounds, and an “other tissues” compartment to assist in mimicking the circulatory pattern in nonmetabolizing, nonaccumulating tissues. Micromanufactured HμREL® biochips are placed in an appropriate CO_2 environment and incubated at 37ºC. Microfluidic channels between the compartments permit a culture medium that serves as a “blood surrogate” to re-circulate as in a living system. Drug candidates of interest are added to the culture medium and allowed to re-circulate through the device. The effects of drug compounds and their metabolites on the cells within each respective organ compartment are detected by measuring or monitoring key physiological events.

HμREL® was used to test the cancer chemotherapeutic pro-drug tegafur. Tegafur itself is inactive, and requires metabolic activation by cytochrome P450 enzymes present in the liver to generate the cancer cell-killing metabolite 5’-fluorouracil (5-FU) [70-71]. Both tegafur and, separately, 5-FU were tested in a HμREL® device containing hepatocytes cultured in the liver compartment and colon cancer cells cultured in the "target tissue" compartment. Tegafur was added to the recirculating culture medium and pumped through

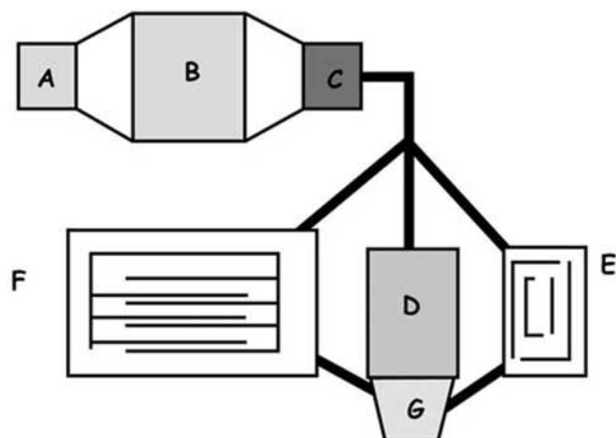

Fig. (4). Schematic representation of the HμREL microfluidic cell circuit. (A) Input, (B) gas exchange champer, (C) target tissue culture chamber, (D) liver cell culture chamber, (E) adipose cell culture chamber, (F) other tissues, (G) output (modified from [69]).

Hurel™ devices at various concentrations for 24 hrs. Following drug exposure, the Hurels™ were treated with the membrane-permeable nucleic acid dye Hoechst 33342 and the membrane-impermeable nucleic acid dye ethidium homodimer. Using these stains, all cells appear fluorescent blue, but dead cells are marked by the fluorescent red ethidium homodimer. Both tegafur and 5-FU were found to be cytotoxic to colon cancer cells in a dose-dependent fashion. However, tegafur was ineffective when tested using a traditional static cell-based assay. It was also proven that, if no hepatocyte culture was included in the system, tegafur had no effect on the colon cancer cells, whereas 5-FU caused significant cell death. In other words, the HμREL® liver compartment was necessary for bio-activation of tegafur. Moreover, although 5-FU triggered cell death in the traditional assay, HμREL® demonstrated cytotoxicity much more rapidly and strongly with either 5-FU or tegafur than did the traditional assay with 5-FU [72].

Immobilized cultured systems are even more suitable for large-scale, routine drug screening than cell suspension culture-based approaches. This is due to a drastic reduction of technical requirements associated with the maintenance of a controlled environment (CO_2 enrichment, 37ºC). In addition, and in a strictly scientific sense, three-dimensional culture systems are far more realistic representations of actual tissue segments than two-dimension ones (including microfluidic cell circuits). As an example, a novel, miniaturized biosensor system was recently created by combining the electrophysiological response of immobilized cells with superoxide-sensing technology and optical and fluorescence microscopy [73]. Cells were immobilized in a calcium alginate matrix and a miniature square piece of cell-containing gel matrix was aseptically adhered on a glass microscope slide bearing a microfabricated gold electrode array (Fig. (**5**)). This configuration allows for the continuous monitoring of the cell membrane potential, while cell division is assayed with an optical microscope.

