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Cell-based biosensors: Recent trends, challenges and future perspectives

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ABSTRACT

Existing at the interface of biology and electronics, living cells have been in use as biorecognition elements (bioreceptors) in biosensors since the early 1970s. They are an interesting choice of bioreceptors as they allow flexibility in determining the sensing strategy, are cheaper than purified enzymes and antibodies and make the fabrication relatively simple and cost-effective. And with advances in the field of synthetic biology, microfluidics and lithography, many exciting developments have been made in the design of cell-based biosensors in the last about five years. 3D cell culture systems integrated with electrodes are now providing new insights into disease pathogenesis and physiology, while cardiomyocyte-integrated microelectrode array (MEA) technology is set to be standardized for the assessment of drug-induced cardiac toxicity. From cell microarrays for high-throughput applications to plasmonic devices for anti-microbial susceptibility testing and advent of microbial fuel cell biosensors, cell-based biosensors have evolved from being mere tools for detection of specific analytes to multi-parametric devices for real time monitoring and assessment. However, despite these advancements, challenges such as regeneration and storage life, heterogeneity in cell populations, high interference and high costs due to accessory instrumentation need to be addressed before the full potential of cell-based biosensors can be realized at a larger scale. This review summarizes results of the studies that have been conducted in the last five years toward the fabrication of cell-based biosensors for different applications with a comprehensive discussion on the challenges, future trends, and potential inputs needed for improving them.

1. Introduction

The advent of point-of-care and bench-to-bedside devices in recent years has made biosensors a familiar name to the scientific community. Research in biosensors has gained a major impetus in the last 5 decades owing to their applications in many fields of study such as environmental monitoring (Cai et al., 2018), clinical diagnosis (Jiang et al., 2015; Pandey et al., 2018), food safety (Jiang et al., 2015; Zou et al., 2016), geo-exploration (Zammit et al., 2013), etc. It is the flexibility in design of biosensors that has allowed the emergence of such a diverse array of applications. The functional strategy of a biosensing device is, to a large extent, dependent upon the type of biorecognition molecule (bioreceptor) chosen for the detection of a target analyte (Kumar et al., 2017; Singh et al., 2012). Biosensing parameters, such as response time, sensitivity, and specificity can be determined by the type of bioreceptor used in a biosensor. Conventionally, biomolecules such as enzymes, antibodies, DNA, etc. have been utilized as bioreceptors in biosensing due to their high specificity to target molecules (Kumar et al., 2017; Pandey et al., 2017; Singh et al., 2012). However, living cells, with their vast array of biomolecular mechanisms, offer an interesting alternative to these molecular bioreceptors.

Living cells express a wide variety of molecules (receptors) in different proportions, hence cells can not only yield quantitative response to specific stimuli in a given condition but they can also help in quantitatively analyzing more than one analyte while requiring less effort and expenses. This was the primary motivation behind utilization of cells as bioreceptors in the earlier days (Corcoran and Rechnitz, 1985). While using cells as bioreceptors, the enzymes and other molecules required for biosensing are present in their native environment and hence display optimal activity and specificity against the target analyte. Thus, cells offer ease in designing the functional strategy of biosensors in a way that was not possible previously with molecule-based biosensors. In addition, cell-based biosensors enable analysis and monitoring of drug-ligand interactions, effect of bioactive agents,

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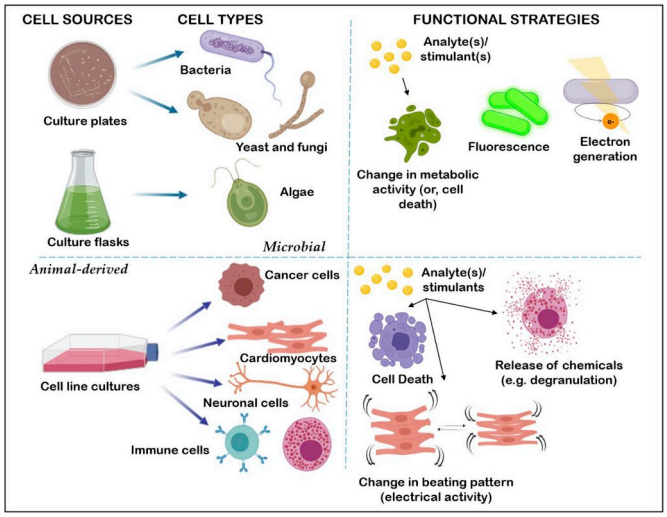

Fig. 1. Schematic showing the different cell types (left) and functional strategies (right) utilized in cell-based biosensors.

environmental toxicity studies, etc. in a more physiologically relevant way (Cevenini et al., 2016; Liu et al., 2014b; Pan et al., 2015). With the use of suitable substrates, it is possible now to use cell-based biosensors for *in situ* (*ex vivo* in some cases) monitoring with living cells in their native environment.

Different cell types such as bacteria, yeast, fungi, algae, and other higher eukaryotes including fish, rat and human cells have been utilized for biosensor fabrication (Brennan et al., 2016; Liu et al., 2014b; Su et al., 2011) (Fig. 1). Microbial cells, including bacteria, fungi, yeast and algae, have largely been utilized in biosensors for water quality monitoring and toxicity assessment (Gao et al., 2017; Vopálenská et al., 2015; Yang et al., 2016). On the other hand, higher eukaryotic cells have prominent applications in the study of basic cellular functions and disease pathogenesis. Furthermore, living cells in a biosensor are coupled to external transducers in order to yield a quantified and readable signal. The choice of such transduction mechanisms is often dependent upon functional strategy and the types of cells utilized for biosensing. The electrochemical and optical readouts are the most commonly used methods in most of the transducers employed for microbial biosensors. In contrast, for higher eukaryotic cells more advanced techniques such as electrical cell-substrate impedance sensing (ECIS), light addressable potentiometric sensor (LAPS), etc. are the preferred transduction/detection methods (Liu et al., 2014c). However, fluorescent imaging and common electrochemical methods such as amperometry have also been utilized for development of some of these biosensors (Cevenini et al., 2016; Jiang et al., 2015).

Lying at the intersection of different scientific disciplines, the field of cell-based biosensors has recently been witnessing exciting new developments. Advances in synthetic biology have enabled precise manipulation of genetic circuitry thus paving the way to bacterial cells operating on Boolean logic for quantitative detection of multiple analytes (Courbet et al., 2015). Study of bacterial motion at nanometer scale, analysis at single-cell resolution and online monitoring are some of the concepts that have been made possible because of advances in the fields of microfluidics, lithography and material sciences (Pasternak et al., 2017; Shukla et al., 2018; Syal et al., 2015; Tucci et al., 2019). Thus, from being mere electrodes attached to immobilized cells, cell-based biosensors have evolved into powerful bioelectronic systems that can not only detect specific analytes but can also provide physiologically relevant functional information even at the single cell level. Some of these developments have attempted to address the prevailing challenges for cell-based biosensors like regeneration, heterogeneity in the cell population and high interference. However, considerable efforts are still required for addressing the issues such as degradability and insulating nature of cell culture matrices, genetic instability in cell lines (particularly cancerous cell lines), leaky genetic 'switches', lack of broad range genetic constructs, and high costs of instrumentation, etc. There is also the need of streamlined efforts towards devising an internationally accepted set of standardized protocols for utilizing and validating a particular cell type or population for a particular application. Thus, more efforts are required towards unlocking the vast potential of cell-based biosensors, which may, in the future, play a significant role in shaping the field of health care diagnostics and physiological monitoring systems.

This review summarizes the results of various investigations reported in literature in the last about five years on the development of cell-based biosensors for different applications. The need of immobilization and different techniques utilized for this purpose are discussed. Thereafter, the review has been divided into microbial and animal cell-based biosensors owing to the difference in the approach towards both types of biosensors. For each of the two sections, the types of transducers used have been briefly discussed along with a critical analysis of different studies conducted on cell-based biosensor development based on their applications. Finally, some of the recent trends and future prospects of cell-based biosensors have been discussed with focus on addressing the challenges faced by current biosensing technologies.

2. Immobilization strategies

2.1. Is immobilization really needed?

Living cells consist of a number of sub-cellular organelles, macromolecules and supramolecular assemblies that function in a well-coordinated manner under tight genetic control to bring about various life-sustaining metabolic and self-defensive activities. In other words, living cells can be seen as consisting of a number of molecular sensor arrays that can detect a wide variety of stimuli in and around their environment and, in return, generate a number of different responses via energy transduction (Wang et al., 2013a). Thus, by definition, a living cell can, in itself, be termed as a 'biosensor'. This simple fact affirms that living cells can, in themselves, be utilized as biosensors against specific target analytes.

Many biosensors have been developed over the years that utilize microbial cells in suspension as biosensors or 'bioreporters,' as they are commonly referred as, for environmental monitoring (Amaro et al., 2014; Teo and Wong, 2014; Wang et al. 2013a, 2013d). Such 'biosensors' have also found other applications towards detection of explosives (Kabessa et al., 2016), detection of pathological biomarkers (Courbet et al., 2015), geochemical exploration (Zammit et al., 2013), screening of genetic variants and molecular libraries for industrially-relevant enzymes and other reagents (Mustafi et al., 2014; Rogers et al., 2016; Sana et al., 2017; Siedler et al. 2013, 2017), cell culture monitoring (Goers et al., 2017) and determination of antimicrobial activity (Chan et al., 2013). In most cases, the genetic circuitry of the cells is modified such that exposure to the target analyte leads to the expression of a 'reporter' gene, which is often located downstream to a specific gene promoter against the target analyte (Chan et al., 2013). The products of such genes are often fluorescent or luminescent proteins (e.g., green fluorescent protein, yellow fluorescent protein, luciferase, etc.) (Kabessa et al., 2016; Roggo and van der Meer, 2017; Sana et al., 2017; Siedler et al., 2013; Wang et al., 2013a) and/or enzyme-mediated production of colored or electrochemically active compounds (Zammit et al., 2013) (Fig. 2). Such genetically-encoded biosensors have been reported in mammalian cell lines mainly for applications pertaining to pathogen detection (Jiang et al., 2016), live-cell imaging (Chereddy et al., 2015; Hanna et al., 2014; Krebs et al., 2012), cell signaling pathways (Warren et al., 2015; Willoughby and Cooper, 2008), and protein-protein interactions (Mo et al., 2017) in their native

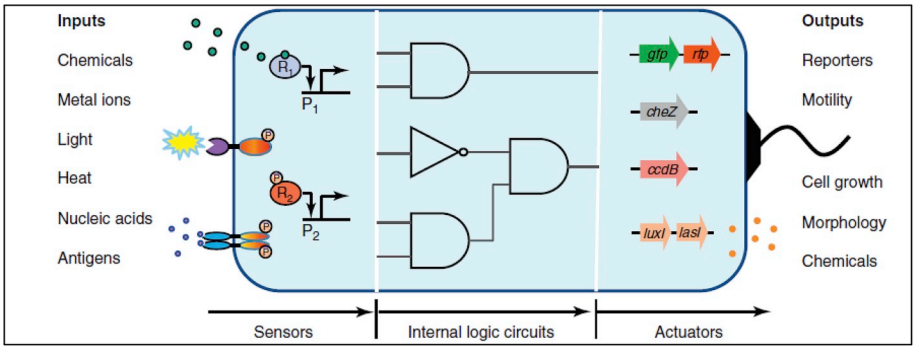

Fig. 2. Design of a genetically engineered synthetic 'bioreporting' cell-based biosensor consisting of the input sensors, the internal genetic circuits designed as logic gates and the output 'actuators' yielding specific responses. Reproduced with permission (Wang et al., 2013a), Elsevier Inc.

environment.

However, these studies do not entirely obviate the need for immobilization of cells on a transducing/detecting platform. It has been shown that immobilization enhances performance parameters of biosensors, including response time, better correlation to target concentration and stability of the response with respect to time (Asif et al., 2016; Yoetz-Kopelman et al. 2016, 2017). Moreover, immobilization or 'adherence' of cells directly onto transducer/detecting electrode arrays instead of passive culture flasks may enable the measurement of many cellular parameters in a precise and quantifiable manner (Yoetz-Kopelman et al., 2016). Hence, in this review we have focused on those cell-based biosensors wherein living cells (genetically modified or otherwise) have been immobilized and/or integrated with transducer/detector substrates for detection/analysis of specific target analytes/cellular responses and other parameters.

2.2. Types of immobilization

2.2.1. Passive immobilization

Several types of immobilization strategies have been explored for attachment of living cells onto transducer/detecting platforms (Fig. 3). In general, the factors that help in determining a particular immobilization strategy for a biosensor include: 1) the cell types used as bioreceptors, 2) the type of material/substrate onto which cells are to be immobilized, 3) toxicity of the substrate, 4) cell viability and growth requirements, and 5) the application of the biosensor. For example, applications such as monitoring of biochemical oxygen demand (BOD) and cell migration rely on the viability and metabolism of the cells used (Reshetilov et al., 2013), hence it is imperative that immobilization should not hinder the cell growth or viability.

