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Mammalian Cell-Based Sensor System

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Abstract Use of living cells or cellular components in biosensors is receiving increased attention and opens a whole new area of functional diagnostics. The term "mammalian cell-based biosensor" is designated to biosensors utilizing mammalian cells as the biorecognition element. Cell-based assays, such as high-throughput screening (HTS) or cytotoxicity testing, have already emerged as dependable and promising approaches to measure the functionality or toxicity of a compound (in case of HTS); or to probe the presence of pathogenic or toxigenic entities in clinical, environmental, or food samples. External stimuli or changes in cellular microenvironment sometimes perturb the "normal" physiological activities of mammalian cells, thus allowing CBBs to screen, monitor, and measure the analyte-induced changes. The advantage of CBBs is that they can report the presence or absence of active components, such as live pathogens or active toxins. In some cases, mammalian cells or plasma membranes are used as electrical capacitors and cell–cell and cell–substrate contact is measured via conductivity or electrical impedance. In addition, cytopathogenicity or cytotoxicity induced by pathogens or toxins resulting in apoptosis or necrosis could be measured via optical devices using fluorescence or luminescence. This chapter focuses mainly on the type and applications of different mammalian cell-based sensor systems.

Keywords Biosensor, Pathogens, Toxins, Cytotoxicity, Rapid detection

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1 Introduction

The cell-based biosensor (CBB) systems that incorporate whole cells or cellular components have an obvious advantage of responding in a manner that can offer insight into the physiological (at organelle, cellular, or tissue levels) effect of an analyte [1]. Cell-based assays (CBA) are emerging as dependable and promising approaches to probe the presence of pathogens in clinical, environmental, or food samples [2–6]. Living cells are extremely sensitive to modulations or disturbances in "normal" physiological microenvironment. Therefore, CBBs are employed to screen and monitor "external" or environmental agents capable of causing perturbations of living cells [5, 6]. Some of the CBAs utilize the metabolic responses of cells (like cyanobacteria) to detect biological or chemical entities, like oxygen and herbicides in water [7]. In another type, mammalian cells or plasma membranes are used as electrical capacitors. The mechanical contact between cell–cell and cell–substrate is measured via conductivity or electrical impedance [8, 9]. Also, mammalian cells are used to measure biochemical and metabolic end-products delivered from cultured cells to the medium [6]. The CBAs can also measure the direct electrical response of electrogenic cells such as neural cells, heart muscle cells, pancreatic beta cells, or a neural cell network [6].

Pathogenic microorganisms must interact at a molecular proximity with cellular components of the host cells in order to elicit the pathogenic response. The mammalian plasma membrane is the first cellular interface for such an encounter with a microbe or microbial products like toxins. Most often, as a result of this encounter, the molecular and structural integrity of the plasma membrane is altered [10, 11].

Plasma membranes of mammalian cells may release membrane-bound markers when they are damaged by the action of a pathogen or toxin [12–14]. This interaction of host cell membrane and pathogen is archetypal [15]. The damage caused by a pathogen on plasma membrane bears a signature of the pathogen itself [15, 16].

In recent years, applications of functional detection of toxic and hazardous agents using CBBs integrating CBAs are reported extensively [2, 3, 17–20] and offer significant promise. Cell-based systems have proved their potential and emerged as some of the most significant approaches in drug development, toxicology, and high-throughput screening (HTS) [21]. Development of detection systems based upon the physiological action of pathogens and toxins (pathogenicity) is receiving increased attention in clinical pathology, as well as in food diagnostics, environmental monitoring, and biosecurity applications. Therefore, a biosensor capable of rapid and functional screening of toxins and pathogens in food samples is in great demand to cater to the contemporary needs of food safety and biosecurity.

The changing scenario of threats and issues of biosecurity and screening of classified agents related to food, agricultural, or environmental safety necessitates not only a sensitive detection regimen, but also requires a rapid, broad spectrum screening tool that can be employed as the "first line of defense." The nature and capability of the detection systems must conform to challenges associated with emerging pathogens and for unknown or little known biohazards. The present methods of detection depend mostly on known chemical characteristics or molecular recognition of target organisms, toxins, or substances [5]. In this regard, conventional detection systems fail to detect or identify unknown or emerging analytes, since most of these methods are specifically designed to identify a particular organism or toxin. In this context the need for a "functional detection" system is of utmost importance [22]. A functional detection system is capable of reporting the presence of threat agents or substances in a physiologically relevant manner. A living system can provide some of the key functional information about an analyte [5, 23] which is listed below.

1.1 Toxicity of the Analyte

This can be assessed by using living systems as sensing elements, such as bacteria, higher eukaryotic or mammalian cells, or living animals. The physiological responses, such as membrane damage, apoptosis, and necrosis of the sensing elements as a result of exposure to test substances can be deduced to gain information about the nature of toxicity of an analyte.

1.2 Impact on Cellular Metabolism

This can be evaluated by the alteration of signal transduction, second messenger pathways, or enzyme pathways.

1.3 Receptor–Ligand Interaction

A test substance might act as agonist or antagonist to a particular cell-type or groups of cells. Therefore, a living cell containing such receptors could serve as a better sensing probe to evaluate the agonistic or antagonistic potential of test substances.

1.4 Changes in Gene Expression

The interaction with foreign substances may alter the gene expression profiles of sensing cells, tissues, or organs as a result of exposure. Functional genomics of cultured cells can discern and classify agents or analytes based on their capabilities to intervene gene expression of the sensing cells.

2 Animals as Sensor

Ideally, the best sensing system with the capability of functional detection of human or animal threat agents would obviously be the living animals themselves. Different animals have been used as "sentinels" for detection of toxic and harmful agents (Table 1). In their two comprehensive reviews of the scientific literature from 1966 to 2005, Rabinowitz et al. [24] revealed evidence that animals can potentially provide early warning of an acute bioterrorism attack as well as serve as markers

Table 1 A time-line of examples of animals as sentinels of environmental toxicants

Sentinel incidents			Date	References
Species	Toxicant	Country		
Canaries	Carbon monoxide	England (Wales)	1870s	[168, 169]
Cattle	Smog	England	1910s	[170, 171]
Cattle	Fluoride	England		[26]
Horses	Lead	USA		[26]
Cattle	TCE	Scotland		[172, 173]
Cats	Mercury	Japan	1950s	[174]
Birds	DDT	USA		[26]
Chickens	PCB	Japan	1960s	[175]
Sheep	OP agents	USA		[176]
Horses and other animals	Dioxin	USA	1970s	[177, 178]
Dairy cattle	PBBs	USA	1980s	[179, 180]
Sheep	Zinc	Peru		[26]
Alligators	DDT, dicofol	USA	1990s	[181]
Fish	*Pfiesteria* toxins	USA		[26]

TCE trichloroethylene, *PCBs* polychlorinated biphenyls, *OP* organophosphate, *PBBs* polybrominated biphenyls
Source: van der Schalie et al. [26]

for ongoing human environmental health hazard exposure risks [24, 25]. The sentinel animal species have been successfully used for environmental monitoring applications, hazard and risk assessments, and detection and screening of deleterious changes in animal and human ecosystems [26].

