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Recent progress on cell-based biosensors for analysis of food safety and quality control

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ABSTRACT

Food quality and safety has become a subject of major concern for authorities and professionals in the food supply chain. Rapid methods, particularly biosensors, have exceptional specificity and sensitivity, rapid response times, low cost, relatively compact size, and are user friendly to operate. Cell-based biosensors are portable, and provide the biological activity of the analyte suitable for an initial screening of food. In this overview, the utilization of cell-based biosensors for food safety and quality analyses, such as detecting toxins, foodborne pathogens, allergens, and evaluating toxicity and function are summarized. Our results will promote the future development of cell-based biosensors in the food field.

1. Introduction

Assuring food quality and safety has become a subject of major concern for authorities and professionals in the food supply chain (King et al., 2017). Food quality relates primarily to characteristics, such as appearance, texture, taste, smell, and nutritional value, which must be ensured to guarantee nutritional quality and acceptability by consumers. A food analysis is also of uppermost importance to protect consumers against adulteration, spoilage, and contamination. However, food is exposed to a myriad of environmental chemical substances, many of which are hazardous to humans and other lives. Furthermore, modern societies have great concerns regarding the presence of harmful compounds in foodstuffs owing to the increasing complexity of food supply chains, environmental constraints, and the changing patterns of consumer choice and food consumption (Malik et al., 2010; King et al., 2017).

Notably, totally eliminating food contaminants and perfecting analytical tools for food quality are impossible (Marvin et al., 2009). However, suitable analytical methods for checking the safety and quality of foods are required. One of the key challenges to control foodstuff composition and detect chemical or biological contaminants is the availability of selective, sensitive, and reliable analytical methods (King et al., 2017). The conventional analysis of risk-associated residues in foodstuffs provides limited sample analysis capacity, particularly for a high-throughput requirement (Maragos and Busman, 2010). Most analyses are now performed by skilled personnel using instrumentation (such as chromatography, spectroscopy, and mass spectrometry) or animal models (Fatma et al., 2017). However, sophisticated operations with significant expenses require long procedures and skilled staff, which limit their use during the initial research stage in the food field (Zhu et al., 2017; Frankel and Mayer, 2000). Issues, such as ease of use, low maintenance, and continuous operation, are also becoming of practical importance (Singh et al., 2013).

Rapid methods, particularly biosensors, have exceptional specificity and sensitivity, rapid response, low cost, relatively compact size, and are user friendly to operate. Biosensors have gained interest as analytical tools in medicine, agriculture, food safety, bioprocessing, industry, and environmental monitoring (Dominguez et al., 2017; Bahadır and Sezgintürk, 2015a, 2015b). In particular, cell-based biosensors (CBBs) are portable and provide the biological activities of the analyte that are suitable for an initial screening of food (Sharma et al., 2015; Banerjee and Bhunia, 2009).

The present review mainly focuses on the recent advances in the growth of CBBs in the food field, including detection of pathogens, allergens, toxins, active compounds, additives, and food processing control. The perceptions discussed in the current review will promote the application of CBBs for food safety and quality operations. The prevailing challenges and future outlook on improved CBBs are also highlighted.

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2. Cell-based biosensors

2.1. Cell-based biosensor and its properties

A CBB is an analytical device that uses living cells as the recognition elements with a suitable physicochemical transducer to detect physiological changes in cells, which has been rapidly developed in the past decade (Ziegler, 2000; Banerjee and Bhunia, 2009; Stenger et al., 2001). The first microbe-based or CBB was actualized by Diviès (1975). CBBs represent the next revolution in analytic science, offering a number of unique advantages, including rapid response, high sensitivity, low cost and excellent selectivity, noninvasiveness and long-term measurements (Gray et al., 2001). More importantly, CBBs are likely to have improved stability and higher biocatalytic activity to provide physiologically relevant data in response to an analyte and to sense their functionality or biological activity (Banerjee and Bhunia, 2009; Kintzios et al., 2007). An immobilized cell sensing system is common to use in a CBB, extensive information on the use of immobilized cell culture systems and surface biofunctionalization of sensors has been reviewed by Kisaalita (2010), Banerjee and Bhunia (2009) as well as Kintzios (2007) and Liu et al. (2014) and is not discussed in detail here.