In addition, immobilized cell viability, RNA and calcium concentration, radical oxygen species (ROS) and glutathione accumulation are assayed by fluorescence microscopy after provision of an appropriate dye. Using this system, it is possible to correlate seven different cell physiological parameters to each other and formulate a model for ROS-mediated signaling function on cell apoptosis. Experiments with doxorubicin (adriamycin) demonstrated how the sensor could be used for investigating the mode of action of a pharmaceutical compound. Previous studies [74, 75] indicated that this anticancer agent exerts its cytotoxic activity *via* $O_2^{\bullet-}$ formation and a putative intracellular H_2O_2–generation mechanism. Doxorubicin also interferes with $O_2^{\bullet-}$ formation by plasma membrane NADH-quinone reductases [76]. Consequently, the biosensor was used for the quantitative detection of superox-

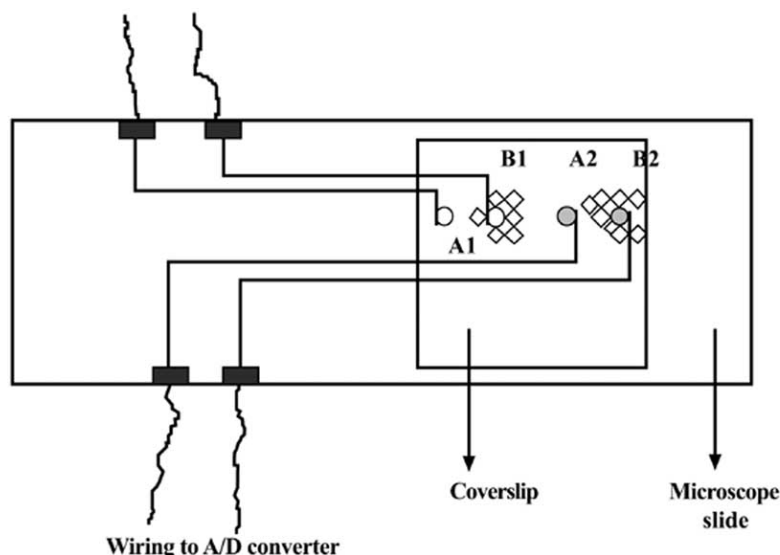

Fig. (5). Schematic representation of the immobilized cell biosensor. The reference electrodes **A** lie in a cell-free gel matrix, while the measuring electrodes **B** lie in the immobilized cell-matrix system, which has a surface area of 0.5 x 0.5 cm^2. Both electrodes are covered with a glass cover slip, which is aseptically sealed with Parafilm®. Measurement and reference electrodes are connected *via* wiring to the Advantec Adam-4017 PC I/O data converter. Cells (✧) are not shown in scale (modified from [73]).

ide produced after treating the immobilized cell cultures with doxorubicin. The sensor was able to rapidly (5 sec) detect as low as 5 nM of superoxide and this response was linear at a 5-15 μM concentration range of doxorubicin (Fig. (**6**)).

CONCLUSIONS

Increasing our knowledge of cellular physiology at the molecular level has allowed the replacement of empirical diagnostic methods with the advanced analytical tools of late 20th century medicine. In this context, immunoenzymic assays were developed after breakthroughs in immunology took place. The in-depth understanding of nucleic acid chemistry spurred the revolution of nucleic acid technologies, including the reverse transcriptase-polymerase chain reaction (RT-PCR) and gene sequencing.

As the analytical environment, in particular the testing conditions at the point of care, becomes more and more demanding, novel diagnostic tools must satisfy both scientific and social economic standards. Emerging technologies are by definition hybrids of different scientific disciplines. To exploit the intricate advantages of cellular sensors to the fullest degree, analytical systems based on cells must represent an optimized amalgam of cell biology, physical chemical sensing science and information technology.

It is not possible to compare the performance characteristics of cellular sensors with those of various nucleic acid technologies, such as nested PCR or real-time PCR, which exhibit a superior sensitivity (with the ability, for example, to detect a single virus particle in a sample). The latter technologies, however, require sophisticated laboratory infrastructure and trained technicians, while they detect only nucleic acids. On the contrary, cell sensors may respond to target molecules belonging to a vast repertoire of different chemical groups.

At their current status, cell-based technologies could directly compete with immunoenzymic assays and other immunoanalytical systems for a respectable range of the available blood and urine tests. In fact, assays such as CANARY or BERA could efficiently replace ELISA or radioimmunoprecipitation (RIA) assays in the detection of antigenic molecules derived from pathogens or disease (such as tumor markers). Of course, the selectivity of cell sensors strongly depends on the specificity of membrane antibodies that are either inserted or expressed onto the cell surface. It appears that cell sensors share some of the limitations present in enzyme immunoassays. On the other hand, the sensor response is independent from factors narrowing the applicability of plate-trapped antigen (PTA) immunoassays, such as the duration of incubation time [77].