Even though the use of animals could provide useful information about physiological risks associated with a particular agent or groups of agents for a prolonged period of time, the limited portability and robustness for practical large scale application for in-field or military applications preclude the use of large living animals for biosensing. The application of living cells to monitor and screen pathogens or toxic substances offers many advantages over using a whole animal for functional biosensing. Li et al. [27] listed several advantages of using cultured cells over using whole animals, such as, when cells are used for evaluation of different analytes. The key factors of cellular function affected by those analytes can be singled-out without interference from more complex, whole-organism or whole-organ responses. In addition, the cell types distributed or grown in a thin layer provide better optical observation capabilities using microscopes or other optical devices. The time necessary to raise an animal can be avoided and, most importantly, a wide range of medium formulations and cell lines originating from most tissue types are commercially available [27]. In addition to these factors, some other advantages of using cells can be attributed to their abilities of responding to external stimuli in a way that is physiologically relevant. Moreover, culturing and maintenance of cells are less expensive compared to animal maintenance. Also, CBAs provide much broader and more complex functional information, such as global information about protein synthesis and apoptotic or necrotic cell death when compared to nucleic acid and immunochemical methods. Information obtained by a CBA can provide insight into the mechanism of toxicant or pathogenic action, which in turn facilitates not only detection but also agent classification. Considering all these factors, "cell-based biosensors" (CBB) incorporating living cells, tissues, or cellular components have become very popular and useful in functional biosensing [2, 28]. As such, cell-based detection or screening of hazardous agents is an attractive approach, since it avoids the impracticalities associated with the use of whole animals for function-based detections or limitations of conventional biochemical or molecular detection strategies, such as nucleic acid or antibody-based methods (Fig. 1).

3 Cell-Based Biosensor

When used in detection systems, living cells or cellular components demonstrate unique capability of functional evaluation of the analyte and, therefore, physiological effect of the analyte can be deduced from the response [5]. Biosensors which incorporate CBAs have become a reliable and promising approach to investigate the presence of hazardous agents (such as pathogens or toxicants) in clinical, environmental, or food samples [5, 6]. The CBB systems can screen and detect

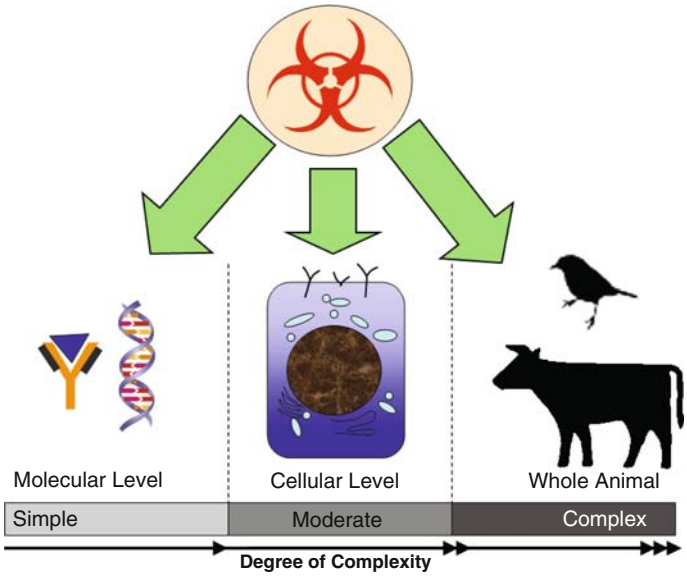

Fig. 1 Assessment of hazardous agents utilizing biological entities. Cell-based assays offer function-based diagnostics with optimal complexity

anomalies, disturbances, or changes in "normal" physiological activities of mammalian cells initiated by an "external" or environmental agent [5, 6].

In some of the CBB applications, mammalian cells or plasma membranes derived from cells have been used as electrical capacitors or the mechanical contact between cell–cell and cell–substrates has been measured by evaluating the conductivity or electrical impedance [8, 9, 29]. Biological products in water, such as herbicides or oxygen (as a metabolic end product) were detected using the physiologic responses of cells (e.g., cyanobacteria) [7]. Biochemical and metabolic end-products released by cultured cells into the medium in response to analytes were measured by CBBs connected to pH sensors or potentiometers [6]. Electrogenic cells, such as neural cells, heart muscle cells, pancreatic beta cells, or neuronal cell networks are used extensively in CBB applications to measure the direct electrical response of these cells when exposed to different agents [5, 6].

To overcome issues with stability and robustness of using living cells in sensor applications, cellular components such as lipid membranes are being used in CBB. Cellular components derived from whole cells are more stable than the whole cell itself and they eliminate the need for maintaining expensive cell culture setup [30–33]. Components of cell membranes such as receptors, e.g., G-protein-coupled receptors (GPCR), ion channel receptors, or cell surface proteins such as heat-shock proteins are immobilized onto functionalized sensor surfaces for cell-based detection assays [4, 34, 35].

3.1 Bacterial Cells vs Mammalian Cells

The term "cell-based biosensor" has been equally implicated in the description of detection systems employing bacterial or prokaryotic cells as well as higher eukaryotic or mammalian cells [1, 6, 36]. There have been several reports where whole cell microorganisms, such as bacteria and algae, were used as the recognizing elements in detection of environmental hazards such as heavy metal ions [37, 38]. Using photosynthetic green algae and cyanobacteria, Sanders and co-workers reported sensitive detection of the nerve agents tabun (GA), tributylamine, and dibutyl sulfide [39]. A microbial amperometric biosensor based on yeast cells was used for detection of lactate [40]. In another type of bacterial CBB, Immonen and Karp [41] employed two genetically engineered *Lactobacillus lactis* ssp. *cremoris* strains for detection of nisin in food samples. A rapid and selective method for the determination of free short-chain fatty acids in milk was developed by Schmidt and co-workers using a microbial sensor based on thick film oxygen electrode technology [42]. Several groups have used bacterial bioluminescence-based optical biosensors or electrochemical sensors containing recombinant or wild type microbial cells in rapid environmental monitoring systems for onsite biochemical oxygen demand or toxicant evaluation [20, 43–47].

The advantages of using single-cell microorganism-based sensors include rapid growth and ease of culturing. Culturing of bacterial cells is usually less expensive than that of mammalian cell culture, and bacteria are more robust and have better viability than mammalian cells. Despite these advantages, the mammalian cells can report information such as bioavailability [48], cellular metabolism, and physiologic responses relevant to human and animals [9, 17, 49–52] or cytotoxic responses [3, 12, 17, 19, 53, 54]. This type of information is important for functional biosensing. The ability of mammalian cells to simulate the organism's physiologic function and interact with toxicants has become an essential tool for screening, sensing, and evaluating environment, food, or clinical hazards [3, 5].

4 Classification of Mammalian CBBs

A variety of mammalian CBBs exist which differ widely not only in types of cells utilized, but also in detection applicability and methodology used. Therefore, the employment of mammalian CBBs is applicable across many fields from medical diagnostics and drug discovery to pathogen and toxin testing. The great diversity in this field coupled with innovative advances in the engineering of detection platforms allows for a great heterogeneity in the composition and use of any individual mammalian CBB. CBBs are broadly classified based on the function and mechanism of action of the biosensing (Table 2).

Table 2 Classification of cell-based biosensors

Function/ mechanism at cellular levels	Cell types	Primary signals derived from cells	Device/methods used for secondary transduction of signals
Excitable/ electrogenic	Neuron, cardiac cells, neuronal network	pH, flow of ions	LAPS, microelectrodes
Electrical responses	Epithelial cells, cardiomyocytes, neuron	Electric current, flow of ions	Impedance, IDES
Cellular receptors	Epithelial cells, hepatocytes, stem cells, mononuclear cells, T- or B-cells	pH, alteration of molecules within cells	Cell-signaling molecules, LAPS, optical methods
Cellular metabolism	Epithelial cells, hepatocytes, stem cells	pH, ion channel, molecular flux	pH-sensitive ISFETs, LAPS, ion-sensors
Cytotoxicity	Epithelial cells, endothelial cells, macrophages, myeloma, mononuclear cells, T- or B-cells	Changes in membrane integrity, cellular morphology	Optical methods, potentiometric methods
Genomic responses	Epithelial cells, endothelial cells, macrophages, myeloma, T- or B-cells	Changes in gene expression	Reporter gene assay, optical methods, cytometry

LAPS light-addressable potentiometric sensor, *IDES* interdigitated electrode structures, *ISFET* ion-sensitive field effect transistor

4.1 CBBs Utilizing Electrical Measurements

4.1.1 CBBs Utilizing Excitable or Electrogenic Cells

Bioelectric and chemical responses of excitable cells like cardiac myocytes [48], neurons [5], glial cells, cultured neuronal networks [5], brain tissue slices, or vertebrate retina [55, 56] can be utilized to deduce functional information of an analyte [1]. In this class of CBBs, stimuli (for example, chemicals, such as toxins, drugs, or physical stimulant, such as light or electronic signal) are added into a detection chamber containing cells cultured on a chip. As a result of stimulus, the cells produce action potential resulting from physico-chemical changes, such as pH of culture medium, which is detected by an electrical device through a thin layer of electrolyte [57, 58]. By evaluating the changes in electrophysiological activities of the cardiac myocyte cells from rats, Liu et al. [59] demonstrated sensitive detection of heavy metals, such as mercury, lead, cadmium, etc., using a light-addressable potentiometric sensor (LAPS) [48].