Electrochemical and optical techniques are most widely used in the development of CBBs. Among them, an electrical cell-substrate impedance sensor (ECIS) is one of the initial breakthroughs that brought the concept of a mammalian or higher eukaryotic whole-cell-based biosensing to commercial and research diagnostics (Liu et al., 2014; Silva et al., 2018; Giaever and Keese, 1993). Fig. 1A and B presents a fabrication process and schematic of the ECIS system proposed by X. Zhang et al. (2017) and Giaever and Keese (1993). Detailed information about the theories, structure and operation of the ECIS is reviewed by Liu et al. (2014) and Zhang et al. (2015). The majority of CBBs measure optical properties of cellular metabolites or intracellular enzymes (Banerjee et al., 2007; Gray et al., 2005; Curtis et al., 2008). Optical CBBs have been applied successfully in the food industry to rapidly detect potentially harmful contaminants. Fig. 2 illustrates the principle of optical biosensing in cellular study schemes (Méjard et al., 2014).

2.2. Cell types for CBBs and their characteristics

2.2.1. Mammalian cells

Mammalian cells with excitable cell membranes are used as the sensor in cell-based sensing, as they contain many highly evolved biochemical pathways (Pancrazio et al., 1999). Mammalian cells provide more comprehensive and complex functional information (such as signaling events, protein synthesis, apoptotic, or necrotic cell death) than nucleic acid and immunochemical methods (Banerjee and Bhunia, 2009). Mammalian cell sensors can work as an early warning system for food risk assessment. Indeed, they represent the living materials that eventually become the designated target of all food ingredients.

Mammalian cells reflect toxicological, physiological and cellular responses relevant to humans and animals, which circumvent the limitations compared to those of bacterial biosensors. Thus, biosensors based on engineered human cells are most appropriate when genotoxicity data are used to predict *in vivo* genotoxicity and potential carcinogenicity of chemicals for humans. The strengths and weaknesses of human cell- and bacterial cell-based biosensors for detecting genotoxicity should be regarded in terms of their applications (Blagus et al., 2014). In contrast, mammalian cells are much more demanding in terms of culture, growth, robustness, and genetic manipulation compared to bacteria.

2.2.2. Microorganismal cells

In general, every type of cell can be used to develop a CBB, but the overwhelming majority of sensing systems are based on microorganisms, such as bacteria and yeast (Kintzios and Banerjee, 2015; Keenan et al., 2007). The advantages of bacterial cells for the development of CBBs are their ubiquitous presence, rapid growth, ease of culture, low cost and ease of genetic manipulation, and their ability to metabolize a wide range of chemical compounds (D'Souza, 2001).

Microbial biosensors based on light emissions from luminescent bacteria are being applied as a sensitive, rapid, and noninvasive assay to evaluate the safety of food. These biosensors are usually equipped with reporter genes fused with a DNA response element for environmental stress-induced targets, such as the *lux* bacterial luciferase and the *gfp* reporter genes. The reporter protein exhibits specific luminescence, fluorescence, or color development as the detectable signal (Kisaalita, 2010). Although bioluminescent bacteria are found in nature, many bioluminescent cell sensors have been developed using genetically engineered cells. In contrast to biosensors that use pure enzymes or other molecular biorecognition elements, bioavailability and environmental parameters (temperature and pH), which influence cell sensitivity, are integrated into the biosensor response.

The overall performance of mammalian cell- and microorganismal cell-based biosensors differentiate themselves from other types in a number of traits, as summarized in Table 1 (Kintzios and Banerjee, 2015).