Unfortunately, the still limited background research on cellular sensors has not helped the commercial presentation of automated analytical devices, as seen in considerable variety for ELISA or other conventional assays. This particular development has also been delayed by the absence of sophisticated software that could automatically recognize and classify the sensors´ responses. This is essential, because the pattern of the response is complicated by non-specific interferences, due both to analyte and to environmental variations (such as temperature and pH). Therefore, a complete elimination, at the signal processing stage, of the potential noise sources is required in order to determine the sensitivity of any cell-based detection system to a particular chemical agent. Quite recently, a multi-net classifier system for the detection of plant viruses, using BERA biosensors and Artificial Neural Networks (ANNs) was reported. The key feature of the method is the combination of specialized ANNs that are trained to recognize plant viruses according to biosensors' responses, therefore replacing the previous empirical examination of the biosensor´s response data curve [78].

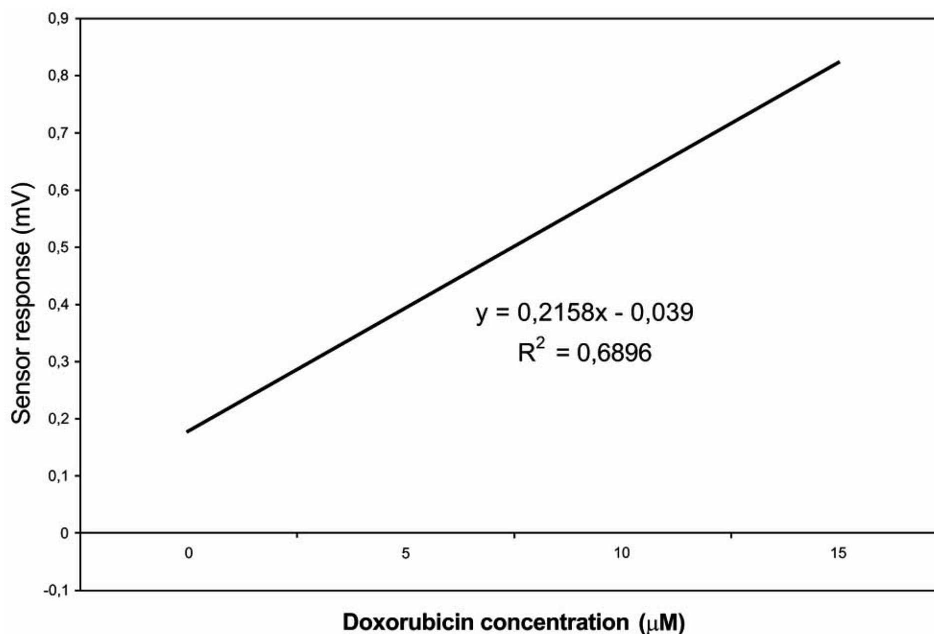

Fig. (6). Relationship between the response of the immobilized cell sensor and doxorubicin (adriamycin) concentration (mean values, n=15). Adriamycin-mediated cytotoxicity is associated with the formation of superoxide anion in target cells, which is then determined by using the cellular biosensor system (source: author's own research, unpublished results).

The transduction of the sensors′ responses in electric signals and their instant evaluation by means of specific software either on site (stand alone devices) or *via* an Internet site would allow for implementation of cellular biosensors to small clinic and doctors' offices at a minimum cost. Further advances in microfluidic cell circuits (like HμREL®) and the development of generic methods for the specific detection of target analytes, like CANARY and BERA, along with an increasing investment in automated equipment and user-friendly software, pave the way for the final destination of cell biosensors: the clinical diagnostic laboratory.

ACKNOWLEDGEMENT

I wish to express my warmest thanks to Mrs. Angela-Lu Petrou-Gini (Greek Ministry of Foreign Affairs) for proofreading the text in English.

ABBREVIATIONS

BOD	=	Biochemical oxygen demand
HBV	=	Hepatitis Virus B
HCV	=	Hepatitis Virus C
BERA	=	Bioelectric Recognition Assay
CANARY	=	Cellular Analysis and Notification of Antigen Risks and Yields
ELISA	=	Enzyme linked immunosorbent assay
ANNs	=	Artificial Neural Networks
RIA	=	Radioimmunoprecipitation assay
POC	=	Point of care

REFERENCES

[1] Simonsen, M. Med. Device Daily: State of the Industry Report, **2004**, 201.