The use of microelectrodes for extracellular recording from electrogenic cells has become a popular technique for different applications ranging from toxicant evaluation to drug screening [60, 61]. To conduct extracellular recordings, commercial systems are available from different manufacturers, such as Plexon Inc. (Dallas, TX), Multichannel Systems GmbH (Reutilingen, Germany), and Alpha MED Sciences, Co. (Tokyo, Japan) [60, 62]. Researchers at the US Naval Research Laboratory in collaboration with University of North Texas reported the development of microelectrode array using cultured neuron for quantitative measure of

synchronization in neuronal impulses [63–66]. The typical characters of neuronal spikes under exposure to different chemicals were correlated with type of exposure leading to a specific pattern for a specific group of agents.

4.1.2 CBBs Utilizing Changes in Impedance

Living cells grown on a surface (adherent cell types) function similar to a simple circuit, since they can be considered an electrical system containing conductive fluid packaged within a membrane surrounded by another conductive fluid. The conductive fluids make up the resistance elements of the circuit, while the membrane acts as a capacitor. Impedance methods have been used to monitor tissue cultures online and in real time [67]. Impedance-based CBBs utilize the ability of adherent cells to change electrical impedance due to the dielectric properties of membranes of biological materials including cells. Impedance-based measurements rely on the phenomenon that intact living cells are excellent electrical insulators at low signal frequencies. As cells grow or migrate to increase surface area of coverage over an electrode surface, the effective electrode impedance rises. Adherent cells grown on planar electrodes can be used as a CBB for impedance measurements to indicate cell adhesion, spreading, and motility, since it has been found that the changes in cellular motility and spreading can be associated with exposure to different analytes [9, 29].

In a particular type of impedance-based CBB, cells derived from a male monkey's kidney were adherently grown on interdigitated electrode structures to monitor impedance changes associated with cell growth [68, 69]. The cellular behaviors of nonexcitable cells like endothelial cells [70], fibroblasts [9], or macrophages [71] were monitored using impedance measurements. In another type of impedance-based CBB, Kovacs and colleagues at Stanford University and at the US Naval Research Laboratory achieved functional agent sensing by monitoring changes in membrane impedance [72, 73]. Prolonged cellular growth period and morphological changes have been implicated as disadvantages of impedance-based biosensing.

4.2 CBBs Utilizing Biological and Physiological Measurements

4.2.1 CBBs with Cellular Receptors

One of the salient features of CBBs is that the cellular events initiated by analytes or agents can be interpreted and explicated both for detection as well as to deduce functional information about the mechanism of action of the analyte. A typical cell–analyte event is initiated by the intimate interaction of cellular receptors (most often referred to as "membrane receptors") with the foreign agents (analyte or ligand). Receptors may serve as the "entry port" for the foreign materials into the cell. For

example, mammalian membrane proteins act as receptors for bacterial surface proteins and aid in the internalization of the bacteria [74] or may induce downstream intracellular signaling pathways upon binding to membrane receptors (for example, GPCRs) [75]. In-depth information about biosensing strategies using natural cellular receptors are reported elsewhere [76, 77]. Since most CBBs directly or indirectly exploit cellular receptors as the first biorecognition element, the receptors warrant significant attention in the field of development of cell-based biosensing strategies. A "rational design" of CBBs can become much easier when enough information about types of receptors expressed by a particular cell-type is available, and that information can effectively be utilized for pathogen or hazardous agent detection.

4.2.2 Cellular Receptors Used for Biosensing and Their Types

Cellular receptors are proteins (glycoproteins or lipoproteins) typically associated with cell membrane (or within the cytoplasm or cell nucleus) that bind to molecules or chemical entities (ligands) such as proteins and hormones, and upon binding the structural conformation of the receptor changes, resulting in the initiation of the cellular response. As such, ligand-induced changes in the behavior of receptor proteins results in physiological changes in the cell that represent the biological effect of the ligand. In a receptor-based biosensor, the typical cellular responses initiated by the ligand (in this case the analyte) can be interpreted to identify the analyte (detection), since receptor–ligand interactions are often specific or at least provide information about structural similarities about a group of analytes. For example, estrogen receptors on a cell bind to estrogen-like foreign substances called xenoestrogen (having structural similarity to estrogen and considered as environmental pollutants) and elicit estrogenic responses in estrogen responsive cell types [78].

Based on structural and functional characteristics, membrane receptors were classified into four major categories, namely, ion channel receptors, G-protein-linked receptors, receptors with a single transmembrane domain, and enzyme-linked receptors [76, 79], and are summarized in Table 3.

Ion Channel Receptors

Ion channel receptors, ionotropic receptors, or ligand-gated ion channels (LGIC) are responsible for the physiological activities which coordinate information flow in the brain and control behavioral activities. Any disturbance in the control of this balance leads to deleterious outcome, resulting in abnormal activity (e.g., epilepsy), behavioral disorders (e.g., anxiety), or even neuronal cell death (excitotoxicity) [80]. The LGICs activated by extracellular ligands may be divided into four superfamilies: (1) *Cys-loop superfamily*, (2) *glutamate receptors* (NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; and

Table 3 Classification of membrane receptors

Characteristics	Receptor types			
	Ion channel receptors	G-protein-linked receptors	Receptors with a single transmembrane domain	Enzyme-linked receptors
Endogenous ligands	Neurotransmitters	Neurotransmitters, hormones, autoacoids, chemotactic factors	Growth factor hormones, cytokines	Atrial natriuritic peptide ligands, growth factors
Structure	Several proteins with a pore	1–2 proteins	1–2 proteins with catalytic domain	Individual protein linked with enzyme
Transmembrane segments	Four	Seven	One	Single-pass transmembrane proteins
Function	Regulation of ion transport	Activation of G-proteins, regulation of cellular functions and expression of proteins	Catalytic	Suppress proliferation, stimulate synthesis of extracellular matrix, stimulate bone formation, attract cells by chemotaxis
Cellular responses	Depolarization/ hyperpolarization	Depolarization/ hyperpolarization	Regulation of cellular functions, proliferation, and differentiation	Regulation of cyclase, production of cyclic GMP, cell signaling and regulation of cell cycle

Source: Subrahmanyam et al. [76]

kainite), (3) *TRP (transient receptor potential) channels*, and (4) *ATP-gated channels*. In mammals, the Cys-loop superfamily comprises cationic receptors (nicotinic, 5-HT, and zinc activated) and anionic receptors [γ-aminobutyric acid (GABA) and glycine receptors]. The members of this superfamily exhibit important physiological functions and mutations may lead to a range of pathological states. Ligands specific for this class of receptors include endogenous chemicals such as neurotransmitters. Typical examples of these include GABA, glycine, serotonin, and ATP.