The functional strategy of a biosensor is also an important determinant of immobilization method. For example, bioluminescence-mediated detection of target analytes (e.g. heavy metals) necessitates the microbial cells to be metabolically active and expressing the bioluminescent gene in a stable manner. Thus, such biosensors often involve immobilization strategies that do not affect cell viability, such as physical adsorption (Fang et al., 2016; Yamashita et al., 2016), hydrogel/sol gel entrapment/encapsulation (Axelrod et al., 2016; Gao et al. 2016b, 2017) and biofilm formation (Catal et al., 2018; Chouler et al., 2018; Webster et al., 2014). Since such techniques rely on the cells' natural ability to adhere to surfaces, they are collectively referred to as passive immobilization techniques. Bioelectrochemical biosensors, particularly microbial fuel cells (MFCs) rely mostly on biofilm formation for applications such as water toxicity determination, biochemical oxygen demand (BOD) monitoring (Chouler et al., 2018; Xu et al., 2016b; Yang et al., 2016; Yi et al., 2019), antibiotic monitoring (Catal

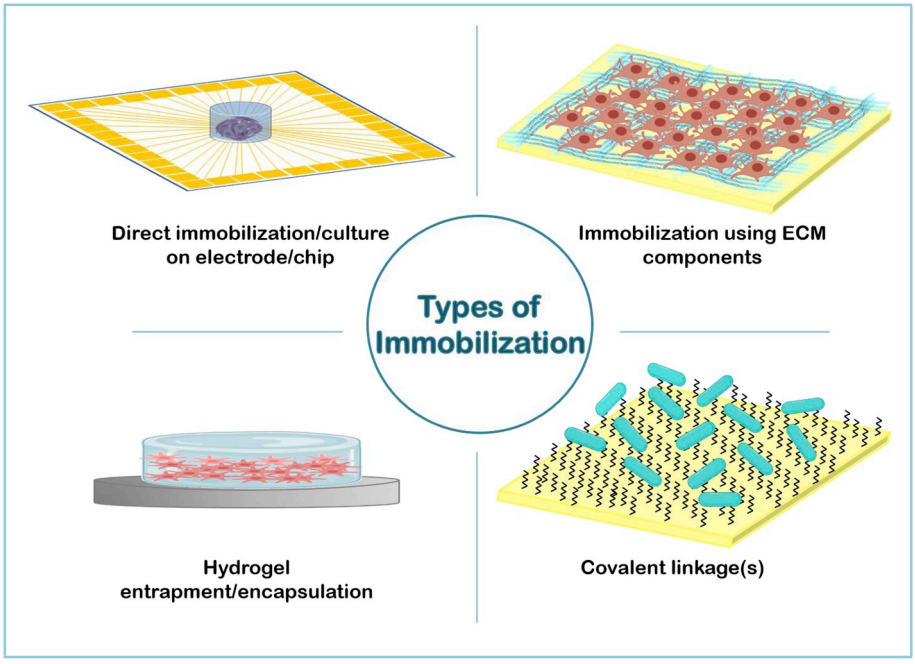

Fig. 3. Some of the prevalent methods used for immobilization of cells.

et al., 2018), bioprocess monitoring (Kaur et al., 2014; Kretzschmar et al., 2017), etc. Additional advantage of utilizing physical adsorption and biofilm formation is that the cells remain in direct contact with the transducing surface, thus enhancing the sensitivity. Conventional passive supports such as cellulose nitrate and polycarbonate films have also been utilized in the recent years for efficient immobilization (Chee, 2016; Qiao et al., 2015; Wei et al., 2017). Other studies have reported on the utilization of packed bed reactors and silicone beads for biofilm formation and adsorption of different green algae species (Haigh-Flórez et al., 2014; Tahirbegi et al., 2017).

Entrapment in sol gel/hydrogel is also a prominent cell immobilization technique. Some of the most commonly used gel matrices are calcium alginate (or, alginate-starch), agarose and chitosan. In addition to maintaining viability, such matrices provide a 3D tissue-like environment to cells for growth and proliferation, which is crucial for applications related to monitoring physiologically relevant parameters such as proliferation rate, cell migration, adhesion and chemosensitivity. Even though these matrices provide strong inert support to mimic tissue like structure, they often do not participate in signal transduction and may lead to signal loss or noise due to leaching and diffusion of cells out of the gel. Uniformity of biofilms and stability in terms of shelf life are other significant issues that limit the utility of passive immobilization techniques. Efforts have been made to incorporate conductive materials (e.g., graphene oxide, carbon nanodots, etc.) into hydrogels to enhance signal transduction, particularly in electrochemical biosensors (Fang et al., 2016; Jiang et al., 2017a). Nanomaterials have emerged as promising candidates for cell immobilization. Magnetic Fe_3O_4 nanoparticles have been employed for labeling *Bacillus subtilis* cells electrostatically and subsequently immobilizing them on ultramicroelectrode array (UMEA) using external magnetic field (Wang et al., 2017). This modified UMEA was subsequently utilized as a BOD microsensor and shown to be regenerable and reproducible with better sensitivity than dissolved oxygen (DO)-based and mediated BOD sensors. Another study has been reported on the immobilization of *E. coli* cells on nanoporous gold for selective detection of sulfide (Liu et al., 2017). Silicon-based nanostructures have been utilized for both immobilization of breast cancer cells and improving sensitivity of the biosensor in terms of monitoring drug effects at very low concentrations (2 nM) (Shashaani et al., 2016; Zanganeh et al., 2016). Thus, nanomaterials have shown potential as robust substrates that serve the dual purpose of cell immobilization and signal enhancement. However, the toxicity of certain nanomaterials to microbial cells and high costs of synthesis presently limit their widespread use in cell-based biosensors.

2.2.2. Active immobilization

Active immobilization techniques are an efficient alternative to passive immobilization since these techniques involve the utilization of chemical linkers or other agents for immobilization of living cells onto a suitable substrate, e.g. glutaraldehyde crosslinking. These methods allow tighter binding of the cells to transducer surface leading to enhanced sensitivity. A recent study by Mishra et al. (2017) highlights the significance of covalent (or active) immobilization of cells (*Sphingomonas* sp.) for improving the stability and sensitivity of an optical biosensor against methyl parathion. Biomolecules that interact directly with receptors and other moieties present on the cell surface have been used to immobilize bacterial cells on various substrates. Leonard et al. (2017) developed an antimicrobial susceptibility testing (AST) platform by immobilizing *E. coli* cells on Si micropillar arrays via wheat germ agglutinin (WGA). Bacteria-specific antibodies have also been utilized for efficient immobilization on plastic and glass surfaces (Safavieh et al., 2017; Syal et al. 2015, 2017).

Some immobilization strategies are unique for higher eukaryotic cells, such as utilization of the extracellular matrix (ECM, a semi-solid network of various proteins and fluids to which cells are bound in a multicellular organism) and its components for immobilization. It has been reported that surface modification by ECM components enables uniform immobilization of cells while maintaining their viability, which is a crucial factor in many biosensing applications (Liu et al., 2014c). Collagen, fibronectin and laminin together with peptides such as poly-L-lysine have been predominantly used for the immobilization of various cell types including mouse germ cells, taste receptor cells, myocytes and MCF-7 breast cancer cells (Anh-Nguyen et al., 2016; Chowdhury et al., 2018; Hu et al. 2016, 2017). Recently, Lee et al. (2018) demonstrated a 'bio-artificial tongue' by culturing neonatal mouse taste cells in a 3D matrix constituted by decellularized tongue ECM (TEM). They argued that the utilization of this TEM-based platform enabled the simulation of signal transmission between taste cells and adjacent neuronal cells and thus paved the way for *in vitro* modeling of taste sensing and related mechanisms. Another study utilized the adhesion of NIH/3T3 fibroblasts on natural ECM components such as vitronectin and laminin for analysis of adhesion properties of different biomaterials (Ivanova et al., 2017). Thus, ECM components have not only proven to be a powerful approach toward immobilization of viable cells on rigid, hostile surfaces, but they can also be helpful in development of electronics-integrated functional tissue models. Such systems may prove useful in regenerative medicine where real time monitoring of growth and functionality of the regenerating tissues can be crucial.

Some applications, however, require complete absence of an intermediate immobilizing layer. Li et al. (2015) studied the thermodynamics and binding kinetics of protein-carbohydrate interactions at cancer cell surface by culturing unfixed adenocarcinoma cells on a polystyrene-coated QCM chip. In this context, microfluidics technology has demanded special attention. The integration of microfluidics with biological sciences has led to the development of specialized microfluidic chips particularly designed for the culture of living cells in micro-sized wells, thus enabling growth and proliferation of cells (Popova et al., 2016). The use of microfluidic culture chips has, to a large extent, obviated the need for specialized immobilization techniques. Evidently, many recent studies have demonstrated the culture and growth of different microbial and animal cells in microfluidic chips, including algae, bacterial cells, and cancer cells. These chips have been integrated with different types of transducers to capture the cells' response to specific bioactive analytes and parameters. This has further led to the inception of cell microarrays for high throughput molecular analyses (Weingeist et al., 2013; Wu et al., 2014). The powerful concept of lab-on-chip has also emerged for analysis and monitoring of various cellular parameters, with some platforms achieving single cell resolution (Shukla et al., 2018; Wang et al., 2018). Recent years have seen an increased focus on the development of 'soft' substrates and conductive hydrogels that serve as both immobilization matrices and conductive electrodes for transduction. 3D bioprinting has emerged as a powerful tool for micropatterning of cells on different surfaces in the desired alignment and orientation. Furthermore, the relatively newer technique of 4D printing, with its powerful dynamic approach, may bring new functional roles to cell-based biosensors. These developments are discussed in Section 5.

3. Microbial biosensors

Many different types of microorganisms have been utilized for biosensor fabrication over the years, including bacteria, photosynthetic algae (Tahirbegi et al., 2017; Tsopela et al. 2014, 2016) and yeast cells (Pham et al. 2013, 2015; Vopálenská et al., 2015) with prominent applications in environmental monitoring and antimicrobial susceptibility testing. Some of these biosensors exploit the natural ability of a particular microorganism to sense a specific analyte while several others are dependent on the introduction of specifically designed genetic constructs in a host microorganism for detection of bioavailable analytes.

Different types of transducing/detecting systems have been utilized in microbial biosensors, electrochemical and optical being the most

Table 1
Electrochemical microbial biosensors for environmental monitoring.

Target Analyte(s)	Microorganism(s) used	Immobilization Method	Transducer/detector	Linear range (Sensitivity, LOD[a])	Reference
Water toxicity (DCP)	*Shewanella oneidensis* MR-1	–	Bioelectrochemical	–	Yang et al. (2018)
Wastewater toxicity (Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, 3,5-dichlorophenol, 4-chlorophenol, phenol)	*Saccharomyces cerevisiae* S288C	Physical adsorption on boron-doped nanodiamond (BND)-chitosan hydrogel polymer on glassy carbon electrode (GCE)	Electrochemical (chronoamperometry) with mediators: menadione and ferricyanide	–	Gao et al. (2017)
Biochemical oxygen demand (BOD)	Activated sludge	Entrapment in chitosan-bovine serum albumin (Chi-BSA) cryogel with graphene	Electrochemical with mediator: methylene blue	1.0–100 mg O_2 L^{-1} (LOD: 0.1 mg O_2 L^{-1} at flow rate of 100 μL min^{-1})	Niyomdecha et al. (2017)
BOD	*Bacillus subtilis*	Labeled with Fe_3O_4 nanoparticles (external magnetic field-mediated immobilization)	Electrochemical (chronoamperometry)	2–15 mg L^{-1} (59.8 nA mm^{-2}/mg L^{-1}, 0.8 mg L^{-1})	Wang et al. (2017)
Wastewater toxicity (Heavy metals: Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+})	*Escherichia coli* ATCC 25922 (cell surface modified with thionine via electrostatic interactions)	Physical adsorption on chitosan-carbon nanodot modified GCE	Electrochemical	–	Fang et al. (2016)
Wastewater toxicity (Cu^{2+}, Cd^{2+}, DCP, pesticides: Ametryn, Acephate)	*E. coli* ATCC 25922, *Bacillus subtilis* CGMCC 1.1086, *S. cerevisiae* S288C	Entrapment in calcium alginate on a polyethylene terephthalate (PET) sheet	Electrochemical (chronoamperometry)	–	Gao et al. (2016b)
Biochemical oxygen demand (BOD)	Mixed culture (including *Geobacter*)	Biofilm formation	Bioelectrochemical	14–100 mg L^{-1}	Yamashita et al. (2016)
BOD	*Chromobacterium violaceum* R1	Entrapment in calcium alginate (enclosure with polyamide membrane)	Electrochemical (chronoamperometry) with mediator: ferricyanide	20–225 mg O_2 L^{-1} for standard glucose-glutamic acid (GGA) solution	Khor et al. (2015)
Arsenic (As^{3+})	*S. oneidensis* (transformed with plasmid-encoded encoded *mtrB* integrated with arsenic-inducible promoter)	Biofilm formation	Bioelectrochemical	LOD: 40 μM	Webster et al. (2014)
Trichloroethylene	*Pseudomonas* sp. ASA86	Cellulose nitrate membrane	Electrochemical (chloride ion electrode)	0.05–4 mg L^{-1} (LOD: 0.05 mg L^{-1})	Chee (2016)
Sulfide	*E. coli* BL21 (overexpressed Sulfide:quinoneoxido reductase (SQR))	Nanoporous gold (NPG)	Voltammetry	50 μM–5 mM (18.35 μA mM^{-1} cm^{-2}, 2.55 μM)	Liu et al. (2017)
Hydrogen sulfide (H_2S)	*Thiobacillus thioparus*	Alginate and agarose bed	Titrimetric	LOD: 0.5 ppm	Vosoughi et al. (2015)
Herbicide (Atrazine and diuron)	*Anabaena variabilis*	Entrapment in alginate	Electrochemical (amperometry) with mediator p-benzoquinone	Sensitivity: −7.7 μA $μM^{-1}$ cm^{-2}, LOD: 64 nM (atrazine)	Tucci et al. (2019)
Herbicide (Diuron)	*Chlamydomonas reinhardtii* (green algae)	Culture in electrochemical lab-on-chip microfluidic device	Electrochemical (Oxygen electrode)	0–1 μM (0.48 nA s^{-1} $μM^{-1}$, 0.1 μM)	Tsopela et al. (2016)
Herbicide (Diuron)	*Chlamydomonas reinhardtii*	Tungsten/Tungsten oxide (Ti/TiO_2) based ultramicroelectrodes in algal suspension	Electrochemical (Chronoamperometry)	LOD: 0.2 μM	Tsopela et al. (2014)
Pharmaceuticals (Omeprazole, lansoprazole, naphthoflavone and methylcholanthrene)	*Arxula adeninivorans* G1212/YRC102-hAhR-hARNT-phyK (genetically transformed)	–	Electrochemical (amperometry)	–	Pham et al. (2015)

[a] Limit of detection.