[2] Dodds, M.; By, P.; Michelmore, S.; Young, T.; Nam, S.; Kamen, E. Medical Supplies: and Devices: Industry Overview 2004, SG Cowen, NY, **2004**.

[3] Pagana, K.D.; Pagana; T.J. Mosby′s Diagnostic and Laboratory Test Reference, 7th Edition, Elsevier Mosby: St. Louis, **2005**.

[4] Marquette, C.A.; Blum, L.J. *Biosens. Bioelectron*., **2006**, *21*, 1424.

[5] Castro, F.J.; Sauleda, S. ; Esteban, J.I.; Viladomiu, L.; Martell, M.; Dragon, E.; Esteban, R.; Guardia, J. *J. Virol. Methods*, **2001**, *91*, 51.

[6] Vernet, G. *J. Clin. Virol*., **2004**, *31*, 239.

[7] Roubaud, V.; Sankarapandi, S.; Kuppusamy, P.; Tordo, P.; Zweier, J.L. *Anal. Biochem*., **1997**, *247,* 404.

[8] Barbacanne, M.A.; Souchard, J.P.; Darblade, B.; Iliou, J.P.; Nepveu, F.; Pipy, B.; Bayard, F.; Arnal, J.F. *Free Radic. Biol. Med*., **2000**, *29*, 388.

[9] Kuthan, H.; Ullrich, V.; Estabrook, R.W. *Biochem. J*., **1982**, *203*, 551.

[10] Yao, D.; Vlessidis, A.G.; Gou, Y.; Zhou, X.; Zhou, Y.; Evmiridis, N.P. *Anal. Bioanal. Chem*., **2004**, *379*, 171.

[11] Benov, L.; Sztejnberg, L.; Fridovich, I. *Free Radic. Biol. Med*., **1998**, *25*, 826.

[12] Kintzios, S. Proc. 6th Biodetection Conference, Knowledge Foundation: Washington, DC, **2006**.

[13] Kintzios, S. In: Encyclopedia of Sensors, Grimes C., Ed.; American Scientific Publishers, CA, **2005**, Vol. *2*, pp. 1-15.

[14] Bickerstaff, G.F. Immobilization of Enzymes and Cells, Humana Press: Totowa, **1997**.

[15] D'Souza, S.F. *Curr. Sci*., **1999**, *77,* 69.

[16] Daunert, S.; Barrett, G.; Feliciano, J. S.; Shetty, R. S.; Shrestha, S.; Smith-Spencer, W. *Chem. Rev.*, **2000**, *100,* 2705.

[17] Choi, S.H.; Gu, M.B. *Biosens. Bioelectron*., **2002**, *17*, 433.

[18] Gu, B.; Chang, S.T. *Biosens. Bioelectron*., **2001**, *16*, 667.

[19] Min, J.; Kim, E.J.; LaRossa, R.A.; Gu, M.B. *Mutat. Res*., **1999**, *442*, 61.

[20] Knight, A.W.; Keenan, P.O.; Goddard, N.J.; Fielden, P.R.; Walmsley, R.M. *J. Environ. Monit*., **2004**, *6*, 71.

[21] Kaiser, K.L.E.; Palabrica, V.S. *Water Pollut. Res. J. Can.,* **1991**, *26*, 361.

[22] Van der Lelie, D.; Regniers, L.; Borremans, B.; Provoost, A.; Verschaeve, L. *Mutat. Res*., **1997**, *389*, 279.

[23] Riska, P.F.; Su, Y.; Bardarov, S.; Freulich, L.; Sarkis, G.; Hatful, G.; Carriére, C.; Kumar, V.; Chan, J.; Jacobs, W.R. Jr. *J. Clin. Microbiol*., **1999**, *37*, 1144.

[24] Delehanty, J.B.; Shaffer, K.M.; Lin, B. *Biosens. Bioelectron.,* **2004**, *20*, 773.

[25] Andrews, J.S.; Mason, V.P.; Thompson, I.P.; Markx, G.H. 8th World Biosensor Congress; Granada, **2004**.

[26] Falsey, J.R.; Renil, M.; Park, S.; Li, S.J.; Lam, K.S. *Bioconj. Chem*., **2001**, *12*, 346.

[27] Ziauddin, J.; Sabatini, D.M. *Nature,* **2001**, *411*; 107 .