G-Protein-Coupled Receptors

A vast array of cellular signaling pathways are mediated through GPCRs, interacting with numerous signaling molecules of diverse structure and function, including hormones, neurotransmitters, and local mediators. GPCRs consist of a single polypeptide chain spanning the lipid bilayer which includes several transmembrane segments. Endogenous ligands belonging to this class that are important target analytes for sensor technology include all neurotransmitters, most hormones and autocoids, chemotactic factors, and exogenous stimulants such as odorants [76]. For example, Whitaker and Walt [81] recently developed a fiber-optic single-cell array-based biosensing method using Chinese hamster ovary (CHO) cell line ectopically expressing different human GPCRs to detect and analyze real-time Ca^{2+} responses resulting from exposure to various agonists.

Receptors with Single Transmembrane Segments

Some of the crucial cell-signaling pathways are regulated by a group of receptors with single transmembrane segments. The prominent members of this group include growth factors, such as epidermal growth factors, platelet-derived growth factors, fibroblast growth factors, and nerve growth factors. Structurally these receptors are segmented into three domains, an extracellular domain responsible for ligand binding, a single transmembrane segment responsible for signal transmittal and conformational events, and cytoplasmic domains eliciting cellular responses by activating signal transduction pathways. Proteins such as phosphorylase C, GTPase-activating protein, and phosphatidylinositol 3-kinase are some classical examples of this receptor type.

Enzyme-Linked Receptors

Enzyme-linked receptors are probably the most studied group of receptors pertaining to pharmacological and clinical applications. These receptors are also transmembrane proteins having ligand-binding domains on the outer surface of the plasma membrane, while the signaling elicitor component may or may not be

physically associated with the transmembrane domain. Broadly, these receptors are grouped into five major categories (adopted from Subrahmanyam et al. [76]) as follows. (1) *Receptor guanylyl cyclase* – ligand specificity is peptide hormones secreted by heart muscle cells. (2) *Receptor tyrosine kinase* (RTK) – this is a high affinity cell surface receptor containing a large transmembrane domain and a glycosylated extracellular domain. There are approximately 20 RTK families based on their affinity to different ligands [82], such as epidermal growth factor family, insulin receptor family, fibroblast growth factor family, vascular endothelial growth factor family, and others. (3) *Cytokine receptor superfamily* – this group includes growth hormone prolactin and antigen-specific receptors on T- and B-lymphocytes that regulate proliferation and differentiation in the hematopoietic system [83]. (4) *Tyrosine phosphatases* – these interact with phosphotyrosines on a particular type of protein and play significant roles in cell signaling. (5) *Serine/threonine protein kinases* – these perform various functions such as suppression of proliferation and stimulation of synthesis of extracellular matrix (ECM). Phosphacan, a chondroitin sulfate proteoglycan of nervous tissue, is a typical example of ligand for this class of receptors [84].

4.2.3 Receptors for Host–Pathogen Interaction

Multicellular organisms express specialized surface receptors called "cell adhesion receptors" so as to form tight associations with neighboring cells and with ECM to facilitate building cell layers, tissues, and organs. Cell adhesion receptors are constituted by different protein families, most important of these are the integrins, the cadherins, the immunoglobulin superfamily cell adhesion molecules (IgCAMs), the selectins, and the syndecans [85]. It is well established that, in addition to their structural role, adhesion receptors in most cases also play a crucial role in signal transduction from the exterior to the interior of the cell [86]. As a result of these multiple functions, the expression of adhesion receptors on the cell surface is critical and integrities of these proteins are conserved in multicellular organisms. However, due to the nature of the surface exposure, signaling capacity, and conservation, these receptor proteins have evolutionarily became ideal targets for pathogen interaction, communication, and invasion [85, 87].

Several ECM proteins and adhesion receptors are targeted by different pathogenic microorganisms (Table 4). Three major classes of these receptors – integrins, cadherins, and IgCAMs – are discussed below with regard to their interactions with pathogenic microorganisms.

Integrin Receptors and Interacting Pathogenic Bacteria

Integrins are cell surface receptors that are glycoprotein in nature and interact with ECM proteins or recognize membrane-bound counter receptors and elicit intracellular signal transduction. Some outer membrane proteins expressed by enteropathogens

Table 4 Pathogens targeting cell adhesion molecules

Species	ECM protein/receptor
Borrelia burgdorferi	FN/β1 integrins
Mycobacterium leprae	FN, LN/β1 and β4 integrins
Mycobacterium bovis BCG	FN/β1 integrins
Neisseria gonorrhoeae and *N. meningitidis*	FN, VN/β1 and β3 integrins
Porphyromonas gingivalis	β1 integrins
Shigella flexneri	β1 integrins
Staphylococcus aureus	FN, LN, Col/β1 integrins
Streptococcus pyogenes and *S. dysgalactiae*	FN/β1 integrins
Yersinia pseudotuberculosis and *Y. enterocolitica*	β1 integrins
	Immunoglobulin-related cell adhesion molecules (IgCAMs)
Haemophilus influenzae	CEACAMs
Moraxella catarrhalis	CEACAMs
Neisseria gonorrhoeae and *N. meningitidis*	CEACAMs
	Cadherins
Listeria monocytogenes	E-cadherin

CEACAMs carcinoembryonic antigen-related cell adhesion molecules, *Col* collagen, *FN* fibronectin, *LN* laminin, *VN* vitronectin
Source: Hauck et al. [85]

like *Yersinia* species, *Y. pseudotuberculosis* and *Y. enterocolitica*, and *Shigella* (IpaC protein) function as ligands for mammalian β1-integrin receptors. Gram-positive bacteria such as *Staphylococcus aureus* or *Streptococcus pyogenes* interact with integrins by recruiting ECM proteins. These ECM proteins are adhesins in nature and include fibronectin-binding proteins A and B (FnBP-A and -B) of *S. aureus* or Sfb1 (also called F1) of *S. pyogenes* [88, 89]. Enteropathogenic *Escherichia coli* (EPEC) interacts with β1-integrins through its surface intimin adhesin [90]. EPECs insert a protein into the host cell membrane called transepithelial intimin receptor (Tir) which serves as the counter receptor for the bacteria-associated intimin [91]. Evidence from in vitro experiments using cultured cells indicates that the region of Tir that is recognized by intimin displays homologies to β1-integrin receptors, leading to the belief that intimin might also bind to certain cellular integrins [92].

Immunoglobulin Superfamily Cell Adhesion Molecules and Associated Pathogenic Bacteria

Cell adhesion molecules of the immunoglobulin superfamily are characterized by the presence of at least one immunoglobulin-like domain in their extracellular part. The immunoglobulin domain functions as a site for binding and recognition. IgCAMs appear as integral membrane proteins or coupled to the membrane via a glycosyl-phosphatidylinositol anchor. The prominent pathogen exploiting IgCAMs is found in the genus *Neisseria*, where pathogenic *N. gonorrhoeae* and *N. meningitidis* both express adhesins, the colony opacity-associated (Opa) proteins that bind to

members of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family, a subgroup of IgCAMs that elicits the pathogenesis of this microorganism [85, 87, 93].

Cadherins and Interacting Pathogens

Cadherins are transmembrane glycoproteins that mediate tight homotypic cell–cell association [94]. Cadherins possess five cadherin-motif subdomains in their extracellular segment which allow them to dimerize with neighboring cadherin molecules in the presence of Ca^{2+} [95]. *Listeria* is considered to be the most prominent example of pathogenic bacteria engaging cadherins. *Listeria monocytogenes*, a Gram-positive, facultative intracellular pathogen, expresses a family of adhesins termed internalins that mediate the invasion of this pathogen into different cell types [96]. Cossart's group at the Pasteur Institute, France has led the way in understanding the *Listeria* surface proteins, such as internalins and their interactions with host cell receptors. Two types of internalins are proposed to be the key virulence factors playing important roles in the host cell adhesion–invasion mechanism for *Listeria*: Internalin B (InlB) aids in the invasion into a number of different cell types and seems to associate with different receptors such as Met receptor tyrosine kinase [97, 98], while internalin A (InlA) allows the bacteria to penetrate efficiently human epithelial cells [99]. This property is based on InlA binding to human E-cadherin [100]. Interestingly, like the homophilic interaction between cadherins, the binding of InlA to E-cadherin is Ca^{2+} dependent. As cadherins are linked to the actin cytoskeleton, it is not surprising that InlA-mediated invasion can be blocked by agents that disrupt the integrity of the actin cytoskeleton such as cytochalasins [101, 102].