Table 2
Optical biosensors for environmental monitoring.

Target Analyte(s)	Microorganism(s) used	Immobilization Method	Genetic Modification(s)	Transduction mechanism	Linear range (Sensitivity, LOD[a])	Reference
BOD	*Saccharomyces cerevisiae*	– (sequential injection analysis)	–	Chemiluminescence with mediators: ferricyanide and quinone	10–315 mg L^{-1} for standard GGA solution (LOD: 9.53 mg L^{-1})	Costa et al. (2018)
Water toxicity (mercury, formaldehyde and ammonium hydroxide)	*E. coli* TV1061	Calcium alginate matrix	*lux*CDABE integrated with heat-shock *grpE* promoter	Bioluminescence	–	Axelrod et al. (2016)
Water contaminants (heavy metals, endocrine destructive compounds and organic solvents)	*E. coli* DPD2794, DPD2544 and TV1061	Calcium alginate matrix (entrapment)	*recA* (DPD2794), *fabA* (DPD2544) and *grpE* (TV1061) promoters with *luxCDABE* reporter operon	Bioluminescence	–	Eltzov et al. (2015)
Water quality (DNA damaging agents: Nalidixic acid, oxidative stress agents: paraquat, Cd^{2+}, hydroquinone)	*E. coli* (different strains)	Porous aluminum oxide (PAO) culture chips in a flow cell	Several modifications for fluorescence (GFP) and/or bioluminescence (luciferase system)	Fluorescence and/or Bioluminescence	0–200 mg L^{-1}	Yagur-Kroll et al. (2015)
Lead (Pb^{2+})	*E. coli* DH5α	Microbial culture in a microfluidic device	Plasmid pT7cadO1945 that expresses GFP on exposure to Pb^{2+}	Fluorescence	–	Bae et al. (2018)
Arsenic (As^{3+}), Cadmium (Cd^{2+}), Lead (Pb^{2+}), Zinc (Zn^{2+})	*E. coli*	Microbial culture in a microfluidic device	CadC-controlled T7 RNA polymerase circuits	Fluorescence	–	Kim et al. (2016)
Lead (Pb^{2+}), Cadmium (Cd^{2+})	*E. coli* DH5α	Microbial culture in a microfluidic device	Plasmids pHK194 and pHK200 expressing GFP	Fluorescence	–	Kim et al. (2015)
Copper (Cu^{2+})	*S. cerevisiae*	Entrapment in alginate beads	Modification of AMP pathway (purine synthesis)	Colorimetric	1–100 μM (LOD: 1 μM)	Vopálenská et al. (2015)
Methyl parathion	*Sphingomonas* sp. JK1	Adsorption on PEI-functionalized silica beads followed by immobilization on optical plates via glutaraldehyde crosslinking	–	Optical	0.1–1 ppm	Mishra et al. (2017)
Pesticides (Diuron, Simazine, Atrazine)	*Chlamydomonas reinhardtii* (green algae)	Cultured biofilm in a packed bed reactor microfluidic device	–	Fluorescence	–	Tahirbegi et al. (2017)
Herbicide (Simazine)	*Dictyosphaerium chlorelloides* strain Dc1M	Adsorption on ImmobaSil™ porous silicone disks	–	Fiber optics (luminescent O_2 transducer)	50–800 μg L^{-1}; (LOD: 12.2 μg L^{-1}; LOQ: 40.8 μg L^{-1})	Haigh-Flórez et al. (2014)
Endocrine-disrupting agents (17β-estradiol (E2), 17α-ethynylestradiol (EE2), diethylstilbestrol (DES) and estrone (E1))	*S. cerevisiae*	Entrapment in alginate gel supplemented with 10% trehalose	yeast codon-optimized variant of NanoLuc luciferase (yNLucP) under the regulation of human estrogen receptor α activation	Bioluminescence	LOD: 0.08 ± 0.02 nM (E2)	Cevenini et al. (2018)

[a] Limit of detection.

prominent. Electrochemical readouts exploit the activation/production of redox active species by the microbes for biosensing (Su et al., 2011). In some cases, redox-active mediators may be externally supplemented to improve electron transfer across the electrode. Thus, genetic modification is not always required in electrochemical sensing (Table 1). However, electrochemical systems are often vulnerable to high noise and background due to interfering species. Optical readouts, on the other hand, rely heavily on genetic modification for the microbes to produce a visual/fluorescent response against a specific stimulus (Table 2). Most genetic modifications involve the introduction of an analyte-specific promoter upstream to a 'bioreporter' gene in the host microbial cells. Upon exposure to the target, the promoter gets activated and the bioreporter gene expresses fluorescent proteins. Thus, optical biosensors are generally more specific than electrochemical ones, however they require a number of additional steps involving gene modification and hence, increase the cost of the device considerably. Moreover, the biosensor response in optical systems is largely dependent on expression of the fluorescent protein which may vary considerably for different batches of cell populations, thus causing variability in the sensitivity every time the cell population changes. And long-term stable expression within a cell population is another big feat to achieve.

3.1. Water toxicity/quality monitoring

Different microbial sensors have been developed for the detection of various environmental contaminants and physicochemical parameters including biochemical oxygen demand (BOD), heavy metals (Axelrod et al., 2016; Bereza-Malcolm et al. 2017, 2018; Cortés-Salazar et al., 2013), plasticizers (Bohrn et al., 2013), toxic gases (Eltzov et al., 2015; Lu et al., 2015), pharmaceuticals (Bayram and Akyilmaz, 2016), and endocrine-disrupting agents (Cevenini et al., 2018).

BOD is one of the most commonly used parameters to determine water quality in terms of total biodegradable organic content in water bodies (Jouanneau et al., 2014; Reshetilov et al., 2013). The most common method to determine BOD requires incubation of water samples with activated sludge (mixture of microorganisms) in specialized chambers for five days, which limits its use for on-site and continuous monitoring. Thus, microbial biosensors have been considered to be excellent alternatives to this approach as they have short processing times, are more portable, and enable online monitoring of water samples. Research in microbial BOD sensors, in the last few years, has been largely focused on electrochemical mediator-type biosensors and self-powered microbial fuel cell (MFC)-based biosensors. Mediator-type biosensors, in contrast to conventional dissolved oxygen (DO)-electrode based systems, utilize redox-active chemicals in place of DO for electron transport from biocatalytic machinery of microorganisms to the electrode (Table 1). Such biosensing systems are potentially insensitive to pH changes, independent of the partial pressure of oxygen in the system, and have high current response (Reshetilov et al., 2013). Different mediators have recently been studied for use in BOD biosensing, such as methylene blue (MB) (Niyomdecha et al., 2017; Zaitseva et al., 2017), ferricyanide (Hu et al., 2015a; Khor et al., 2015), and ferrocene (Zaitseva et al., 2017). A recent study demonstrated that two-mediator systems, particularly ferrocene-methylene blue, respond better than one-mediator systems in terms of stability (43 days), sensitivity (detection limit of 2.5 mg O_2/dm^3) and assay time (10 min for one sample) for a *Debaryomyces hansenii*-based biosensor (Zaitseva et al., 2017). MB has been shown to be a better mediator in combination with graphene than ferricyanide in terms of reproducibility of an activated sludge-based BOD biosensor (Niyomdecha et al., 2017). Self-powered microbial fuel cells (MFCs) have also emerged as potential BOD monitoring devices (Abrevaya et al., 2015; Sun et al., 2015; Veerubhotla and Das, 2018) (Table 3). MFCs are a type of bioelectrochemical systems (BES) that utilize microbial biofilms growing anaerobically at an electrode surface (usually anode) to oxidize organic compounds (e.g., waste water) and produce electricity (Yamashita et al., 2016). Thus, the type of microbial cells utilized for formation of anodic biofilm greatly affects the sensitivity of MFC biosensors. Biosensors based on mixed cultures have been shown to yield wider response range and higher sensitivity (3.86 times) when compared to pure culture of *Shewanella loihica* PV-4, an electrogenic bacterial strain (Yi et al., 2018). This might be due to the higher bioactivity and substrate consumption rate of mixed culture. More studies are required to compare and validate the effect of different microbial strains on biosensor response and sensitivity. Recently, Khor et al. (2015) developed a rapid redox-mediated BOD biosensor by immobilizing *Chromobacterium violaceum* R1 cells on platinum ultramicroelectrodes (UMEs). This mediated biosensor was shown to be unresponsive to DO and displayed a linear range of 25–230 mg O_2 L^{-1} for synthetic wastewater. A similar linear response (8–240 mg L^{-1}) was obtained by a mediator-less MFC biosensor utilizing anaerobic sludge for formation of anodic biofilm (Hsieh and Chung, 2014). But the BOD values obtained for industrial wastewater by this biosensor did not correlate well with those obtained by standard BOD method. This study highlights a major limitation faced by MFC biosensors: non-correlation with standard BOD methods. Due to the dynamic nature of MFCs, many factors may influence their output and hence interfere with biosensing, such as pH and temperature, presence of heavy metals, presence of competing microbial cells, etc. Thus, real wastewater samples often need to be diluted many times before they can be analyzed by MFC biosensors. These issues pose a major roadblock to the development of online BOD monitoring devices as well. Though a recent study by Pasternak et al. (2017) has demonstrated a self-powered, autonomous and floating biosensor for online monitoring in off-grid locations. This device produced sound and visual cues when BOD changed upon addition of urine in water. In a proof-of-concept study, Di Lorenzo et al. (2014) demonstrated a small scale, 3D printed single-chambered MFC for monitoring of chemical oxygen demand (COD) in the range 3–164 ppm and a remarkable response time of 2.8 min. Efforts to increase the upper detection limit of BOD sensors have also been made by devising a hydraulically-linked MFC array (Spurr et al., 2018). A linear range of up to 720 mg L^{-1} was obtained using this device. Most MFC devices have been based on anaerobically-grown anodic biofilms, which restricts their use to deaerated and polluted samples only. A novel study by Yamashita et al. (2016) demonstrated an open-type membrane-less BES for *in situ* BOD monitoring in an aerated tank. More such studies are required to elucidate the full potential of MFC biosensors in monitoring the quality of natural water bodies and drinking water supplies. Development of optical biosensors for BOD determination has somewhat lagged behind the more popular electrochemical devices. However, Costa et al. (2018) have recently demonstrated a microfluidic chemiluminescence device utilizing reaction between luminol and oxygen produced by *S. cerevisiae* for automated BOD monitoring. This system enabled a 75-times reduction in reagent consumption and effluents production when compared to standard 5-day test.

Electrochemical toxicity biosensors, much like the BOD biosensors discussed previously, necessitate the presence of externally supplemented redox mediators for electron transfer from within the cell to the electrode surface. However, unlike BOD sensors, the mediators are often co-immobilized, internalized or labeled on the surface of microbial cells being used as bioreceptors (Fang et al., 2016; Gao et al. 2016b, 2017). In addition to redox mediators, carbon nanomaterials have been utilized to enhance sensitivity and electron transfer in biotoxicity sensors. Recently, Gao et al. (2017) utilized a double-mediator system co-immobilized with *S. cereviseae* cells on a chitosan hydrogel film with boron-doped nanocrystalline diamond (BND) particles for determination of biotoxicity of heavy metal ions (Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}) and phenolic compounds (3,5-dichlorophenol (DCP), 4-chlorophenol and phenol) in wastewater. In another study, *E. coli* cells were wrapped with redox mediator thionine and then subsequently immobilized with chitosan-entrapped carbon nanodots on a glassy carbon electrode (GCE)

Table 3
Microbial fuel cell (MFC)-based biosensors for various applications.