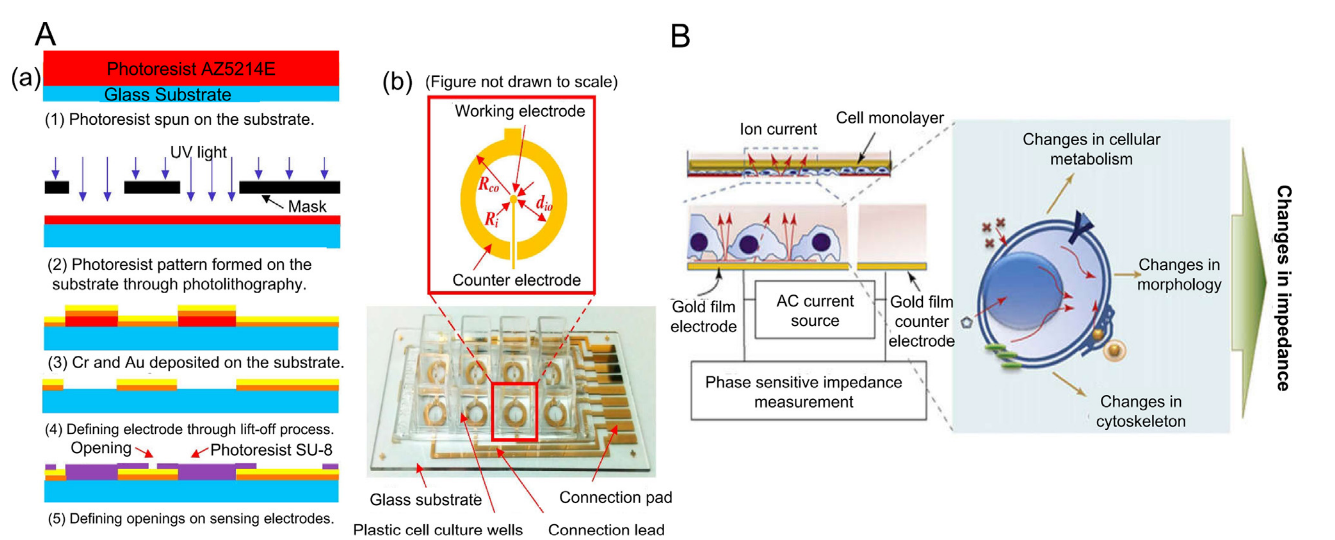

Fig. 1. A. (a) Illustration of ECIS sensor fabrication. (b) The array of eight ECIS sensors (X. Zhang et al., 2017). **B.** Schematic of the ECIS system (Giaever and Keese, 1993).