Other Mammalian Surface Proteins

The intimate physical interaction between host–pathogen is mediated by several other surface expressed molecules on mammalian cell membrane. Prominent examples of this are the group of proteins called heat-shock protein (Hsp). Our group has discovered that under thermal stress conditions *Listeria monocytogenes* can over-express a 104 kDa surface alcohol acetaldehyde dehydrogenase designated Listeria adhesion protein (LAP), which acts as a ligand to a ubiquitous mammalian heat-shock protein (Hsp60) and aids in bacterial adhesion and translocation in intestinal epithelial cells [103–105]. Interestingly, physiological stressors and nutritional status can up- or downregulate the expression of LAP, and thereby its interaction with its receptor Hsp60 [106, 107]. Viruses also utilize receptors to gain entry into host cells. These may include one or more attachment receptors and at least one entry receptor [108]. Interaction with attachment receptors increases infectivity but may be an optional requirement [109, 110]. Examples of attachment receptors include cell-bound heparan sulfate molecules for HIV [110] and herpes

simplex virus [111]. The entry receptors for HIV are CD4 and members of the extended chemokine receptor (CCR) family (also known as co-receptors) [112]. Some viruses use multiple alternative receptors to enter a host cells. For example, HIV uses CCR5, or CXCR4, CCR2b, CCR3, CCR8, and CCR9 to enter host cells. The repertoire of host cell surface receptors available to a given virus comprises the receptor array. Specific interaction of virus with the host cell depends on the availability of a minimal set of receptors from a series of available receptors in that host cell surface [108].

4.2.4 Application of Receptors in Functional Biosensing

The application of receptors for cell-based sensing is a technique which has been used for a long period of time. CBAs for HTS are a widely used technique in drug discovery and in clinical/pharmacological applications [21]. In recent years, interfacing of electronics and optical technologies has aided in technological advancement and instrumentation for CBAs. Some examples of pioneering technologies using receptor-based HTS, utilizing GPCRs [113], and ion channels [114, 115] are FLIPR (Molecular Devices) and Photina (PerkinElmer). Both are automated systems for measuring calcium concentrations in cellular compartments. The former uses fluorescence imaging while the latter utilizes luminescence assays. Other optical tools such as confocal imaging and laser scanning microscopy and cytometry enable acquisition of real-time and time-lapse cellular and sub-cellular images. Laser scanning imaging utilizing fluorescence imaging techniques is now routinely used for HTS and to evaluate receptor–ligand interactions [116].

Several automated systems are now available to monitor receptor–ligand interaction. FMAT 8100 HTS (Applied Biosystems) is a system which employs a macroconfocal scanning platform to visualize and quantify protein–peptide or protein–protein interactions in real-time [117]. ArrayScan (Cellomics) is used to perform high-content cell-based imaging assays to monitor cellular localization of molecules, cell–cell interactions, and cytotoxicity or receptor (GPCR) internalization [118–121].

4.3 CBBs Based on Metabolic Measurements

The metabolic activities of a cell are a function of its interactions with surrounding environment or agents. The changes to the extracellular environment due to cell metabolism (as a result of exposure to physical or chemical factors) can be measured. Manifestation of cellular metabolism is associated with changes in the rate of acidification of the media and changes in medium composition or characteristics, such as changes in extracellular pH or concentrations of different ionic species initiated by secreted cellular metabolites, such as glucose, lactate or ammonia [122]. Other common methods for metabolic sensing using living cells include

oxygen and/or carbon-dioxide consumption or production and microcalorimetry [23]. Well-established biochemical sensors such as glass electrodes, pH-sensitive ISFETs (ion-sensitive field effect transistors), or LAPS can be utilized to monitor pH-changes, Clark electrodes for oxygen, and amperometric (enzyme sensing) devices for glucose and lactose monitoring [5, 6, 67]. Ion-selective ISFET can be used to monitor the concentrations of the ionic species. Such a potentiometric sensor was used to detect the effect of histamine as a model toxin on human umbilical vein endothelial cells [123]. With the advent of metabolomics, CBAs are receiving increased attention to screen various toxicants. For example, hepatocytes express high levels of drug-metabolizing enzymes (both phases I and II) and have been widely used in CBA platforms to evaluate the hepatotoxic potentials of various drug molecules or environmental toxicants [124, 125].

In another type of cell-based sensing platform, stem cell-based systems are used to evaluate agents which affect cell metabolism. Stem cell-based systems offer a promising and innovative alternative for obtaining large numbers of cells for early efficacy and higher toxicity screening, allowing improved screening of drug or toxicant molecules. The applications of adult and embryonic stem cell-based screening are popular choices in several areas of HTS including cardiotoxicity [126], hepatotoxicity [127], genotoxicity/epigenetic [128] and reproductive toxicology [124]. In another study, embryonic stem cells derived from a mouse were induced to differentiate in vitro to cardiomyocytes or neurons and were cultured on the surface of LAPS in order to monitor changes in extracellular ion concentrations [48]. The metabolic measurements offer functional information relevant to the analyte. At the same time, cross-talks between metabolic pathways may preclude specific detection of a particular analyte, since different toxicants or pathogens evoke different cellular responses, such as signaling pathways or targeting of different organelles.

4.4 CBBs Based on Optical Measurements

Optical assays utilizing the properties of various probing molecules such as color, fluorescence, or luminescence are utilized to acquire real-time and in-situ information about cell–cell or cell–analyte interaction. With the advent of novel organelle specific fluorophores it is possible to visualize a particular cellular component such as nucleic acids, mitochondria, or lipid membranes [129]. CBAs based upon fluorescent or luminescent properties for HTS application are probably the most powerful tools available to cell-biologists today [116, 120, 121, 130]. There have been several other optical methods employed to detect biological agents by monitoring cell death events. Cytotoxic agents which cause cell damage can be screened by using suitable markers for cytotoxicity. The hallmarks of cell death are membrane damage or nucleic acid degradation which eventually leads to either apoptotic or necrotic cell death [131, 132]. The quantification of cell death can be done by using appropriate enzyme substrate or fluorescent dyes [3, 12]. Several bacteria or

mammalian cells have been genetically engineered to emit light (luminescence) when exposed to toxic compounds or pathogens [133–136]. In another type of optical assay cell viability was measured by monitoring respiration by optical oxygen sensing using a fiber-optic phosphorescent phase detector [137].

4.5 Genomic Biosensors

Genomic biosensors perform functional analysis of agents or drugs based on gene expression profiles in target cells [138]. A detailed review on genomic biosensors can be found elsewhere [139]. A genome based biosensor, such as GenomeScreen (developed by Aurora Biosciences Corp.), scans the genome broadly and identifies relevant genes in live cells. A library of cell clones is generated with a randomly integrated promoterless fluorescent reporter gene (such as β-lactamase) throughout the genome. A fluorescence activated cell sorting step is performed on this "Gene-Tag" library to isolate clones (of cells) based on the expression levels of individual tagged genes. This method is very specific and can provide information at the gene level; however, limited choice of appropriate cell lines with intact signal transduction pathways precludes wide use of this type of CBB.

4.6 CBBs Based on Cytopathogenicity

CBAs incorporating cytotoxicity measurements are gaining increasing importance in sensor design and detection strategies. The iniquitous distinction of "pathogens" is designated to the microorganisms which have the capability of inducing diseased conditions in other organisms such as humans, animals, and plants. The hallmark of the pathogenesis by a pathogen is the disruption of a normal physiological condition of the host. Such pathogenic organisms can be detected by damage inflicted upon the host system. Several pathogens are known to cause damage of varying severity to human and animal cells or tissues. A comprehensive list of such pathogens and their target cells is illustrated in Table 5.