Application	Target Analyte(s)/Parameter(s)	Microorganism(s) used	Device configuration	Linear range (sensitivity, LOD)	Reference
Environmental Monitoring	Heavy metals (mining rock drainage)	Biofilms (source not mentioned)	Membrane less air-breathing cathode MFC	–	Adekunle et al. (2019)
	Toxicity (avermectins (AVM), ivermectin (IVM), tetracyclines, heavy metals)	Mixed culture (*Azospirillum*, *Acinetobacter*, *Pseudomonas*, *Ocillibacter* among others)	Dual-chambered separated by Nafion 117 PEM	–	Yi et al. (2019)
	Toxicity monitoring (Hg^{2+}, AVM, chlortetracycline (CTC) hydrochloride)	Enriched biofilms (source not mentioned)	Sequential flowing membrane-less device with bioanode and biocathode sensing elements	–	Zhao et al. (2019)
	Neomycin sulphate	Mixed bacterial culture from wastewater treatment plant	Mediator-less, single chambered with air-cathode; 4 devices placed in parallel	–	Catal et al. (2018)
	Toxicants in water (Formaldehyde)	Mixed culture from anaerobic sludge and wastewater	Paper-based device fabricated by screen printing of conductive ink	–	Chouler et al. (2018)
	Cu (II)	Microbes from domestic wastewater	Single chambered with air cathode separated with proton exchange membrane (PEM)	–	Tan et al. (2018)
	BOD, Biotoxicity (Cd^{2+})	*Shewanella loihica* PV-4, mixed culture	Dual-chambered separated by Nafion 117 PEM	0–65.25 mg L^{-1} (mixed culture) and 0–43.50 mg L^{-1} (*S. loihica*)	Yi et al. (2018)
	Carbon monoxide (CO)	Enriched exoelectrogenic microbes from wastewater	Dual chambered separated by cation exchange membrane (CEM) (carbon brush anode)	10–70% CO	Zhou et al. (2018)
	Water Toxicity (formaldehyde)	Mixed culture	Dual chambered separated by CEM (biocathode utilized as sensing element)	0.0005–0.005% (7.4 ± 2.0 to 67.5 ± 4.0 $mA\%^{-1}$ cm^{-2} (for biocathode as sensing element)	Jiang et al. (2017b)
	BOD, online water quality monitoring	Pre-cultured electroactive bacteria adapted to use urine as fuel	Single-chambered (four devices connected in parallel)	–	Pasternak et al. (2017)
	Wastewater toxicity (chromium, hypochlorite, acetate)	Mixed culture (wastewater)	Paper-based device fabricated by coating carbon ink on filter paper (multi-anode assembly) and PTFE-coated carbon cloth as cathode	–	Xu et al. (2016b)
	Water toxicity (formaldehyde)	*Shewanella oneidensis* MR-1	Single-chambered three electrode system with an air-bubble trap	0.001–0.1% (LOD: 0.001%)	Yang et al. (2016)
	Water toxicity (Cu (II))	Mixed culture from effluents of acetate-fed MFCs	Dual-chambered separated by CEM; three-electrode system (saturated calomel electrode (SCE) as reference) with flow through/flow by anode	–	Jiang et al. (2015b)
	BOD	*Thermincola carboxydiphila*, *Pseudomonas aeruginosa*, *Ochrobactrum intermedium*, *Shewanella frigidimarina*, *Citrobacter freundii*, *Clostridium acetobutylicum*	Dual-chambered separated by Nafion 117 CEM	8–240 mg L^{-1}	Hsieh and Chung (2014)
	Wastewater quality (Cr^{6+}, Fe^{3+}, nitrate, sodium acetate)	Mixed culture	Single-chambered batch-mode cube-shaped device (one face of cathode facing air)	–	Liu et al. (2014a)
	COD, Cd^{2+}	Mixed bacterial culture	Layer-by-layer 3D printed single chambered device with air-cathode	COD: 3–164 ppm (Sensitivity: 0.05 μA mM^{-1} cm^{-2}) Cd^{2+}: 1–25 μg L^{-1} (LOD: 1 μg L^{-1})	Di Lorenzo et al. (2014)
Clinical	Antibacterial effects of beta-lactam antibiotics (Antibiograms)	*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213	Microfabricated dual-chambered device separated by a Nafion N115 PEM	–	Schneider et al. (2015)

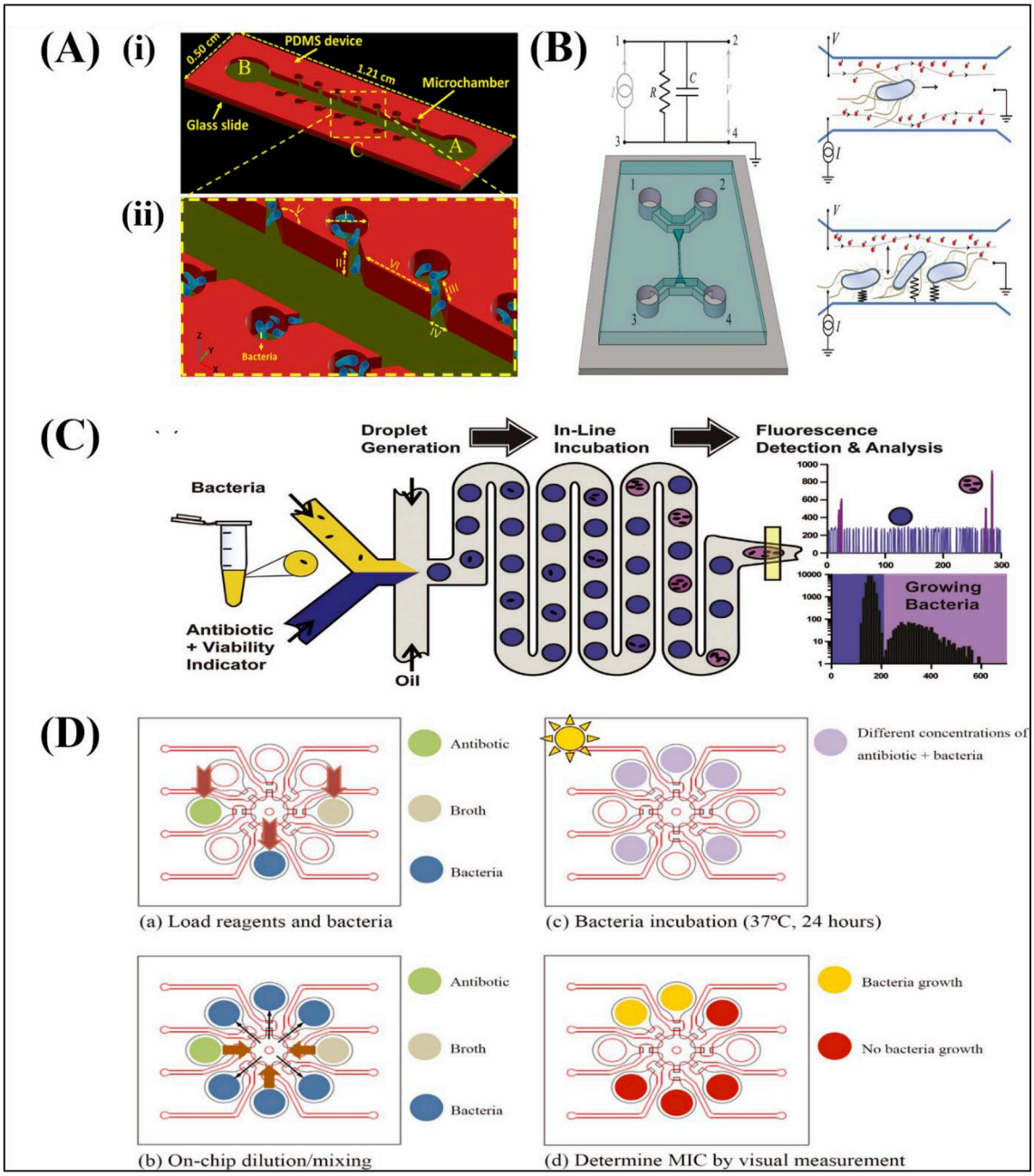

Fig. 4. Cell based biosensors for antimicrobial susceptibility testing. (A) (i) Schematic of nanoliter-sized microchamber/microarray-based microfluidic (N-3M) platform for rapid AST assay, and (ii) magnified version of AST testing zone. Reproduced with permission (Azizi et al., 2018), ACS Publications. (B) Schematic (lower left) and circuit diagram (upper left), and (ii) Detection principle (right) of microfluidic transducer chip for electrical monitoring nanomechanical motion of *E. coli* cells toward AST assay. Reproduced with permission (Kara et al., 2018), Royal Society of Chemistry. (C) Illustration depicting the working principle of the *drop*FAST microfluidic system for AST in picoliter droplet format. Reproduced with permission (Kaushik et al., 2017), Elsevier Inc. (D) Illustration of experimental procedure for AST using automated broth dilution method. Reproduced with permission (Lee et al., 2017), Elsevier Inc.

for the detection of heavy metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}) (Fang et al., 2016). In both the cases, the carbon nanomaterials led to increase in the conductivity of the bioelectrode, however no direct assessment was made to determine the effect on biosensor sensitivity. BES-based biosensors have emerged as potential biotoxicity assessment devices. A *Shewanella oneidensis*-based BES biosensor has been recently developed to determine the biotoxicity of 3,5-dichlorophenol (DCP) (Yang et al., 2018). The half maximal inhibitory concentration (IC_{50}) of DCP was found to be 14.5 mg L^{-1}, which is surprisingly similar to the value (17.1 mg L^{-1}) obtained by another electrochemical biosensor based on mixed microbial consortium (Gao et al., 2016b). In a different approach, Webster et al. (2014) demonstrated a BES biosensor based on genetically-encoded *S. oneidensis* cells for the detection of arsenite. The biosensor, designed to yield increased current upon exposure to arsenite, displayed a linear range of upto 100 μM with detection limit of 40 μM. Electrochemical biosensors utilizing genetically encoded bacterial and yeast cells for sensitive detection of pharmaceuticals and sulfide in wastewater have also been reported (Liu et al., 2017; Pham et al., 2015). In contrast to conventional electrochemical microbial sensors, the tolerance of microbial cells to toxic shock is higher in genetically encoded biosensors and the current output generally increases upon addition of the target analyte. However, the process of genetic modification is highly laborious, incurs higher costs and regeneration of the biosensor is problematic.

Online monitoring systems for heavy metals and other pollutants have gained special importance in the recent years. In this context, a membraneless, air-breathing cathode MFC biosensor has been reported for long term, real time monitoring of mining rock drainage-associated heavy metal toxicity (Adekunle et al., 2019). Another study reported on the development of an *Anabaena variabilis*-based biosensor for online monitoring of herbicides (Tucci et al., 2019). The biosensor displayed a sensitivity of $-24.6\,\mu A\,\mu M^{-1}\,cm^{-2}$ against atrazine up to 0.56 μM, which was enhanced to 131 μM upon addition of activated carbon. Unlike BOD sensors, many MFC-based biotoxicity sensors have aimed at utilizing cathode as the sensing element. The sensitivity of an MFC-based toxicity biosensor was shown to be greatly enhanced when biocathode was utilized as the sensing element in place of bioanode (Jiang et al., 2017b). This biosensor displayed a remarkable detection limit of 0.0005% for formaldehyde detection. In a recent study led by Zhao et al. (2019), both bioanode and biocathode were utilized as sensing elements for the determination of combined organic matter/toxic agent (OM/TA) shock. The inhibition ratios (IRs) found by this unique MFC biosensor was twice of that found by biocathode and bioanode alone. New concepts are being applied in MFC-based toxicity sensors with promising results. A multi-anode paper-based MFC toxicity biosensor has been devised that displayed shorter acclimation time and higher power output (0.33 mW) when compared to carbon-paper based MFC (0.06 mW in 3 h) (Xu et al., 2016b). The primary advantage of using paper devices is that simple folding of paper electrodes over each other may yield higher power output and sensitivity to toxic shock (Chouler et al., 2018; Xu et al., 2016b). However, such devices may not be suitable for long term (months-years) monitoring purposes.

Various microbial biosensors with optical readouts (bioluminescence or fluorescence) have recently been reported (Table 2). Most of these biosensors have either relied on luciferase-based luminescence or GFP fluorescence, which are induced by introduction of related genes in the host microbial cells. However, some recent studies have relied on the autofluorescence of bacterial and algal species for the detection of pesticides and herbicides (Haigh-Flórez et al., 2014; Mishra et al., 2017; Tahirbegi et al., 2017). Optical biosensors, though more specific than electrochemical biosensors in some respects, are highly laborious and incur high costs. There is also the need for common genetic constructs that can be inserted in a broad range of microbial species to yield the desired response.

3.2. Clinical applications

Antimicrobial susceptibility testing (AST) has emerged as one of the most significant clinical applications of microbial biosensors in the recent years owing to the rise of multidrug resistant 'superbugs.' Previous attempts towards fast AST platforms have mostly depended upon identification of resistance-associated genes (Leonard et al., 2018). Such platforms require additional DNA-processing steps, costly instrumentation and cannot be utilized for a broad range of microbial cells and resistance types. These limitations have further necessitated the development of cell-based AST platforms that are rapid and accurate in nature. In general, most cell-based AST platforms are based on phenotypical assessment such as bacterial motion, growth, electrical changes at cell surface, etc. Interestingly, microfluidics technology seems to have had a major impact on the development of such AST platforms (Fig. 4). Different studies have reported the fabrication of microfluidic platforms for assessment of the antimicrobial susceptibility in a number of different bacterial species towards various antibiotics (Azizi et al., 2018; Brosel-Oliu et al., 2019; Kara et al., 2018; Kaushik et al., 2017; Lee et al., 2017; Mohan et al., 2013). Azizi et al. (2018) demonstrated a nanoliter-sized microchamber/microarray-based microfluidic (N-3M) platform for rapid determination of minimum inhibitory concentration (MIC) of ampicillin, kanamycin, and gentamycin against wild-type clinical isolates of *E. coli*, *Klebsiella pneumonieae* and *Enterrococcus faecalis* (measured by resazurin reduction-mediated fluorescence). Similarly, Kaushik et al. (2017) fabricated a single-cell microfluidic biosensor, *drop*FAST, that utilized resazurin-based fluorescence to assess phenotypic growth and antimicrobial susceptibility (ASc) of single *E. coli* cell confined in 20 pL droplets. In a different approach, Kara et al. (2018) investigated the nanomechanical motion of bacterial cells by measuring voltage drop across microfluidic channels filled with electrolyte and correlated it with ASc. A microfluidic device that can automatically perform AST by broth dilution within 9 min has also been reported (Lee et al., 2017). New functional strategies are under investigation for sensitive ASc assessment. AFM cantilevers integrated to imaging (Stupar et al., 2017), direct use of microscopes to monitor motion of bacteria tethered to glass slides (sub-μm range) (Syal et al., 2017), and plasmonic imaging (nanometer range) (Syal et al., 2015) are just some of the functional strategies recently devised for ASc monitoring. However, the practical utility of such biosensors is under question as the techniques used herein are highly expensive and may require bulky instrumentation. Moreover, these studies have been performed only on *E. coli* as subject microorganisms. In a different approach, Reyes et al. (2017) evaluated the performance of a magnesium zinc oxide (MZO) nanostructure-modified QCM towards monitoring of antimicrobial action on *E. coli* and *S. cereviseae* cells. They found that the fabricated biosensor was 10 times more sensitive than regular QCM and highly accurate in determining drug effects. New advanced techniques have been introduced for AST. For example, Leonard et al. (2017) fabricated biofunctionalized silicon micropillar arrays for MIC determination by intrinsic phase-shift spectroscopy (PRISM assay). Flexible plastic microchips have been developed that can isolate *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) from whole blood and then determine ASc via electrical changes upon exposure to antibiotics (Safavieh et al., 2017). Thus, a variety of functional strategies are being adopted and developed for rapid and accurate AST. However, more studies are required to increase the range of microorganisms and antibiotics that can be tested in a cost-effective manner. In addition, a recent study has demonstrated that *in vitro* susceptibility patterns may not reflect the *in vivo* scenario within an infectious area (Jensen et al., 2019). Thus, caution needs to be taken while assessing the utility of the current biosensing technology for AST application.