[28] Rider, T.H.; Petrovick, M.S.; Nargi, F.E.; Harper, J.D.; Schwoebel, E.D.; Mathews, R.H.; Blanchard, D.J.; Bortolin, L.T.; Young, A.M.; Chen, J.; Hollis, M.A. *Science,* **2003**, *301*, 213.

[29] Moschopoulou, G.; Kintzios, S. *Anal. Chimica Acta*, **2006**, *573-574,* 90.

[30] Park, J.C. ; Hwang, Y.S.; Suh, H. *Yonsei Med. J*., **2000**, *41*, 836.

[31] Jones, P. A.; Baker, V.A.; Irwin, A.J.E.; Earl, L.K. *Toxicol. In Vitro,* **1998**, *12*, 373.

[32] Odum, J.; Tittensor, S.; Ashby, J. *Toxicol. In Vitro,* **1998**, *12*, 273.

[33] Aravanis, A.M.; DeBusschere, B.D.; Chruscinski, A.J.; Gilchrist, K.H.; Kobilka, B.K.; Kovacs, G.T.A. *Biosens. Bioelectron*., **2001**, *16*, 571.

[34] Rudolph, A.S.; Reasor, J. *Biosens. Bioelectron*., **2001**, *16*, 429.

[35] Whelan, R.J.; Zare, R.N. *Biosens. Bioelectron*., **2003**, *19,* 331.

[36] Perdikaris, A.; Moschopoulou, G.; Alexandropoulos, N.; Maggana, O.; Nomikou, K.; Kintzios, S., Hepatitis Congress, Mangosee: Dakar, **2006**.

[37] Mochopoulou; G.; Kintzios, S. 9th World Congress on Biosensors. Elsevier: Toronto, **2006**.

[38] Kintzios, S.; Pistola, E.; Panagiotopoulos, P.; Bomsel, M.; Alexandropoulos, N.; Bem, F.; Varveri, C.; Ekonomou, G.; Biselis, J.; Levin, R. *Biosens. Bioelectron*., **2001**, *16*, 325.

[39] Kintzios, S.; Pistola, E.; Konstas, J.; Bem, F.; Matakiadois, T.; Alexandropoulos, N.; Biselis, J.; Levin, R. *Biosens. Bioelectron*., **2001**, *16*, 467.

[40] Kintzios, S.; Bem, F.; Mangana, O.; Nomikou, K.; Markoulatos, P.; Alexandropoulos, N.; Fasseas, C.; Arakelyan, V.; Petrou, A-L.; Soukouli, K.; Moschopoulou, G.; Yialouris, C.; Simonian, A. *Biosens. Bioelectron*., **2004**, *20*, 906.

[41] Kintzios, S. In Frontiers in Drug Design and Discovery, Caldwell, G.W.; Rahman, A.-ur; D'Andrea, M.R.; Choudhary, M.I.; Eds.; Bentham Science Publ., **2006**, Vol. *2*, pp. 225-240.

[42] Slavik, J. Fluorescent probes in cellular and molecular biology, CRC Press: Boca Raton, **1993**.

[43] Peechatinikov, V.A.; Rizvanov, F.F.; Pletnev, V.V. *Stud. Biophys*., **1983**, *93,* 95.

[44] Eddy, A. *Methods Enzymol*., **1989**, *172*, 95.

[45] Dixit, R.; Cyr, R. *Plant J*., **2003**, *36*, 280.

[46] Auerbach, R.; Lewis, R.; Shinners, B.; Kubaim, L.; Akhtar, N. *Clin. Chem*., **2003**, *49*, 32.

[47] Park, J.C.; Hwang, Y.S.; Suh, H. *Yonsei Med. J.,* **2000**, *41*, 836.

[48] Schulte-Hermann, R.; Bursch, W.; Marian, B.; Grasl-Kraupp, B. *IARC Sci. Publ*., **1999**, *146*, 273.

[49] Lind, R.; Connolly, D.; Wilkinson, C.D.W.; Breckenridge, L.J.; Dow, J.A.T. *Biosens. Bioelectron*., **1991**, *7*, 359.

[50] Matsubara, Y.; Murakami, Y.; Kobayashi, M.; Morita, Y.; Tamiya, E. *Biosens. Bioelectron*., **2004**, *19*, 741.

[51] Nicolini, C.; Lanzi, M.; Accossato, P.; Fanigliulo, A.; Mattioli, F.; Martelli, A. *Biosens. Bioelectron*., **1995**, *10*, 723.

[52] Owicki, J.C.; Parce, J.W. *Biosens. Bioelectron*., **1991**, *7*, 255.