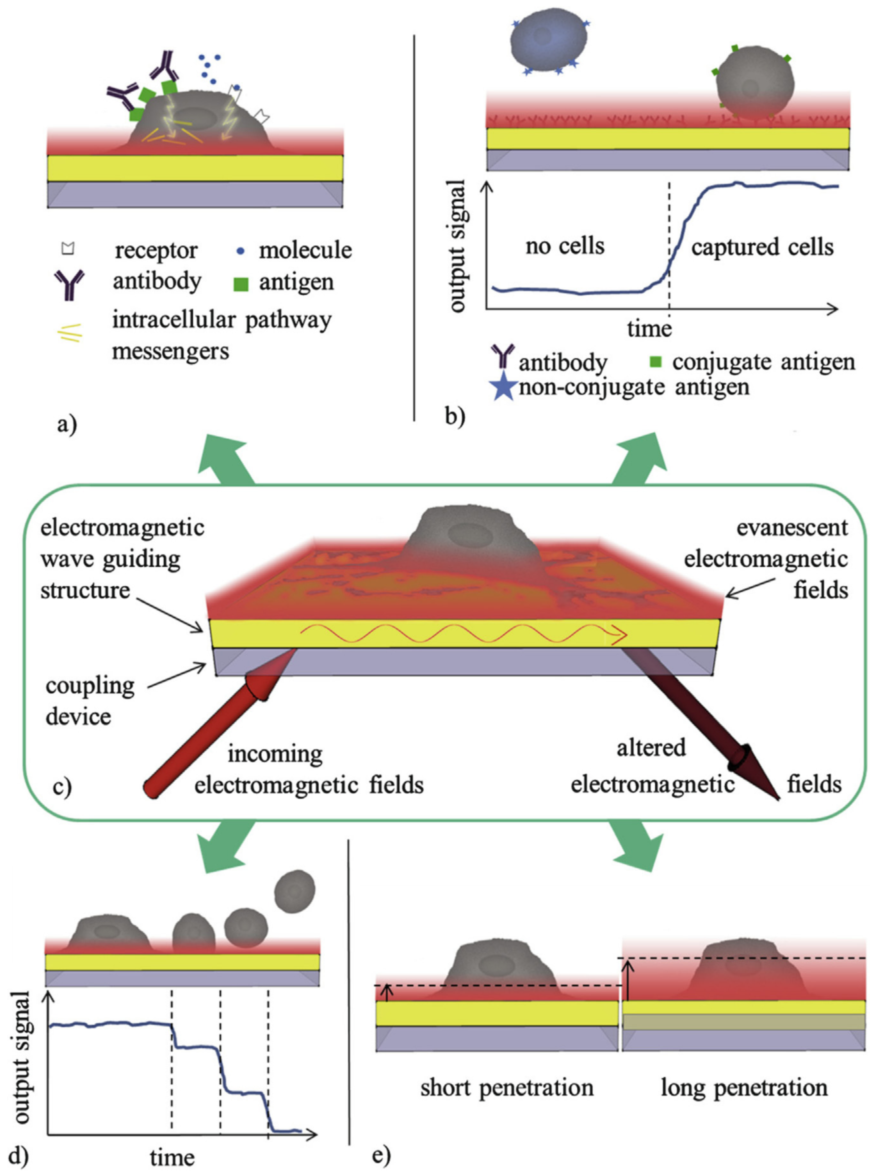

Fig. 2. Optical cellular biosensing scheme and its applications (Méjard et al., 2014).

Table 1
Comparative properties of two types of CBBs (Kintzios and Banerjee, 2015).

Trait	Microorganisms	Mammalian cells
Sensitivity	High	Very high
Specificity	Low	Low
Stability	Medium	High
Reproducibility	Low	Low
Storability	Very high	Low
Speed[a]	Low	Low to very high
Portability	Medium	Low to high
Cost	Low	High
Reusability	Possible	Rare
Online monitoring capability	Yes	Yes
Ease of use	Low	Low
Particular area of application	General toxicity Heavy metals, inorganic pollutants	General toxicity Heavy metals, inorganic pollutants Chemical residues Toxins

[a] The speed of the assay usually depends on the transducer and not the biorecognition element *per se*.

3. Applications of CCBs in food safety control

3.1. Foodborne pathogens analysis

Detecting and identifying foodborne pathogenic bacteria is essential to prevent and recognize problems in the food industry and other fields for health and safety reasons, as bacterial contamination causes serious disease and socioeconomic losses worldwide (Xu et al., 2016; Rohde et al., 2017). Protection against food spoilage has become a task of great social, economic, and public health importance, highlighting the need for rapid, selective, and easy-to-use testing of food substances for pathogens (Wang et al., 2017).

CBBs that identify and quantify pathogenicity induced by various foodborne pathogens have been reported by many researchers (Vidic et al., 2017; Carlyle et al., 2002). Banerjee and Bhunia (2010) presented three prototypes of a B lymphocyte Ped-2E9 CBB (Fig. 3) to rapidly detect a broad range of bacterial pathogens in food and beverages, such as *Listeria monocytogenes*, *enterotoxigenic Bacillus*, and *Serratia*. They used type I collagen mixed with Ped-2E9 cells in a three-dimensional scaffold cell culture to make the analyte-induced response more physiologically relevant. The three prototype devices successfully detected