Apart from AST, there have been few other studies on microbial sensors for detection of other clinically relevant analytes. A microbial paper-based device has been reported for the detection of gold ions in human urine (for monitoring gold accumulation in patients receiving gold-based therapeutics) (Guo et al., 2018). In this study, genetically modified *Cupriavidus metallidurans* CH34 cells were first exposed to different concentrations of gold ions (linear range: 0–750 nM) and then spotted onto a cellulose paper-based microplate for fluorescence analysis using a smartphone. Another study reported on the development of *Bacillus subtilis*-based amperometric biosensor for the detection of paracetamol, an over-the-counter analgesic and antipyretic agent (Bayram and Akyilmaz, 2016).

4. Animal cell-based biosensors

Animal cells (higher eukaryotes) differ from microbial cells in terms of their nutritional requirements, growth rate, and many morphological and structural characteristics. Thus, biosensors based on animal cells face altogether different types of design and fabrication challenges. These biosensors have important applications in the fields of food safety, disease modeling, and drug toxicity assessment.

Electrical cell-substrate impedance sensing (ECIS) and light-addressable potentiometric sensor (LAPS) are the two most prominently used transduction techniques for animal cell-based biosensors. ECIS, developed by Giaever and Keese (1993), utilizes gold interdigitated electrodes (IDEs) for monitoring of impedance (or, capacitance) with changes in cell density. For detailed reports on ECIS for whole cell biosensing, the readers are referred elsewhere (Banerjee and Bhunia,

Table 4
Animal cell-based biosensors for various applications.

Application	Target Parameter/Analyte(s)	Cell line(s) used	Immobilization Method	Transducer/detector	Reference
Cancer Research	Cell viability and proliferation	SGC-7901 (human gastric cancer)	Culture on sensor surface	Electrical 'wound healing' impedance	Wei et al. (2019)
	Cancer staging and pathology	MCF-7, MDA-MB-231 (human breast cancer)	–	Electrical cell-substrate impedance sensing (ECIS)	Wang et al. (2018)
	Effect of N-linked glycosylation on drug sensitivity	SKBR-3, MCF-7, BT474, ZR75–1 (human breast cancer)	Culture on polystyrene-coated sensor surface	Quartz crystal microbalance (QCM)	Peiris et al. (2017)
	Bioelectrical changes upon external electromagnetic (EM) stimulation	QUDB (malignant lung cancer)	Culturing on sensor surface (vertically aligned multiwall carbon nanotubes (VAMWCNT))	Impedance	Rafizadeh-Tafti et al. (2017)
	Cell attachment, spreading and drug-induced apoptosis	MCF-7	Fibronectin mediated attachment to microelectrode arrays (MEA)	Electric cell impedance spectroscopy (ECIS)	Anh-Nguyen et al. (2016)
	Cell proliferation, migration and drug-induced apoptosis	GFP-expressing MCF-7, human mesenchymal cells (hMSCs)	Encapsulation in alginate hydrogel and 3D culture; vertical insertion of electrodes	3D capacitance	Lee et al. (2016)
	Binding of trastuzumab to human epidermal growth factor 2 (HER2) receptor	SKOV3 (human ovary adenocarcinoma epithelial cancer)	Formaldehyde- and/or glutaraldehyde-mediated fixing on sensor surface	QCM	Elmlund et al. (2015)
	Quantification of cell colonies and chemosensitivity	Huh-7 (hepatoma)	Encapsulation in methyl cellulose hydrogel (separate loading on biosensor)	Impedance	Lei et al. (2015)
	Binding thermodynamics of carbohydrate-protein interactions on cancer cell surfaces	KM-12 (colon adenocarcinoma), SKOV3	Culture on polystyrene-coated chips	Quartz crystal microbalance (QCM)	Li et al. (2015)
	Cell proliferation and chemosensitivity	OEC-M1 (oral cancer)	Encapsulation in 3D agarose and culture in microfluidic chamber	Impedance	Lei et al. (2014)
	Cell metabolism	T98G (brain cancer)	Culture on sensor chip	Microphysiometer (Electrical)	Weltin et al. (2014)
	Biomarker characterization	32 cell lines of breast cancer including MCF-7 and MDA-MB-231	Cells spotted on microarray slides	Optical (Cell microarray)	Wu et al. (2014)
	Singer cancer cell migration in 3D matrices	MDA-MB-231, MCF-7	Fibronectin-mediated	ECIS	Nguyen et al. (2013)
Clinical/Health care	Characterization of cardiomyocytes (CMs) and drug effects	CMs derived from CCTL14 human embryonic stem cells (hESCs) and fibroblasts from patient affected with Duchenne Muscular Dystrophy (DMD)	Laminin and fibronectin-mediated	Biomechanical (atomic force microscopy (AFM))/microelectrode array (MEA)	Caluori et al. (2019)
	Bioelectric monitoring of stem cells differentiated to CMs (2D and 3D)	IMR90C01 (human induced pluripotent stem cells (hiPSCs))	–	MEA/Impedance	Fleischer et al. (2019)
	Inflammation (TNFα)	NanoLuc luciferase-expressing HEK293T (human embryonic kidney)	Gelatin-mediated encapsulation of 3D cell spheroids	Bioluminescence (Colorimetric)	Michelini et al. (2019)
	Cell monolayer permeability	Primary porcine aortic endothelial cells (PAECs)	Fibronectin-mediated (microfluidic chips)	Electrochemical (voltammetry)	Wong and Simmons (2019)
	Simultaneous recording of contact electogram and action potential	Neonatal rat ventricular myocytes (NRVMs), human cardiac slices	Collagen-mediated	MEA (& optical mapping)	Chowdhury et al. (2018)
	Cell culture monitoring	AA8 (Chinese hamster ovary fbroblast)	Cell growth on electrodes	ECIS	Pérez et al. (2018)
	Adhesion of biomaterials (PDMS-polyacrylic acid, PDMS-PAA and PDMS-polyvinyl pyrrolidone, PDMS-PVP) (biocompatibility screening)	NIH/3T3 fibroblasts expressing mCherry-vinculin and GFP-tensin	Adhesion on different natural ECM components (fibronectin, vitronectin, laminin-111, laminin-521, collagen)	Optical (fluorescence)	Ivanova et al. (2017)
	Live cell secretion (VEGF)	HeLa	Cultured in microfluidic channels integrated to detection module (plasmonic gold nanohole array)	Optical	Li et al. (2017)
	Study of Spreading depolarization (SD) (with glucose and lactate sensing)	Extracellular space of rat brain	–	MEA	Lourenço et al. (2017)
	Endothelial barrier function	CDC/EU.HMEC-1 (human microvascular endothelial cells (HMECs)), primary human umbilical vein endothelial cells (HUVECs)	Collagen G-mediated	Impedance (xCELLigence®, ECIS®)	Bischoff et al. (2016)
	Cellular differentiation	C2C12 (myoblast)	Matrigel mediated culture on sensor chips	ECIS	Park et al. (2016)
	Biomechanical properties of cardiac cells		Cantilevers submerged in Petri dishes containing cells	Mechanical (Atomic force microscopy)	Pesl et al. (2016)

(*continued on next page*)

Table 4 (*continued*)

Application	Target Parameter/Analyte(s)	Cell line(s) used	Immobilization Method	Transducer/detector	Reference
		CCTL14, CCTL12 (human embryonic stem cells (hESC)) (cardiomyocytes were derived from these lines)			
	Electrophysiological monitoring of skeletal myotubes	Rat skeletal myotubes	Polydopamine and Matrigel-mediated	MEA	Rabieh et al. (2016)
	Glucose	INS832/13 (pancreatic clonal β-cell line), human and mice islets	Matrigel-mediated	MEA	Pedraza et al. (2015)
	Extracellular action potentials	NRVMs	Culture on sensor chip	MEA	Trantidou et al. (2015)
	Extracellular acidification and cellular growth	HeLa	Culture on sensor chips	Light-addressable potentiometric sensor (LAPS), ECIS	Su et al. (2014)
	Guided excitation in patterned CMs	NRVMs	Fibronectin-mediated	MEA	Wang et al. (2013a)
Food safety and technology	Odor (diacetyl), bitter (salicilin) and toxic compounds	SH-SY5Y (human neuroblastoma) expressing ODR-10 (olfactory receptor)	Culture on chip	Impedance, MEA	Gao et al. (2019)
	Tastant molecules (NaCl (saltiness), sucrose (sweetness), saccharin (bitterness), and glycine (umami))	Primary taste cells (neonatal mouse)	2D and 3D hydrogel culture matrix based on decellularized tongue extracellular matrix (TEM) in microfluidic device	Optical (Calcium influx imaging)	Lee et al. (2018)
	N-acyl-homoserine lactones (AHLs) from pathogenic bacteria (*Pseudomonas aeruginosa*)	RBL-2H3 (rat basophilic leukemia mast cells)	Encapsulation in alginate/graphene oxide hydrogel on gold electrode	Electrochemical impedance spectroscopy (EIS)	Jiang et al. (2017a)
	Bitter tastants (sucrose octaacetate, denatonium benzoate, and quercetin)	Porcine tongue epithelium	Entrapment in starch-alginate hydrogel followed by sandwich in polycarbonate membranes and attachment to glassy carbon electrodes (GCE)	Electrochemical	Wei et al. (2017)
	Sour taste (acid)	Rat primary taste cells	Poly-L-ornithine and laminin (PLOL)-mediated	MEA	Zhang et al. (2017a)
	Bitterness (denatonium, PTC, PROP, quinine)	Male mouse germ cells	Attachment via poly-lysine and laminin on electronic plates	Cell impedance sensors (CIS)	Hu et al. (2016)
	Okadaic acid (diarrheic shellfish poisoning, DSP)	HepG2 (liver cancer)	Culture in microfluidic device	Acoustic (Love Wave)	Zhang et al. (2016)
	Okadaic acid (DSP)	HeLa, HepG2	Culture on sensor surface	ECIS	Zou et al. (2016)
	Food allergens (shrimp allergen tropomyosin (Pen a1), fish allergen parvalbumin (PV))	RBL-2H3 (transfected with cationic magnetic fluorescent Fe_3O_4 nanoparticles (CMFNP))	Magnetic glassy carbon electrode (GCE) inserted in cell suspension tube (after detection assay)	EIS	Jiang et al. (2015a)
	Saxitoxin (STX), Brevetoxin (PbTX-2) (marine toxins) (paralytic shellfish toxicity, PSP)	Cardiomyocytes (neonatal Sprague-Dawley rats)	Cells grown on microelectrode array (MEA) modified with gelatin	Electrical	Wang et al. (2015a)
	STX, Tetrodotoxin (TTX) (PSP)	Cardiomyocytes (neonatal Sprague-Dawley rats)	Cells grown on 96-well electronic plate modified with gelatin	ECIS (Real-time cell analysis (RTCA))	Wang et al. (2015b)
	Pungency (Capsaicin and gingerol)	Tongue epithelium (rat)	Entrapment in starch-alginate hydrogel followed by sandwich in polycarbonate membranes and attachment to glassy carbon electrodes (GCE)	Electrochemical	Qiao et al. (2015)
	STX (PSP)	Neuro-2a (Neuroblastoma)	Gelatin-mediated	ECIS	Zou et al. (2015)
	Sweet (Sucrose) and bitter (quinine) tastants	NCI–H716, STC-1 (taste receptor cells)	Cultured on carbon screen printed electrodes (CSPEs)	ECIS	Hui et al. (2014a)
	Tastants (Sucrose/quinine)	NCI–H716, STC-1	Cultured on CSPEs	Double-layered cascaded series stochastic resonance (DCSSR); EIS	Hui et al. (2014b)
	Sweeteners (glucose, sucrose, saccharin and cyclamate)	Taste epithelium	Placed on electrode arrays	Microelectrode array (MEA)	Zhang et al. (2014)
Pharmaceutical	Drug screening (lecithin-based nanoparticles loaded with methotrexate (MTX))	U-2 OS osteosarcoma cells	Culture in microfluidic chamber	Cytotoxicity evaluation by microscopic examination	Mitxelena-Iribarren et al. (2019)
	Anti-cancer drug screening	Human hepatoma HepG2 cells	Encapsulation in Matrigel scaffold for 3D culture	3D electric cell/matrigel-substrate impedance sensing (3D-ECMIS)	Pan et al. (2019)
		Human lung cancer A549 cells	Culture in 96-well plates	Colorimetric (MTT assay)	Chen et al. (2018)

(*continued on next page*)

Table 4 (*continued*)