4.6.1 Cytopathogenicity Assay for Pathogenic Bacteria or Toxins

Impairment of a host cell by pathogenic microbes or toxins often bears a "fingerprint" of that particular pathogen or toxin. These types of assays not only testify the presence of particular pathogens or toxin but also provide information about the functionality of the pathogen or toxin. Cytopathogenicity assays furnish critical information about the biological and physiological interactions occurring between host and pathogen (Fig. 2). Therefore, these cell-based cytotoxicity assays can distinguish a viable pathogen from a nonviable one. This information is often

Table 5 Human or animal cell lines used for cytopathogenicity assay for foodborne bacteria

Bacteria	Cell line	Cell type	Source	Cytopathic effects
Salmonella	CHO	Epithelial	Chinese hamster ovary	Elongation, detachment
	Vero	Fibroblast	Monkey kidney	Lysis, protein synthesis inhibition
	HEp-2	Epithelial	Human laryngeal	Invasion
	JY	B-cell	Human	Invasion
	H9	T-cell	Human	Invasion
	Henle-407	Epithelial	Human jejuna	Intracellular growth
	J774	Macrophage	Mouse	Intracellular growth
	HeLa	Epithelial	Human cervix	Toxicity, actin polymerization
	HT-29	Epithelial	Human colon	Apoptosis
E. coli	Vero	Fibroblast	Monkey kidney	Lysis, protein synthesis inhibition
	CHO	Epithelial	Chinese hamster ovary	Lysis, toxicity
	Henle-407	Epithelial	Human jejuna	Adhesion
	HEp-2	Epithelial	Human laryngeal	Adhesion, toxicity
	MAC-T	Epithelial	Bovine mammary gland	Invasion
	MDBK	Epithelial	Bovine kidney	Invasion
	HeLa	Epithelial	Human cervix	Toxicity, apoptosis
	T84	Epithelial	Human colon	Apoptosis
	Y1	Epithelial	Human adrenal gland	Rounding, detachment, cAMP
	W138	Fibroblast	Human lung	Toxicity
	J774	Macrophage	Mouse	Apoptosis
	HT-29	Epithelial	Human colon	Apoptosis
Shigella	HeLa	Epithelial	Human cervix	Cell death, protein synthesis inhibition
	Vero	Fibroblast	Monkey kidney	Lysis, protein synthesis inhibition
	3T3	Fibroblast	Mouse	Invasion, actin polymerization
	U937	Monocyte	Human	Apoptosis
	Mϕ	Macrophage	Mouse	Invasion, apoptosis
Campylobacter	HeLa	Epithelial	Human cervix	Distended cells (CDT effect)
	Vero	Fibroblast	Monkey kidney	Distended cells (CDT effect)
	AZ-521	Epithelial	Human stomach	Vacuolation
	Ped-2E9	B-cells	Mouse	Toxicity
Yersinia	J774	Macrophage	Mouse	Exocytosis, apoptosis
	HEp-2	Epithelial	Human laryngeal	Invasion, lysis
Vibrio	CHO	Epithelial	Chinese hamster ovary	Elongation, cAMP accumulation
Listeria	Caco-2	Epithelial	Human colon	Adhesion, invasion, apoptosis
	CHO	Epithelial	Chinese hamster ovary	Detachment, lysis
	Henle-407	Epithelial	Human jejuna	Intracellular growth, death
	Vero	Fibroblast	Monkey kidney	Toxicity

(*continued*)

Table 5 (continued)

Bacteria	Cell line	Cell type	Source	Cytopathic effects
	HEp-2	Epithelial	Human laryngeal	Invasion
	J774	Macrophage	Mouse	Intracellular growth
	RAW	Macrophage	Mouse	Intracellular growth
	HeLa	Epithelial	Human cervix	Toxicity
	HUVEC	Endothelial	Human umbilical vein endothelial cell	Intracellular growth
	Hep-G	Epithelial	Human liver	Intracellular growth, apoptosis
	3T3	Fibroblast	Mouse	Invasion, plaque formation, lysis
	L-M	Fibroblast	Mouse	Invasion, plaque formation, lysis
	NS1	Myeloma	Mouse	Lysis, toxicity
	Ped-2E9	B-cell	Mouse hybridoma	Lysis, apoptosis
	RI-37	B-cell	Human–mouse hybridoma	Lysis, apoptosis
	Ramos RA1	B-cell	Human	Lysis, apoptosis

Source: Bhunia et al. [4]

very important from an industrial (especially food manufacturing) point of view, as nonviable bacterial cells typically cannot cause disease and are excluded from the purview of safety regulations. In contrast, immunological and nucleic acid based methods for detecting pathogens and toxins primarily rely on chemical properties or molecular recognition to identify a particular agent. As a result, no functional (biological and physiological) information can be obtained from such assays.

4.6.2 Cytotoxicity Assay

The cytopathogenicity of a pathogen or toxin is revealed by the damage of the target host cells or tissues incurred by host–pathogen interaction. The common outcomes of a bacteria or toxin mediated injury to a eukaryotic cell are manifested by cell membrane damage or pore formation, cell death via apoptosis or necrosis, cell lysis, or detachment of cells from substrata (Fig. 2). The effect conveyed by pathogenic microorganisms or their toxins can be assayed using cytopathogenicity or cytotoxicity assays [4, 67]. A typical cytopathogenicity or cytotoxicity assay can be performed either directly by microscopic methods or by indirect determination of eukaryotic cell membrane damage or pore formation. The direct microscopic evidence, such as rounding of Vero cells, is routinely used to evaluate the presence of certain pathogens or toxins [140, 141]. Membrane damage can be measured by incorporation of dyes like trypan blue or propidium iodide [12, 53, 54, 142]. Diffused enzymes, such as alkaline phosphatase (ALP) or lactate dehydrogenase that are released from eukaryotic cells as a result of membrane damage, can also be assayed colorimetrically or spectrophotometrically to assess the extent of the cell-damage [12, 19]. However, there are limitations to assays probing for released