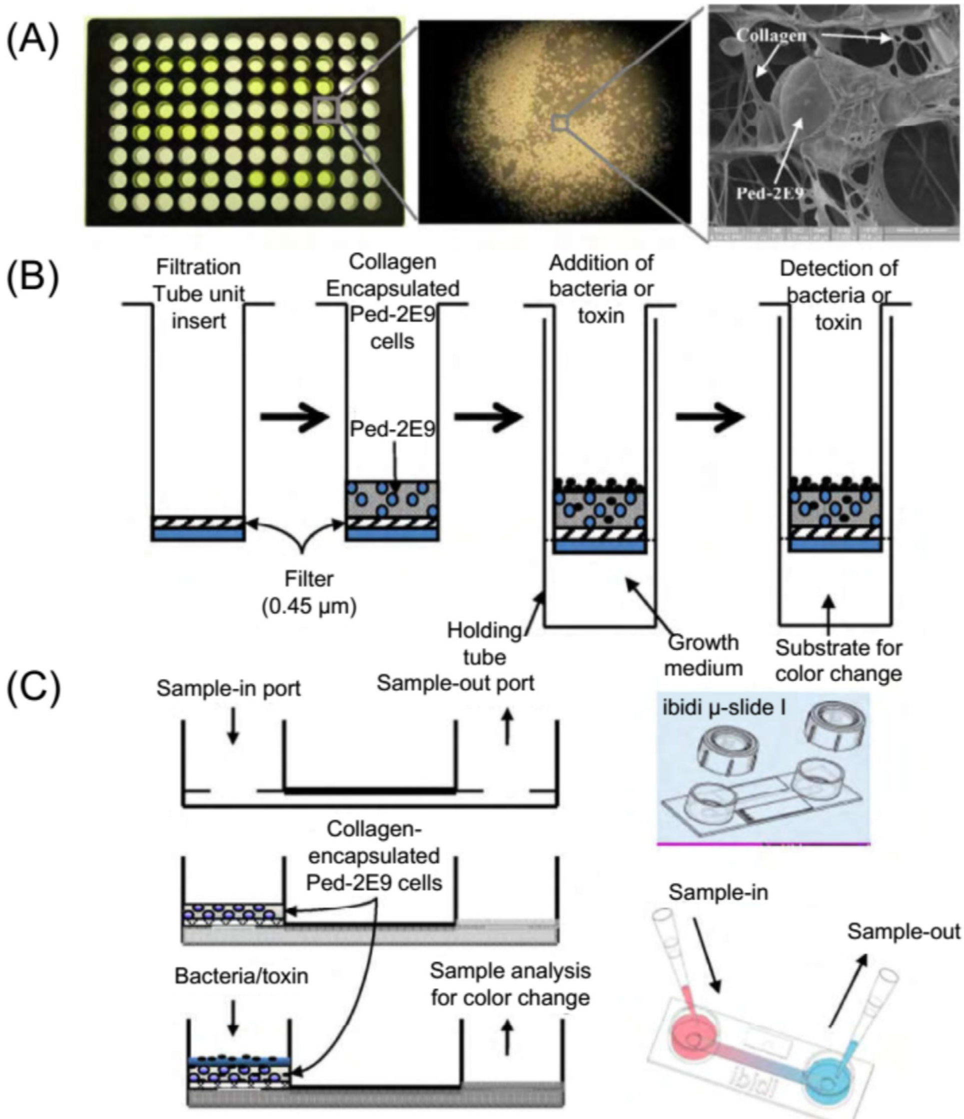

Fig. 3. Design of different cell-based sensor devices: (A) Device I (96-well plate), (B) Device II (Filtration tube), and (C) Device III (μ-Slide) (Banerjee and Bhunia, 2010).

a broad range of pathogens in food and beverages, even in a complex detection background. *L. monocytogenes* and *Bacillus cereus* were detected at a low initial concentration of 10^2–10^4 CFU/g in practical samples.