[53] Lehmann, M.; Baumann, W.; Brischwein, M.; Ehret, R.; Kraus, M.; Schwinde, A.; Bitzenhofer, M.; Freund, I.; Wolf, B. *Biosens. Bioelectron*., **2000**, *15*, 117.

[54] Hediger, S.; Sayah, A.; Horisberger, J.D.; Gijs, M.A.M. *Biosens. Bioelectron*., **2001**, *16*, 689.

[55] Mao, C.; Kisaalita, W.S. *Biosens. Bioelectron*., **2004**, *19*, 1075.

[56] Yang, Y.; Sulé-Suso, J.; El Haj, A.J.; Hoban, P.R.; Wang, R.-K. *Biosens. Bioelectron*., **2004**, *20*, 442.
[57] Armbruster, V.; Gharbi, T.; Viennet, C.; Humbert, P. *Sens. Actuat. A. Physical*., **2004**, *116*, 219.
[58] Kobayashi, A.; Goto, M.; Sekine, T.; Masumoto, A.; Yamamoto, N.; Kobayashi, K. *Artif. Organs*, **1992**, *16*, 564.
[59] Ijima, H.; Matsushita, T.; Nakazawa, K.; Fujii, Y.; Funatsu, K. *Tissue Eng*., **1998**, *4*, 213.
[60] Fukuda, J.; Sakiyama, R.; Nakazawa, K.; Ijima, H.; Yamashita, Y.; Shimada, M. *Int. J. Artif Organs,* **2001**, *24*, 799.
[61] Fukuda, J.; Sakai, Y.; Nakazawa, K. *Biomaterials,* **2006**, *27,* 1061.
[62] Funatsu, K.; Nakazawa, K. *Int. J. Artif Organs,* **2002**, *25*, 77.
[63] Koide, N.; Shinji, T.; Tanabe, T.; Asano, K.; Kawaguchi, M.; Sakaguchi, K. *Biochem. Biophys. Res. Commun*., **1989**, *161*, 385.
[64] Glicklis, R.; Merchuk, J.C.; Cohen, S. *Biotechnol. Bioeng*., **2004**, *86*, 672.
[65] Fukuda, J.; Okamura, K.; Nakazawa, K.; Ijima, H.; Yamashita, Y.; Shimada, M. *Cell Transplant,* **2003**, *12*, 51.
[66] Hurel Corporation Newsletter, **2006** (www.hulercorp.com).
[67] Baxter, G.T.; Freedman, R. *Am. Biotechnol. Lab*., **2004**, *2*, 1.

Received: 17 November, 2006 **Revised: 06 May, 2007** **Accepted: 07 May, 2007**

[68] Sin, A.; Baxter, G.T.; Shuler, M.L. *Proc. SPIE*, **2003**, *4560*, 98.
[69] Park, T.-H.; Shuler, M.L. *Biotechnol. Prog*., **2003**, *19*, 243.
[70] Viraviadya, K.; Sin, A.; Shuler, M.L. *Biotechnol. Prog*., **2004**, *20*, 316.
[71] Gu, W.; Zhu, X.; Futai, N.; Cho, B.S.;Takayama, S. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 15861.
[72] Ma, S.H.; Lepak, L.A.; Hussain, R.J.; Shain, W.; Shuler, M.L. *Lab. Chip*., **2005**, *5*, 74.
[73] Kintzios, S.; Marinopoulou, I.; Moschopoulou, G.; Mangana, O.; Nomikou, K.; Endo, K.; Papanastasiou, I.; Simonian, A. *Biosens. Bioelectron*., **2006**, *21,* 1365.
[74] Yang, M.; Nazhat, N.B.; Jiang, X.; Kelsey, S.M.; Blake, D.R. *Br. J. Haematol.,* **1996**, *95*, 339.
[75] Singal, P.K.; Li, T.; Kumar, D.; Danelisen, I.; Iliskovic, N. *Mol. Cell. Biochem*., **2000**, *207*, 77.
[76] de Grey, A.D.N.J. *Protoplasma,* **2003**, *221*, 3.
[77] Mowat, W.P.; Dawson, S. *J. Virol. Methods,* **1987**, *15*, 233.
[78] Frossyniotis, D., Anthopoulos, Y., Kintzios, S., Perdikaris, A., Yialouris, C. P. *Lectures Comp. Sci.,* **2006**, *4132*, 401.

Copyright of Mini Reviews in Medicinal Chemistry is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.