Application	Target Parameter/Analyte(s)	Cell line(s) used	Immobilization Method	Transducer/detector	Reference
	Drug Screening (Combinations of Celecoxib (Celbx), 5-Fluorouracil (5-FU), Cyclophosphamide (CTX), Doxorubicin (DOX))				
	Proarrythmic potential (E−4031, bepridil and amiodarone)	Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)	Fibronectin-mediated	MEA	Izumi-Nakaseko et al. (2018)
	Proarrythmic potential (28 drugs)	hiPSC-CMs	–	MEA	Blinova et al. (2018)
	Monitoring of single cancer cell against anticancer drugs	MCF-7	Direct attachment on electrode surface	Impedimetric	Gharooni and Abdolahad (2017)
	Chemotherapy effects (evaluation of anti-cancer drugs)	LTEP-P (lung adenocarcinoma), LTEP-P/DDP-1.0 (cisplatin-resistant lung adenocarcinoma)	Cultured on CSPE	ECIS and DCSSR	Guohua et al. (2017)
	Salicin	HEK-293 expressing T2R16 human bitter taste receptor	Poly-lysine and laminin-mediated	CIS	Hu et al. (2017)
	Monitoring of cancer cells in anticancer drug treatments	MCF-7	Hydrodynamic capture in microfluidic chips	Impedimetric	Nguyen et al. (2017)
	Drug-induced cardiotoxicity	hiPSC-CMs and rat primary neonatal cardiomyocytes	Fibronectin-mediated	Complementary metal oxide semiconductor (CMOS)-based MEA	Jans et al. (2017)
	Drug-induced cardiotoxicity	NRVMs	Fibronectin-mediated	Piezoresistive	Kim et al. (2017)
	Insulin secretion suppressors and potentiators (6298 marine natural product fractions)	Secreted insulin-luciferase (Ins-GLuc)-expressing MIN6 pancreating β cells	Culture in 96-well plates	Optical	Kalwat et al. (2016)
	Drug activity (Mebendazole)	MCF-7	Physical interactions with Silicon nanowire electrodes (SiNWEs)	Electrochemical (voltammetry)	Shashaani et al. (2016)
	Effect of MBZ and PTX	MCF-7	Culture on Silicon nanograss (SiNG) electrodes	Electrochemical (voltammetry)	Zanganeh et al. (2016)
	Proarrythmia profiling (Verapamil, Ranolazine, Flecainide, Amiodarone, Ouabain, Cisapride, and Terfenadine)	hiPSC-CMs	Fibronectin-mediated	MEA	Gilchrist et al. (2015)
	Human ether-a-go-go-related gene (hERG) inhibition (astemizole, sertindole, cisapride and droperidol)	hiPSC-CMs	Fibronectin-mediated	Impedimetric (interdigitated electrodes)	Hu et al. (2015b)
	Antiarrhythmic drug evaluation (verapamil and flecainide)	Rat primary neonatal cardiomyocytes	Gelatin-mediated	Impedimetric (xCELLigence® RTCA Cardio system)	Wang et al. (2013b)
Environmental	Water toxicity	Rainbow trout gill epithelial (RTgill-W1) cells	Fibronectin-mediated	ECIS	Brennan et al. (2016)
	Biotoxicity	Luciferase green-expressing human embryonic kidney Hek293-T cells	Mixed with agarose	Bioluminescence	Cevenini et al. (2016)
	Biological activity of water (Water toxicity)	A549, ACHN, HepG2, and SK-N-SH	–	Impedimetric (xCELLigence® RTCA HT system)	Pan et al. (2015)
	Water toxicity	Rainbow trout gill epithelial (RTgill-W1) cells	Fibronectin-mediated	ECIS	Widder et al. (2015)
	Toxicants in water (ammonia, nicotine and aldicarb)	Bovine aortic endothelial cells (BAECs)	Fibronectin-mediated	ECIS with QCM	Liu et al. (2014b)
	Copper sulphate toxicity	Cells derived from Rainbow trout, NIH-3T3, rat skin-derived cells (RAsd), human skin-derived cells (CEsd)	–	Impedimetric (xCELLigence® RTCA DP)	Rakers et al. (2014)
	Chromium ions (Cr (VI)) toxicity	Chinese hamster lung fibroblasts cells (V79)	–	Impedimetric (xCELLigence® SP), LAPS (Cytosensor® Microphysiometer), Bionas® 2500 Analyzing System, CMOS-based impedance chip	Bohrn et al. (2013)

2009; Xu et al., 2016a; Zhang et al., 2017b). LAPS, on the other hand, is a semiconductor based chemical sensor with an electrolyte-insulator-semiconductor configuration (EIS). This technique is most prominently used by microphysiometers that are devices measuring more than one metabolic parameter at a given time. Other than these, electrochemical readouts and quartz crystal microbalance (QCM) have found applications in animal cell-based biosensors. Table 4 summarizes recent developments in animal cell-based biosensors for various applications.

4.1. Cancer research

Cancer cells-based biosensors have enabled non-destructive analysis of various cellular parameters including growth, migration, cell surface interactions, cell-cell interactions, cell-drug interactions, etc. by monitoring the electrical properties of cancer cells (Pan et al., 2019). In addition, these biosensors have allowed the study of cellular parameters and ligand interactions at single cell resolution, thus providing new insights in cancer pathology, biology, and drug development (Pan et al., 2019; Rafizadeh-Tafti et al., 2017; Shukla et al., 2018). Recently, a 3-dimensional (3D) graphene interface has been reported that can impedimetrically (ECIS) differentiate between highly metastatic (MDA-MB-231) and less metastatic (MCF-7) breast cancer cells at single cell level (Wang et al., 2018). This study also highlighted the advantage of 3D graphene bio-interface over conventional planar gold electrodes in terms of capture efficiency and sensitivity. Other studies have reported on the utilization of MCF-7 and MDA-MB-231 breast cancer cells in combination with impedimetric (or, capacitive) electrodes for analysis of cell attachment, migration, spreading and drug-induced cellular mechanisms (Anh-Nguyen et al., 2016; Lee et al., 2016; Nguyen et al., 2013). Anh-Nguyen et al. (2016) demonstrated time-dependent cisplatin-induced morphological changes and subsequent apoptosis of MCF-7 cells cultured on a microelectrode array (MEA) chip via impedance changes. The study measured changes in a 2D cell culture on planar electrodes. However, biosensors for the monitoring of cancer cells in 3D culture environment have become the preferred choice as they represent a more physiologically relevant environment than their planar counterparts (Edmondson et al., 2014; Lee et al., 2016). Lee et al. (2016) demonstrated a capacitive biosensor for monitoring of 3D culture of cancer cells, wherein GFP-expressing MCF-7 cells were encapsulated in alginate hydrogel for 3D culture and then monitored in real time for proliferation and drug-induced apoptosis using vertically placed electrodes. Similarly, Lei et al. (2015) demonstrated impedimetric analysis as a promising alternative to microscopic colony forming assay by showing linear correlation between impedance and colony index of Huh 7 hepatoma cells cultured in 3D environment. The chemosensitivity of these cells was quantified by determining the IC_{50} of doxorubicin (67 μg/mL). Microfluidic devices integrated to 3D cancer cell cultures have been developed for cell proliferation, chemosensitivity, and cell migration studies (Lei et al., 2014; Nguyen et al., 2013). In an interesting study, vertical electrodes were placed at sidewalls of a 3D microfluidic cell culture chamber and linear correlation between density of OEC-M1 human oral cancer cells and corresponding impedance values was demonstrated (Lei et al., 2014). Similarly, another study fabricated a microfluidic system integrated to MEA to study the migration of single metastatic MDA-MB-231 cells in real time in 3D Matrigel matrices with single-cell resolution (Nguyen et al., 2013). These studies verify the significance of impedance analysis toward monitoring different parameters associated with 2D and 3D cancer cell cultures. In contrast to end-point assays, these impedimetric cell biosensors provide real time information and are non-invasive and less time-consuming. An electrical wound-healing impedance assay has been reported recently for rapid assessment of cancer cell viability and proliferation (Wei et al., 2019). A multiparametric microphysiometer has been reported by Weltin et al. (2014) for dynamic monitoring of cancer cell metabolism. Microphysiometers are devices that can monitor at least one metabolic parameter of living cells on a chip non-invasively and are based on LAPS. This study, in contrast to conventional microphysiometers that are based on a silicon chip, demonstrated the fabrication of an optically transparent glass chip-based system for continuous monitoring of pH, oxygen, lactate, and glucose in T98G human brain cancer cells in culture. The sensitivity of this system was found to be $-61.4\ mV\ pH^{-1}$, $-0.735\ \mu A\ \mu M\ cm^{-2}$, $3.3\ nA\ mM^{-1}\ mm^{-2}$ and $2.6\ nA\ mM^{-1}\ mm^{-2}$ for pH, oxygen, glucose and lactate, respectively and alterations in the metabolism were also monitored by disrupting glucose uptake by the cells. Piezoelectric biosensors for analysis of cell surface glycosylation have been recently reported (Li et al. 2013, 2015). In one such study, KM-12 and SKOV-3 cells (colon and ovarian adenocarcinoma, respectively) were cultured on polystyrene-coated QCM chips and thermodynamic parameters of binding events taking place between cell surface carbohydrates and different lectins (e.g. sialic acid and concanavalin A) were deduced from the frequency response of the biosensor (Li et al., 2015). The study indicated that both types of cells have low expression of N-acetyl glucosamine on their surface, while that of sialic acid was very high. A similar study demonstrated real time monitoring and kinetic analysis of lectin-cell (surface carbohydrates) interactions for K562 human acute myelocytic leukemia and Jurkat human acute lymphocytic leukemia cells captured on a modified QCM chip (Li et al., 2013). The study showed that maximum frequency shift was obtained by wheat germ agglutinin (WGA) on Jurkat cells indicating the presence of highly branched glycoconjugates on the cells' surfaces, while a lower but significant shift was observed by the same lectin (WGA) for K562 cells. Kinetic analysis of interactions of trastuzumab monoclonal antibody with human epidermal growth factor receptor 2 (HER2) present on breast cancer cells (SKOV-3, SKBR-3) has been performed using QCM technology (Elmlund et al., 2015; Peiris et al., 2017). These studies reveal that biosensors can provide crucial information pertaining to cancer cell interactions and metabolism in a noninvasive manner and help in deducing biomolecular cell surface 'maps' leading to more efficient diagnostic and therapeutic management. For example, Marzioch et al. (2018) integrated a transparent, electrochemical chip for metabolic monitoring of T-47D breast cancer cells with photodynamic therapy (PDT) and revealed vital information about efficacy of therapy by measuring oxygen consumption of cells in real time. Peiris et al. (2017) used QCM biosensor technology in combination with conventional immune-fluorescent staining to show that reduction of N-linked glycosylation on the surface of SKBR-3 cells enhanced the binding of Herceptin (trastuzumab) with HER2 receptor and cells' sensitivity to doxorubicin, while decreasing their sensitivity to growth factors (IGF-1 and EGF) at the same time.

4.2. Clinical and health care applications

In addition to cancer pathogenesis, cell-based biosensors have interesting applications in monitoring the response of normal living cells toward changes in their microenvironment, including presence of drugs and other bioactive molecules. This section pertains to cell biosensors for assessment and monitoring of normal cell activities and disease models other than cancer. Assessment of electrophysiological and biomechanical properties, particularly of cardiac and neural cells (neurons), is an important parameter to understand pathologies associated with major organs in the body (Denning et al., 2016). Chowdhury et al. (2018) have developed a technique by integrating optical mapping with MEA for concurrent measurement of contact electrogram and action potential (AP) of *ex vivo* human cardiac slices. Further, the advent of induced pluripotent stem cells (iPSCs)-derived cardiomyocytes (CM) has allowed their utilization in the development of noninvasive technology towards the study of electro(bio)mechanics, response of cardiac cells to various stimuli and cardiomyopathies (Blinova et al., 2018; Caluori et al., 2019; Denning et al., 2016; Hu et al., 2015b; Pesl et al. 2016, 2017). Recent studies have reported on the development of MEA-based platforms (with or without impedance monitoring) for long time monitoring of growth and function of 3D cardiac cell cultures in order

to understand cardiomyopathies and drug effects (Caluori et al., 2019; Fleischer et al., 2019; Trantidou et al., 2015). Fleischer et al. (2019) recently demonstrated a new protocol for iPSC-derived 3D CM culture and utilized impedance monitoring with microcavity arrays (3D counterparts of MEA) to evaluate long term stability, maturation and beating patterns of the 3D culture over 80 days. Caluori et al. (2019) integrated atomic force microscopy (AFM) to MEA technology to study cardiac excitation-contraction coupling (cECC) in iPSC-derived 3D CM cultures and found a distinctive beating-force relation for Duchenne muscular dystrophy (DMD) cardiac models. A previous study by Pesl et al. (2016) reported on the utilization of AFM in conjunction with cantilever probes towards biomechanical characterization and effect of stimulants on iPSC-derived CM embryoid bodies (EBs). It is important to note here that ECIS and LAPS have been found to be equally competent in monitoring of CM beating status when compared to each other (Hu et al., 2013). However, no direct comparison of these techniques with CM-based MEA devices has been made, that have gained special interest in the recent past particularly for drug proarrythmic potential investigations (will be discussed in Section 4.4). MEA devices have been utilized for quantification of guided excitation of cardiomyocytes (Wang et al., 2013b). Skeletal myotubes (derived from skeletal muscle) are another type of cells that have been utilized in establishing ECIS and MEA-based devices used for growth monitoring and electrophysiological characterization of skeletal muscles (myoblasts) (Park et al., 2016; Rabieh et al., 2016). Neurological research is another field of investigation where cell-biosensors have proven to be significant. In fact, MEA technology (in addition to patch clamp electrodes) is the primary mode of understanding neuronal circuits, physiology and abnormalities, both *in vitro* and *in vivo* (Seymour et al., 2017; Spira and Hai, 2013). Recently, Lourenço et al. (2017) have developed an MEA based device for simultaneous monitoring of glucose and lactate with neuronal activity towards analysis of spreading depolarization (SD). Another study demonstrated the superiority of glassy carbon (GC) electrodes over conventional Pt electrodes for neural stimulation and recording of brain activity with low background noise (Vomero et al., 2017).