In a biosensor system, mast cells loaded with fluorescent dye (Fluo-4) have been used as indicators of pathogen-induced alkalinization of secretory granules (Curtis et al., 2008). The mast cells activated upon the interaction between *L. monocytogenes* and *E. coli* with the receptor, resulting in the release of fluorescent dye through spontaneous exocytosis. A linear regression to yield a rate of 0.0041 F/F0 per min for addition of *E. coli* within 210 s was obtained. The limitations of the specific environment, poor reproducibility, short self-life, non-specificity, and difficult preservation of CBBs are important. These deficiencies have been overcome with the development of genetically-engineered cell-based biosensors (GECBBs) (Velusamy et al., 2010). Genetically engineered B lymphocytes have been used to rapidly identify pathogens at very low levels based on the mechanism that exposure to a specific bacterium causes light to be emitted within seconds (Rider et al., 2003). As little as 500 CFU/g of *E. coli O157:H7* was detected in lettuce in < 5 min, the corresponding photon counts was about 500 which treated for 40 s. Because of the speed, sensitivity, and specificity, they suggested that this pathogen identification technology would be useful for food quality monitoring and other applications.

3.2. Toxicity evaluation and quantification of toxins

CBB is a novel strategy that has been used to detect or evaluate single toxins or combination of toxins, based on the response of the cells to toxic species, which is similar to the physiological response of a vulnerable subject (summarized in Table 2). The results obtained from toxin-cell interactions reveal functional information about the toxin on mode of action, toxic potency, bioavailability, and target tissue or organ.

Zearalenone (ZEN) and its metabolites were detected in milk and coffee cream using a bioluminescent whole-cell biosensor with a genetically modified *S. cerevisiae* strain (Välimaa et al., 2010). The limit of detection (LOD) varied from 1 (α-zearalanol and α-zearalenol) to 71 nM (β-zearalenol), with the correlated EC_{50} values (the concentrations yielding half of the maximum response) were in wide range of 41–1890 nM. Banerjee and Bhunia (2010) also used a CBB to detect toxins in food and beverages, such as μ-hemolysin, cholera toxin,

Table 2
Application of CBBs in food safety.

Transducer type	Cell line	Food	Target molecule	Linear Range	Limit of Detection	Refers
ECIS	BEL-7402 cells		DON ZEN	0.120 μg/mL 0.1–50 μg/mL	0.03 μg/mL 0.05 μg/mL	Gu et al. (2015)
ECIS	HeLa & HepG2 cells	Shellfish	Okadaic acid (OA)		10.2 μg/L 3.3 μg/L	Zou et al. (2016)
ECIS	Neuroblastoma cell	Shellfish	Saxitoxin, ouabain, veratridine		0.03 ng/mL	Zou et al. (2015)
Colorimetric	HepG2 cells	Shellfish	OA	10–800 μg/L	33.9532 μg/L	Su et al. (2017)
Colorimetric	HepG2 cells THP-1 cells	Shellfish	OA	10–800 μg/L	3.4083 μg/L 13.4456 μg/L	Su et al. (2018)
Potentiometric	Vero cells	Pistachio nut	AFB_1	0.15–30 ng/mL	0.5 ng/mL	Mavrikou et al. (2017)
Colorimetric	Ped-2E9	Milk	*C. perfringens*,		10 ng/mL	Banerjee and Bhunia (2010)
			B. cereus A926, *B. cereus MS1–9*, *B. subtilis*		10 ng/mL 10 ng/mL 40 ng/mL	
		Water	*V. cholerae*, *Stoichactis helianthus*		10 ng/mL 40 ng/mL	
Potentiometric	Vero cell		Aflatoxin M_1		5 pg/mL	Larou et al. (2013)
Optical	*Saccharomyces cerevisiae* strain	Milk	zearalanone α-zearalanol β-zearalanol zearalenone α-zearalenol β-zearalenol		2 nM 9 nM 15 nM 1 nM 1 nM 79 nM	Välimaa et al. (2010)
EIS	Rat basophilic leukemia (RBL-2H3) cell	Shrimp	Shrimp allergen Pen a 1	0.5–2.5 μg/mL	0.15 μg/mL	Jiang et al. (2013)
EIS	Cationic fluorescent magnetic bead-transferred RBL-2H3 mast cells	Crucian carp & brown shrimp	DNP-BSA, shrimp allergen Pen a 1, PV	1×10^{-3}-10^{4} ng/mL 0.5–4.5 ng/mL 0.1–0.5 ng/mL	3.3×10^{-4} ng/mL 0.16 ng/mL 0.03 ng/mL	Jiang et al. (2015)
EIS	RBL-2H3 mast cell		DNP-BSA			Jiang et al. (2016)
Potentiometric	*Escherichia coli* ATCC 11303	Milk	7 quinolones, 3 tetracyclines		0.25–0.8 MRL maximum residue limit	Pellegrini et al. (2004)
Bioluminescent	*E.coli*	Milk, egg, kindey, liver, muscle	TET, OTC, CTC		100–600 μg/kg	Kurittu et al. (2000a, 2000b)
Bioluminescent	*E.coli*	Milk	OTC		10 μg/kg	Hansen and Sorensen (2000)
Bioluminescent	*Bacillus cereus* ATCC 11778	Poultry muscle	TET		100 μg/kg	Pikkemaat et al. (2010)
Bioluminescent	*E.coli*	Milk	TET, OTC, CTC, DC, MTC, DCC, MC		2–35 ng/kg	Kurittu et al. (2000a, 2000b)
Bioluminescent	*Escherichia coli pK12* harboring plasmid pRecAlux3	Milk, fish and tissues of livestock	Enrofloxacin, Ciprofloxacin, Danofloxacin, Sarafloxacin, Marbofloxacin, Difloxacin, Ofloxacin, Pefloxacin, Norfloxacin, Lomefloxacin, Orbifloxacin	6.75–200 μg/kg, 6.75–200 μg/kg, 12.5–400 μg/kg, 12.5–400 μg/kg, 9.375–300 μg/kg, 25–1600 μg/kg, 12.5–400 μg/kg, 12.5–400 μg/kg, 12.5–400 μg/kg, 25–800 μg/kg, 25–800 μg/kg	12.5–100 μg/kg in 11 edible tissues	Cheng et al. (2014)
Bioluminescent	*E. coli* K-12	Fish tissue	Tetracycline, oxytetracycline		20 μg/kg 50 μg/kg	Pellinen et al. (2002)
Bioluminescent	*E.coli*	Poultry muscle	DC, CTC, TET, OTC		5, 7.5, 25, 25 ng/g	Virolainen et al. (2008)