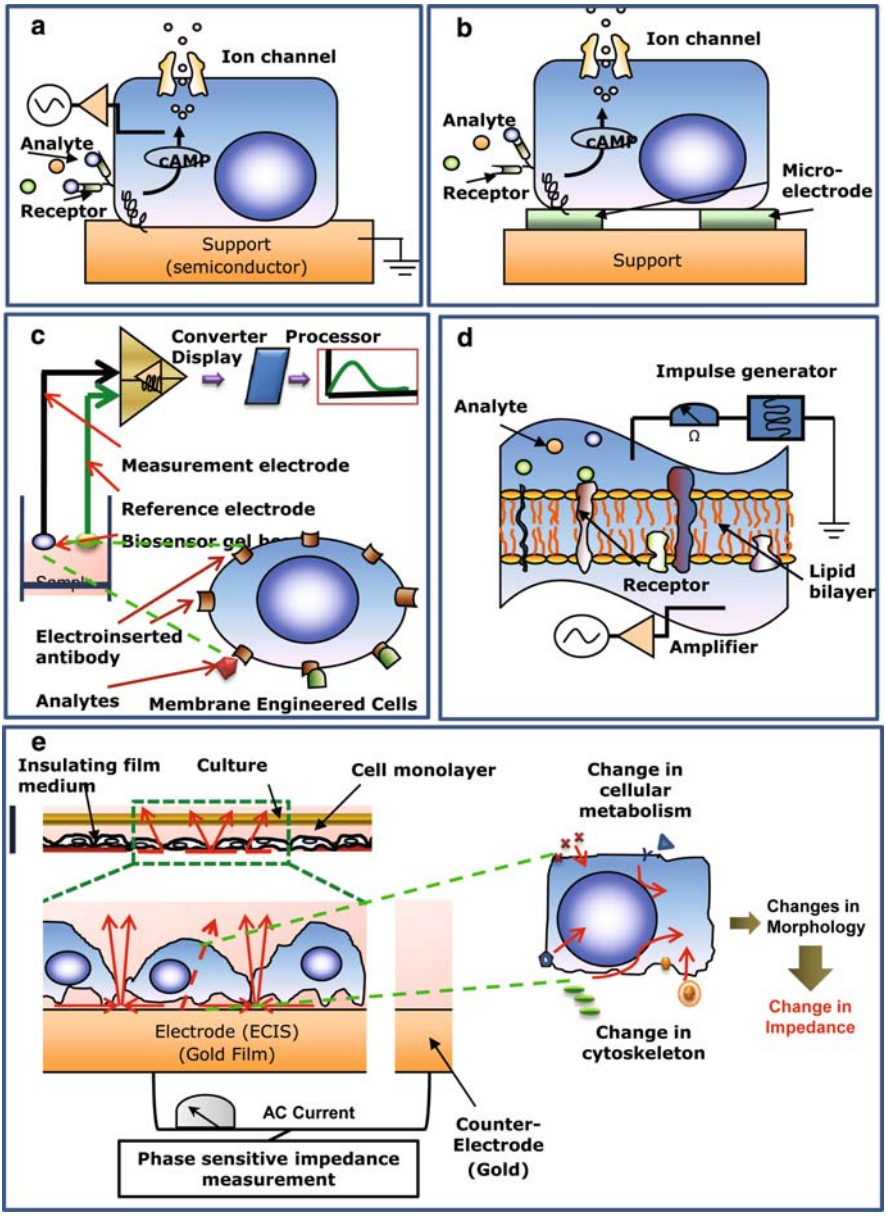

Fig. 2 Schematic diagram of CBB based on cytopathogenicity. Cellular processes in response to pathogens or toxins (external stimuli) can be exploited for evaluation of cytotoxicity. In a CBB system based on cytopathogenicity the cells release extracellular that is enzymes, such as alkaline phosphatase or lactate dehydrogenase or intracellular molecules, such as calcium ions. These marker molecules are detected as estimators of cytotoxicity

enzymes. These enzymes may not be present in all types of cells, and the effect of a certain membrane-degrading pathogen or toxin may not produce pores large enough to release more sizeable proteins. Other assays such as 3-[4,5-dimethyl thiazolyl-2]-2,5-diphenyltetrazolium bromide (MTT) or 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazoliol]-1,3-benzenedisulfonate (WST-1) can report pathogen or toxin mediated cytotoxicity based upon parameters of proliferation, viability, or activation [143–145].

4.7 CBBs Using a 3D Cell Culture System

Mammalian cells cultured in three-dimensional (3D) configurations are considered a major breakthrough in biosensor design. Cells are grown in a biocompatible scaffold such as collagen, matrigel, hydrogel, or aliginate gel to provide a 3D architecture emulating tissues in the body [146]. Cells grown in 3D configuration more accurately represent gene expression, cell differentiation and other biological activities similar to in vivo models than 2D culture system [147–149]. The 3D cell culture system has been used to study host-cell–pathogen interaction for several microorganisms including *Salmonella* Typhimurium [150], uropathogenic *E. coli* [151], *Pseudomonas aeruginosa*, cytomegalovirus, and norovirus [152, 153]. Recently, collagen encapsulated hybridoma B-cells in 3D scaffold were successfully used in an array format in 96-well plate for detection of *Listeria monocytogenes* and *Bacillus cereus* toxins [3]. The 3D cell culture model shows much promise to be an integral part of the cell-based biosensor for HTS in pathogen testing, drug discovery and point-of-care applications [152, 154].

4.8 Current Status of Cell-Based Biosensing

The applications of biosensors that incorporate whole mammalian cells to detect foodborne pathogens are drawing increasing importance. This is because mammalian CBBs have the unique potential of distinguishing viable bacterial cells from those that are nonviable. As mentioned previously, this serves as a critical parameter in the food industry, since nonviable bacterial cells or inactive toxins do not raise any concern. The development of a CBB which could be used in the food industry is still undergoing rigorous laboratory validations. Some of the key developments in this area are listed below.

4.8.1 Electrical Cell–Substrate Impedance Sensing

"Electrical cell–substrate impedance sensing" (ECIS) was developed by Giaever and Keese [9] for the detection of changes in cellular morphology (Fig. 3e). In ECIS,

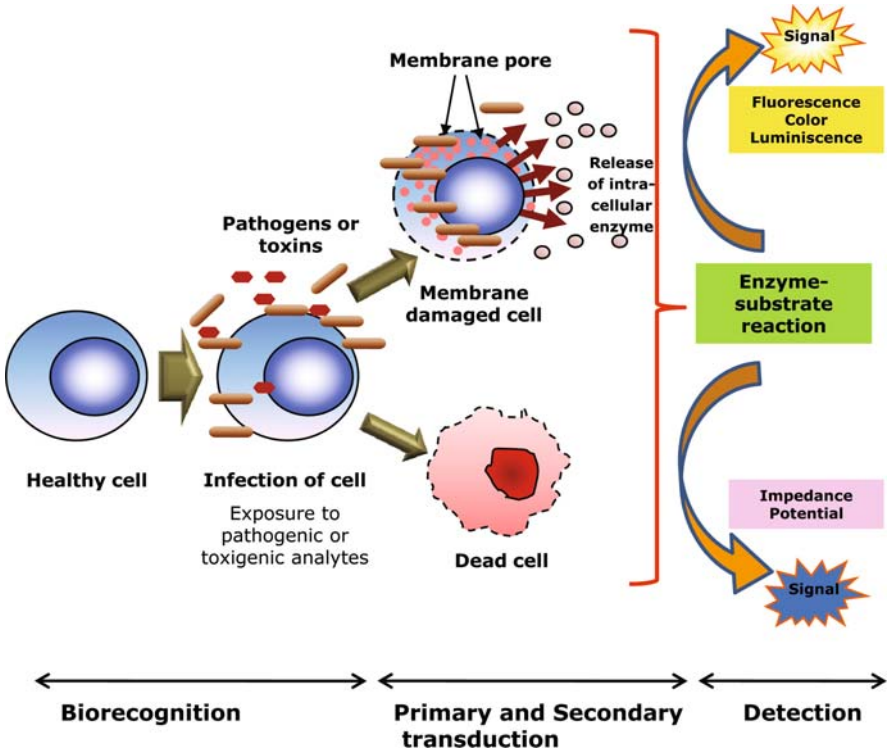

Fig. 3 Different strategies of construction of cell-based biosensors exploiting changes in electrical properties. (**a**) CBB that measures changes in electrical potential. An analyte interacts with a receptor located in the cell membrane. After interaction, an ion influx is caused by a signal cascade involving cyclic-adenosine monophosphate (*cAMP*). Changes in the electric potential of the cell are measured by a silicon-based semiconductor, which is located inside the support. The cell may be stimulated by a microelectrode. (**b**) Same cell as shown in (**a**) but the cell is immobilized on microelectrodes. Semiconductor structures may also be integrated into the support. (**c**) An immobilized CBB based on the bioelectric recognition assay (BERA). The measuring electrode is inserted in the immobilized cell-gel bead, while the reference electrode is inserted in a cell-free gel bead. The cell-gel beads contain mammalian cells those were engineered to carry analyte-specific antibody in the membrane at high density on the cell surface. The assay principle is that attachment of the analytes to their corresponding antibodies will result in structural changes of the cell membrane, which is measured as changes occur in the cell membrane electric potential. Measurement and reference electrodes are connected via wiring to a data converter, which assess the membrane potential changes. (**d**) Isolated receptors may also be immobilized inside artificial cell membranes (lipid bilayer). Electrical current flow through this membrane yields information about the analyte. (**e**) CBB based on measurement of the impedance, i.e., the resistance and the capacitance are deduced by measuring the current and voltage across a small empty electrode. Then adherent cells are grown to cover the electrode. As a result, the cell membranes block the current flow causing changes in impedance. (**e**) Cellular functions measured by electric cell substrate impedance sensor (ECIS), which measures various cellular attributes such as: barrier function, cell membrane capacitance, morphology changes, cell motility, cell–cell proximity, proximity of cells to the surface ((**a**), (**b**) and (**d**) are redrawn from Keusgen [168])

sensing is achieved by measuring the changes in the impedance of a small microelectrode in response to AC current flow. High-resolution recording of ECIS are able to indicate changes in cellular morphology at a nanoscale level. This multichambered biosensing system has been used to detect minute pathogen-induced changes in the cellular response that are normally invisible in conventional microscopic techniques. Equipped with greater sensitivity, ECIS also acquires data in real time and is capable of quantifying intracellular and intercellular changes. This sensor has commercial applications in several areas; including in vitro toxicity testing, signal transduction involving GPCRs for drug discovery, cancer cell invasion, and the testing of endothelial cell barrier function [155]. This was one of the initial breakthroughs to bring the concept of a mammalian or higher eukaryotic whole cell-based biosensing system for diagnostics application.