Other applications where live mammalian cells-based devices have been employed include evaluation of endothelial barrier function and permeability, live cell secretion, and determination of anti-inflammatory properties (Bischoff et al., 2016; Li et al., 2017; Wong and Simmons, 2019). Interestingly, some studies have focused on the development of real time remote monitoring and smartphone-based systems for analysis purposes (Michelini et al., 2019; Pérez et al., 2018). Adhesion properties of different biomaterials have been studied using recombinant NIH/3T3 fibroblast cell line (Ivanova et al., 2017). Another study measured the change in electrical activity of β cells upon exposure to glucose via electrophoresis-mediated biosensing on an MEA device (Pedraza et al., 2015), thus, adding a new dimension to diabetes pathology.

Such biosensors can contribute significantly to the fields of tissue engineering and regenerative sciences, wherein *ex-vivo* growth of functional artificial organs (liver, pancreas, etc.) is an active area of research. Integrated biosensor systems with electronic circuits are needed that can monitor the orchestrated differentiation potential of stem cells in real time and simultaneously change the conditions required to direct the differentiation in a particular direction.

4.3. Food safety and technology

Taste and odor perception and analysis are important parameters in food quality and safety assessment. Several attempts have been made to fabricate efficient 'bioelectronic tongue and nose' that mimic human olfaction and gustation for food quality and safety evaluation (Ha et al., 2015; Son and Park, 2018; Zhang et al., 2017a). Recently, Gao et al. (2019) reported an integrated, biomimetic olfaction, gustation and toxicity biosensor based on human neuroblastoma SH-SY5Y cells. These cells inherently express the T2R16 human bitter receptor while ODR-10 olfactory receptor was transfected on the plasma membrane of the cells. A dose-dependent response was observed by ECIS and MEA for bitterness and odor changes, respectively. Lee et al. (2018) demonstrated simulation of cross-talk between tongue receptors and adjacent neuronal cells for taste sensing. They achieved this by devising a 'bio-artificial tongue' platform consisting of taste cells immobilized in a 3D decellularized porcine tongue extracellular matrix (TEM). A device consisting of intact taste epithelium integrated with MEA has been devised for spatiotemporal analysis of sweeteners (Zhang et al., 2014). Such biosensors can serve as *in situ* tissue models for providing new insights into taste and olfaction mechanisms. Wei et al. (2017) utilized porcine taste bud tissues integrated to GCE to determine the kinetic characteristics of taste receptors. In another study, mouse germ cells expressing bitter receptor T2Rs were integrated with a cell impedance sensing (CIS) chip for specific and sensitive (limit of detection (LOD): 0.125 mM for quinine) detection of bitter compounds (Hu et al., 2016). Similarly, Qiao et al. (2015) quantitatively studied the interaction between rat taste receptors and pungent substances (LOD: 1×10^{-13} mol L^{-1} and 9×10^{-13} mol L^{-1} for capsaicin and gingerol, respectively) by fixing rat taste bud tissues on GCE. Frequency optimization of EIS method and utilization of double-layered cascaded series stochastic resonance (DCSSR) have also been reported for discrimination of various taste elements in complex mixtures (Hui et al. 2014a, 2014b). Though effective, these biosensors vary considerably in terms of the types of cells used, the immobilization methods utilized and the technique used for analysis. Thus, efforts should be directed toward direct comparison and validation of the different techniques and functional designs in order to yield an efficient taste biosensor. Fig. 5 depicts some of the recent innovations in cell-based biosensors for taste analysis.

Detection of food toxins and food borne agents is an active area of investigation where cell-biosensors have yielded fruitful results (Fig. 6). Mast cells (RBL-2H3 cell line), a type of immune cells, have been used for the fabrication of impedimetric biosensors for the detection of quorum signaling molecules from food borne pathogen *Pseudomonas aeruginosa* and food allergens (Jiang et al. 2015, 2017a). Efforts have been made for the detection of marine toxins that may cause diarrhetic and paralytic shellfish poisoning (DSP and PSP, respectively). Two independent studies have reported on the utilization of HepG2 liver cancer cells in the fabrication of Love wave (acoustic) and impedimetric biosensors for the detection of okadaic acid (OA), the toxin responsible for DSP (Zhang et al., 2016; Zou et al., 2016). However, there is no direct comparison between the two different sensors owing to the different transduction techniques. A neuro-2a neuroblastoma cell-based impedance biosensor (CIB) has been fabricated for detection of saxitoxin (STX), the causative agent for PSP (Zou et al., 2015). CM-based impedance and MEA biosensors have been constructed for quantitative detection of marine toxins. In this context, the growth and beating patterns of primary neonatal rat CMs were monitored impedimetrically on exposure to STX and tetrodotoxin (TTX) (Wang et al., 2015b), while in another study, CM electrical activity was monitored as a function of STX and brevetoxin (PbTX-2) concentration (Wang et al., 2015a). In essence, a variety of cells can be applied toward the detection of food borne toxins, allergens and pathogens. However, regeneration of these biosensors and lack of optimized functional strategies pose problems to the development of standardized toxin biosensors.

4.4. Pharmaceutical and pharmacological research

Drug screening and toxicity evaluation are important part of pharmaceutical research. Recently, induced pluripotent stem cell (iPSC)-derived CM-based biosensors have emerged as a powerful tool in assessing drug toxicity (Smith et al., 2017). Several MEA-based CM biosensors have been reported for assessing the potential of drugs to initiate arrhythmia (Gilchrist et al., 2015; Izumi-Nakaseko et al., 2018;

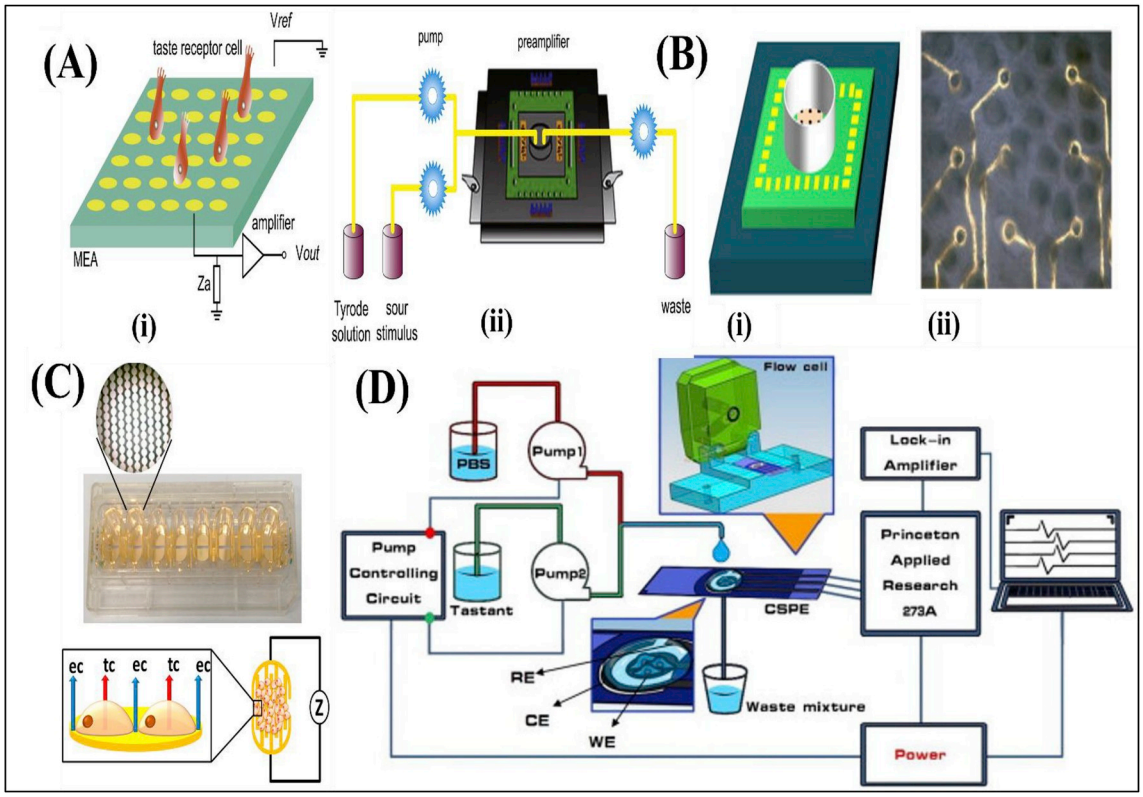

Fig. 5. Design of taste cell-based biosensors for tastant analysis. (A) Schematic of (i) acid-sensing biosensor and (ii) corresponding bioelectronic tongue system for extracellular electrophysiological recordings of taste receptor cell. Reproduced with permission (Zhang et al., 2017a), Elsevier Inc. (B) (i) Diagram of 60-channel MEA device and (ii) gold microelectrodes covered with taste epithelium for sweet analysis. Reproduced with permission (Zhang et al., 2014), Elsevier Inc. (C) 8-well cell impedance sensor (CIS) chip with magnified electrode (upper) and corresponding schematic of impedance measurement for bitter taste analysis. Reproduced with permission (Hu et al., 2016), Elsevier Inc. (D) Diagram of carbon screen printed electrode-based cell biosensor for quantitative taste analysis by stochastic resonance. Reproduced with permission (Hui et al., 2014b), Elsevier Inc. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Jans et al., 2017; Tertoolen et al., 2018; Wang et al., 2013c). Efforts are being made to validate and standardize MEA-based CM sensors for drug proarrythmic potential evaluation (Asakura et al., 2015; Blinova et al., 2018; Gilchrist et al., 2015; Kanda et al., 2016). Recently, a computer simulation technique was developed to analyze the relationship between field potentials measured by MEA and cardiac AP (Tertoolen et al., 2018). It was further validated against primary mouse- and human pluripotent stem cell (hESC)-derived CMs. In addition, ECIS-based methods and piezoelectric cantilevers have been fabricated to monitor changes in beating pattern and mechanical force of CM cells in response to different drugs (Hu et al., 2015b; Kim et al., 2017). A microfluidic platform for multiple drug screening has been devised recently to assess the effect of several chemotherapeutic drugs on osteosarcoma cells (Mitxelena-Iribarren et al., 2019). Effects of multi-drug combinations on A549 lung cancer cells have been studied in a 3D printed microfluidic platform with helical channels (Chen et al., 2018). A secreted insulin-luciferase system (Ins-GLuc) in MIN6 β cells has been developed for high throughput screening of marine bacterial natural products that stimulate glucose-mediated insulin secretion (Kalwat et al., 2016). Apart from this, a 3D electric cell/matrigel-substrate impedance sensing (3D-ECMIS) platform has recently been reported toward improving the accuracy of anti-cancer drug screening platforms (Pan et al., 2019).

4.5. Environmental applications

Cell culture-based assays, particularly based on fish and mammalian cell lines, can prove fruitful for water quality assessment in terms of determination of the effects of different toxicants on cell metabolism and growth (Tan and Schirmer, 2017; Widder et al., 2015). A portable ECIS-based sensor utilizing fish RTgill-W1 gill epithelial cells has been developed for use in the US Army (Brennan et al., 2016; Widder et al., 2015). Human cell lines have been utilized toward development of devices for water toxicity monitoring (Bohrn et al., 2013; Pan et al., 2015; Rakers et al., 2014). Bohrn et al. (2013) presented a comprehensive analysis of Cr (VI) toxicity by human cells (V79) using 3 commercially available cell biosensors (2500 Analyzing System (Bionas), xCELLigence® (Roche) and Cytosensor Microphysiometer (Molecular Devices) and an in-house CMOS based detection device. While Rakers et al. (2014) compared the sensitivity of fish cells and three other vertebrate cell lines for ECIS-based detection of copper sulphate. ECIS has been integrated with QCM for fabrication of a bovine aortic endothelial cells (BAECs)-based sensor for water toxicity analysis (Liu et al., 2014b). In contrast to these studies, Cevenini et al. (2016) have reported on the development of smartphone-interfaced device that utilizes bioluminescent HEK293 human embryonic kidney cells towards water toxicity monitoring. These animal cell-based biosensors have the advantage of providing insights into physiological relevant responses toward water pollutants at the cellular level. However, when compared to microbial cells, the robustness of these cells is limited.

5. Future perspectives

Cell-based biosensors have gained significance in a diverse array of applications ranging from environmental monitoring to pharmaceutical research. The emergence of microfluidics has been a boon for cell-based biosensors as it enables the powerful concept of “cell culture on chip” that can be very helpful in the development of new generation of cell-

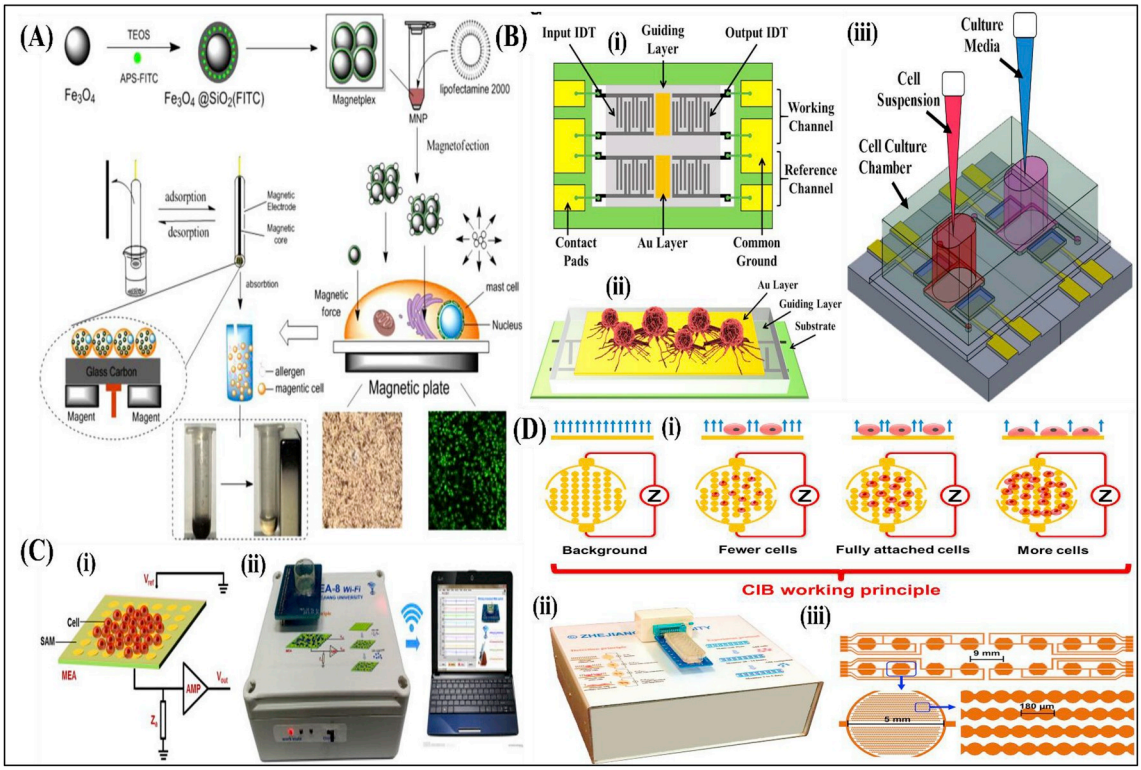

Fig. 6. Recent developments in cell-based biosensors for food toxin detection. (A) Schematic illustration detailing detection principle of RBL-2H3 mast cell-based detection of food borne pathogen. Reproduced with permission (Jiang et al., 2015), Elsevier Inc. (B) (i) Top and (ii) Cross-sectional view of Love wave sensor utilizing HepG2 liver cancer cells for detection of DSP toxin, and (iii) Structure of PDMS cell culture chamber used in the study. Reproduced with permission (Zhang et al., 2016), Elsevier Inc. (C) (i) Schematic diagram and (ii) Portable wireless 8-channel system for MEA based detection of STX and PbTX-2 utilizing CMs as bioreceptors. Reproduced with permission (Wang et al., 2015a), Elsevier Inc. (D) (i) Detection principle of cell-based impedance biosensor (CIB), (ii) Multichannel CIB system, and (iii) Layout of a sensor chip with 16 wells fabricated for detection of PSP toxins using neuroblastoma cells as bioreceptors. Reproduced with permission (Zou et al., 2015), Elsevier Inc.

based biosensors. Bae et al. (2018), for example, reported on the integration of whole cell microbial biosensor with a novel microchemostat platform for heavy metal detection. This integration allowed a uniform cell culture environment along with long term stability and the capability of regeneration by sub-culturing. Smart cell culture systems have been envisioned that enable real time, remote monitoring of various cellular parameters without any external effort (Modena et al., 2018). Self-stabilizing culture systems can be developed by integration of different sensing functionalities with cell cultures. Systems consisting of a consortium of different microbial species/higher eukaryotic cells genetically modified to respond to combinations of a number of different analytes in a well-coordinated manner are just around the horizon. Synthetic living sensors with tailored genetic network are being developed toward diagnosis of various diseases and other analytes (Courbet et al., 2015; Sedlmayer et al., 2018; Skjoedt et al., 2016; Slomovic et al., 2015). In a recent study, the gene expression of bacterial cells (*E. coli* and *B. subtilis*) was controlled using genetic switches so as to yield the desired output in response to biomarkers present in urine or serum (Courbet et al., 2015). These "bactosensors" were designed to respond in line with the Boolean AND gate, and were capable of detecting pathological glycosuria in urine from diabetic patients.

The efficient integration of such synthetic living sensors with rigid electronic circuitry without the loss of function is still a great challenge. Thus, development of bioelectronic surfaces that are functional and at the same time conducive toward cell culture and proliferation is likely to be the focus in the coming years. Visser et al. (2018) recently demonstrated in-air microfluidics for manipulation of microscale liquid streams in the air enabling production of 3D multiscale biomaterials in one step. 'Soft MEAs' have been fabricated by ink jet printing carbon MEAs directly on PDMS, gelatin and other hydrogels for extracellular potential readings of cardiomyocyte-like HL-1 cells (Adly et al., 2018). Efforts towards formulating conductive hydrogels are also being made for bioelectronics applications (Song et al., 2017; Yuk et al., 2019). The use of such hydrogels may improve the sensitivity of electrochemical microbial biosensors considerably. Transparent PEDOT:PSS films having long term stability under water have been developed for direct cellular stimulations and recordings (Kim et al., 2018). Nanostructured electrodes capable of interacting with intracellular environment may have a significant role in improving studies related to electrically-active cells (Rawson et al., 2016).

3D bioprinting is an actively growing field with potential applications in cell-based biosensors due to several advantages like high throughput, digitally controlled patterning, rapid deposition, and highly accurate transfer of various biological factors to the desired locations for numerous applications (Jang et al., 2018; Skardal, 2015). It has been shown that precise placement of mouse myoblasts onto microcantilevers using bioprinting techniques can lead to the formation of myotubes in much less time than by randomly placing myoblasts (Cui et al., 2013). These myoblasts printed cantilevers were further shown to operate as a functional biosensor. Further, layer-by-layer printing technology can arrange various cell types inside a bulk material to produce a signal cascade, where one cell type acts as a transducer and gives feedback to a secondary cell type to perform a desired action. Thus, precise channels can be constructed to direct the movement of an analyte to the transducer (Huh et al., 2015). 3D printing would enable the creation of biomimetic 3D tissues and organoid structures that can prove to be effective models for high-throughput drug screening and toxicity testing (Kang et al., 2015; Mota and Moroni, 2015; Peng et al., 2017).

Recently, cell-based microarrays have gained interest from the

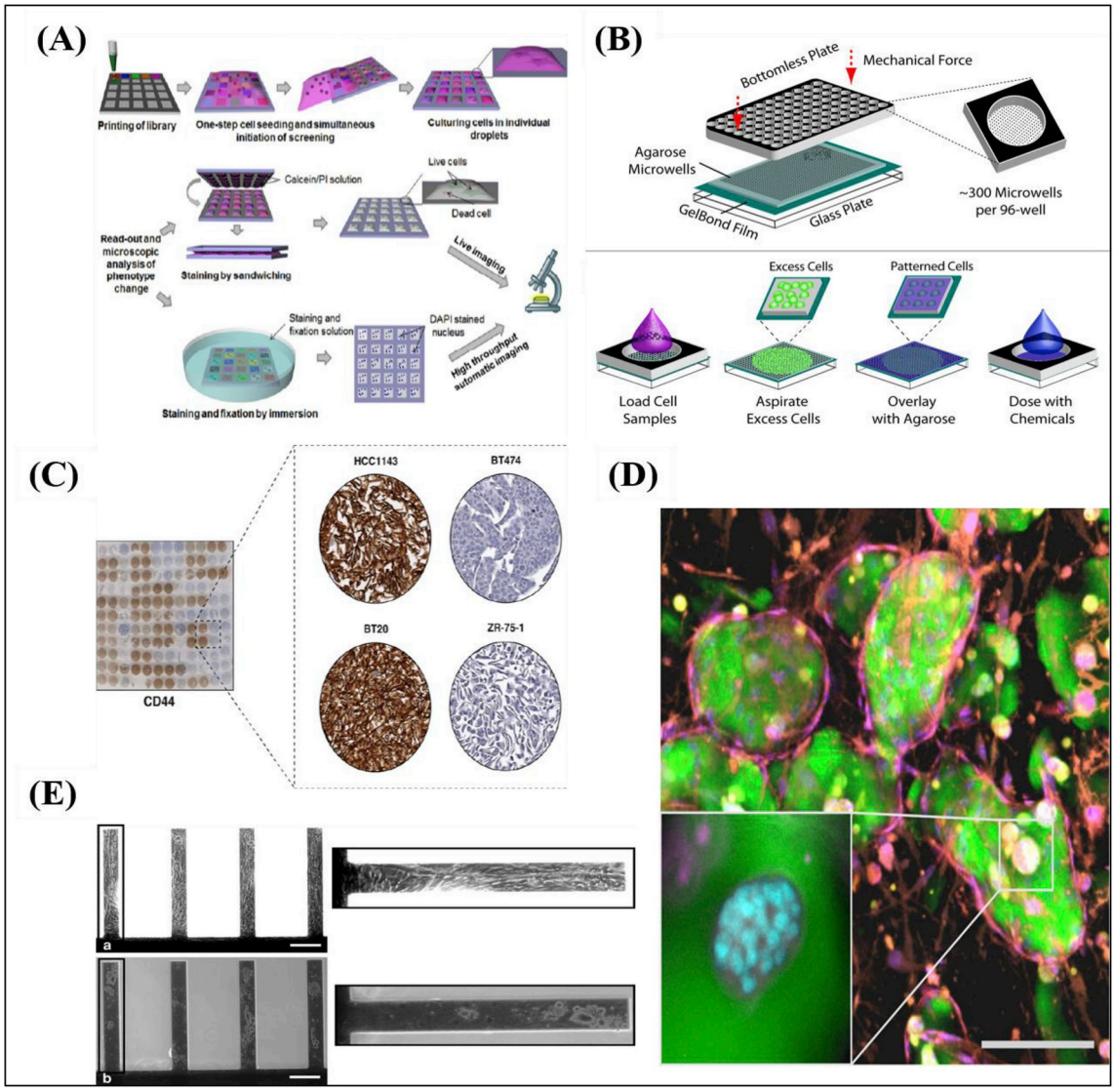

Fig. 7. Emerging concepts in cell-based biosensing technologies. (A) Schematic diagram of reverse cell screening by droplet microarray (DMA) platform. Reproduced with permission (Popova et al., 2016), Royal Society of Chemistry. (B) Assembly of macrowells (upper) and assay procedure (lower) of CometChip for evaluation of double stranded breaks. Reproduced with permission (Weingeist et al., 2013), Taylor & Francis Group. (C) Breast cancer microarray displaying immunocytochemical staining for CD44 marker in different cell lines. Reproduced with permission (Wu et al., 2014), Taylor & Francis Group. (D) Injection molded multiscale tissue construct (pancreatic β-cells (beige with blue nuclei)) encapsulated in alginate (green) within a fibrin network containing human endothelial and stem cells (pink with blue nuclei) fabricated by in-air microfluidics. Reproduced with permission (Visser et al., 2018), AAAS. (E) Cantilevers showing (a) myotube formation by bioprinted C2C12 myoblast and (b) random distribution of nonprinted myoblasts (4 days in culture). Reproduced with permission (Cui et al., 2013), Springer Nature. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

health care and pharmaceutical industry owing to their high throughput capability and miniaturized form (Hong et al., 2017). A Droplet-Microarray (DMA) platform has been developed for reverse transfection and reverse drug screenings by HEK293, HeLa and A549 cells in nanoliter-sized droplets (Popova et al., 2016). Such microarrays may prove helpful in rapid, high-throughput analysis of a number of parameters including cancer biomarkers, dependence of cell properties on microenvironment and cell differentiation (Ghaemi et al., 2016; Orgovan et al., 2014; Wu et al., 2014). Such microarrays would also enable the study of genetic heterogeneity at the single cell level (Weingeist et al., 2013). The fabrication of such microarrays can be made possible by the recent advances made in micropatterning of cells and tissues. Hynes et al. (2014) have demonstrated a novel micropatterning method using quill pen lithography, wherein the Bioforce Nano eNabler™ printing system was modified to enable "quill pen" based printing, and fabrication of a fluorescent biosensor from mammalian cells (adipose derived stem cells, NIH-3T3 fibroblasts, mouse embryonic stem cells) in a 3D hydrogel matrix. More recently, 4D printing, conceived in 2013, has emerged as a powerful tool for fabrication of intelligent multifunctional systems. 4D printed materials can change their functionalities and shapes upon exposure to external stimuli. These systems have already found widespread applications in drug delivery and tissue engineering (Gao et al., 2016a; Kuang et al., 2019). This printing system can enable fabrication of smart devices wherein substrates can change their shape with cellular organization. Conversely, the change in the functionality/shape of a material along with response of immobilized cells presents a new functional strategy for cell-based biosensors. Thus, 4D printing may add a new dimension in the field of cell-based biosensors. Fig. 7 depicts some of the most recent efforts made toward development of cell-based biosensor technologies.

6. Conclusions

In summary, cell-based biosensors have emerged as promising alternatives to traditional conventional techniques over the past about five decades. The convergence of 3D bioprinting, microarray technology and microfluidics has created a new step towards more sensitive, accurate and efficient cell-based biosensing technologies. Genetic manipulation of the cell genome has opened opportunities to visualize and study molecular interactions in their native environment. At the same time, improved cell culture methods and emergence of new 3D culture matrices can lead to the integration of cell culture with electronic devices. With the introduction of new techniques such as tissue reengineering, 3D and 4D printing, growing cells onto 3D multifunctional platforms would become easier leading to the development of a new generation of cell-based biosensors. Such biosensors would allow the studies of cellular mechanisms as they occur in the living systems. Cell microarrays would further help in high throughput screening of drugs and monitoring of cell culture parameters that would significantly lower the costs and labor involved in drug development. Thus, from biofilms to 3D cultures, cell-based biosensors have made a long journey in terms of their design, function, and applications with new advances in bioengineering and chemical sciences, which are expected to continually evolve in the coming times.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT authorship contribution statement

Niharika Gupta: Writing - original draft. **Venkatesan Renugopalakrishnan:** Writing - review & editing. **Dorian Liepmann:** Writing - review & editing. **Ramasamy Paulmurugan:** Writing - review & editing. **Bansi D. Malhotra:** Conceptualization, Writing - review & editing.

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