4.8.2 Bioelectric Recognition Assay

Kintzios and colleagues [31] developed a CBB that employed the so-called bioelectric recognition assay (BERA). Animal and plant cells immobilized on BERA sensors are capable of reflecting the electric response to various ligands bound to the cells. This sensor measures the changes to the cell membrane potential due to either cell–analyte interactions or the oxidation of membrane lipids. For example, the positive signals obtained in the BERA system reflects the binding of viruses to surface antibodies, which changes their membrane potential and not the entry of viruses in immobilized cells (Fig. 3c). Further refinement of membrane engineering technology to allow insertion (electroinsertion method) of virus-specific antibodies on the surface of cultured mammalian cells (such as fibroblasts) has improved the specificity of the sensor [156]. This biosensor technology is also available commercially with an intended target market of food manufacturing and clinical diagnostics.

4.8.3 CANARY^{TM}: A B-Lymphocyte-Based Biosensor

One of the first commercially available cell-based sensors having application to food, clinical, and environmental diagnostics or in the biosecurity area is a genetically engineered CBB developed at MIT's Lincoln Laboratory, called Cellular Analysis and Notification of Antigen Risks and Yields (CANARY) [135]. In this system, B-lymphocytes were engineered to express bacteria-specific antibodies (immunoglobulin). Simultaneously, a calcium responsive bioluminescent cytoplasmic protein – "aequorin" from a jellyfish (which emits light when intracellular calcium concentration increases) – was also engineered in such a way that when an antigen (e.g., bacterial cell surface protein) binds to the antibodies on the engineered B-cell surface, a downstream signal transduction cascade triggers intracellular calcium flux. As a result, the jellyfish protein in the cytoplasm of the engineered B-cell will almost instantaneously emit light which can be detected

with a luminometer [135, 157]. As such, this biosensor reports the presence of an analyte, such as *E. coli* O157:H7, as soon as it binds to the biosensor's receptors.

4.8.4 Cell-Based Sensor Using Ped-2E9 Hybridoma B Lymphocyte

In another type of CBB the foodborne pathogens *Listeria monocytogenes* or *Bacillus cereus* (whole bacterial cells or toxins) were detected using murine hybridoma B-cells (Ped-2E9) [3, 12, 17, 19, 53, 54, 158]. These organisms or toxins can infect and produce detectable cytotoxicity to Ped-2E9 cells; as a result, the hybridoma cells release ALP. The released enzyme can be detected colorimetrically in 1–6 h, which is directly related to the virulence potential of *Listeria* or *Bacillus* spp. [12, 17, 19].

4.8.5 Artificial Cell-Based Sensor

An artificial cell-based sensor was developed to detect a pore-forming hemolysin produced by *Listeria monocytogenes* – listeriolysin O (LLO) by using a nanocomposite material of small unilamellar liposomes containing fluorescent dyes. The liposomes were encapsulated in porous silica using alcohol-free sol–gel synthesis methods. The immobilized liposomes act as a cellular compartment containing the fluorescent dyes. The released dyes as a result of LLO mediated pore formation report the presence of the toxin [159]. The real advantage of this type of synthetic cell-based sensor is that it is composed purely of "nonliving" components, so shelf-life is very long and preservation is simple when compared to mammalian cell-based sensors.

5 Limitations and Drawbacks of CBBs

Cell-based sensors have emerged as a promising approach to address several issues such as functional identification of an analyte [1], screening of a group of molecules or chemical species [5], or in drug discovery and development as well as environmental monitoring [6]. However, there are several issues which may limit extensive application of CBBs such as specificity, reliability and robustness, stability, and shelf-life.

Some CBBs lack specificity and fail to identify the analyte type [60]. For example, different cellular events, such as cytotoxicity/cell death events or cell-signaling pathways may manifest a similar outcome. Membrane active toxins such as hemolysins and cytolysins from different microbes may cause similar damage to membrane structure of mammalian cells, or different adrenergic compounds might elicit similar cAMP or cGMP mediated cellular signaling events. The

discriminatory analysis for the specific detection of an analyte may become difficult when only relying on the hallmark of cellular fate.

CBBs may lack robustness because mammalian cells when cultured in vitro are generally fragile and prone to damage even with slight changes in their growth environment. Thus the cellular microenvironment, e.g., media pH or media composition, must be maintained properly during a CBA. The reliability of the assay depends on maintaining a "true" tissue or organ environment, which often requires sophisticated design of platforms. A classical example of such an issue is observed when neuronal networks or neurons are cultured in 3D instead of 2D formats; they tend to provide much better information [5]. Another limitation of using CBBs is receptor desensitization, which can occur during repetitive analysis. Loss of receptor response is a general problem, regardless of whether the biosensor uses the receptor on the intact living cells or on an artificial support [160, 161].

Another drawback of CBBs is lack of stability and prolonged shelf-life, which are considered crucial properties needed for field application. Long-term cryogenic storage of the cells in liquid nitrogen traditionally reduces the viability, and application of dimethyl sulfoxide (DMSO) as a cryo-protectant might also reduce the number of viable cells [162, 163]. Prolonged storage of the mammalian cells in cultured condition requires extensive facilities such as carbon-dioxide, controlled temperature, and humidity which may hinder in-field application of CBBs. Therefore, to overcome these obstacles, efforts are being made to maximize onsite deployability of CBBs by encapsulating cells in 3D protective materials such as collagen [3]. In addition, field-deployable platforms are being made using cell-support components to prolong the self-life of sensor [164].

One of the most desirable goals is to make the sensors affordable to the users. Since mammalian cell culture is expensive, it is imperative to develop low cost CBBs. Therefore, the development of biosensor platforms utilizing bioluminescence or fluorescence-based detection is possible using a simple, hand-held luminometer or spectrofluorometer.

6 Concluding Remarks and Future of CBBs

As is the case with any technology, biosensing using cell-based approaches is presently undergoing developmental phases. There are limitations (discussed in Sect. 5), but concerted efforts are being made to overcome such limitations to make CBBs available for onsite use and for widespread applications. The power of cellular receptors or signaling pathways has been integrated with analyte-specific antibodies on surface of mammalian cells to render specificity [135]. Application of protease inhibitors [165] and cell-cycle inhibitors [166] in growth medium or cells grown under modified growth conditions [17] have shown to extend shelf-life. These strategies may prove to be encouraging in future development in cell-based sensor for onsite application. With the advent of laser scanning imaging devices [3], micro and nano electronics [167], and fluorescence probes, a new paradigm of

cell-based sensing is definitely emerging. The capacity of CBBs to provide useful information concerning physiological responses to a variety of potentially biohazardous analytes coupled with innovative advances in detection platforms substantiates them as the leaders in the next generation of functional biosensing.

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