(*continued on next page*)

Table 2 (*continued*)

Transducer type	Cell line	Food	Target molecule	Linear Range	Limit of Detection	Refers
Potentiometric	*P. aeruginosa*	–	cephalosporin C	0.1–11 mM		Kumar et al. (2008)
Potentiometric	*B.stearothermophilus* var	Milk	β-Lactam			Ferrini et al. (2008)
Potentiometric	Recombinant *E. coli*	Milk	Penicillin	5–30 mM		Galindo et al. (1990)
EIS	*Streptomyces* strain M7		Lindane	0–750 µg/L	120 µg/L	Rodriguez et al. (2015)
Conductometric	*Arthrospira platensis* cells	Water	Paraoxon-methyl Parathion-methyl Triazine Diuron		10^{-18} M 10^{-20} M 10^{-20} M 10^{-12} M	Tekaya et al. (2013)
Optical	MC3T3-E1cell		Glyphosate			Farkas et al. (2018)
EIS	*Streptomyces* strain M7		Lindane		120 µg/L	Rodriguez et al. (2015)
ECIS	BPAECs	Drinking water	Lindane			Curtis et al. (2009)
Amperometric	*P. putida* JS444		Paraoxon, Methyl parathion, parathion		55 ppb 53 ppb, 58 ppb	Lei et al. (2005)
Amperometric	*Moraxella* sp.	Water	Paraoxon		0.1 µM	Mulchandani et al. (2006)
Amperometric	*C. vulgaris*	Water	Atrazine		1 µmol/dm^3	Shitanda et al. (2009)
Amperometric	*C. vulgaris*	Water	Atrazine		2.0 µmol/dm^3	Shitanda et al. (2005)
Potentiometric	Recombinant *E. coli*	Water	Paraoxon Parathion Coumaphos		3 µM 3 µM 5 µM	Mulchandani et al. (1998a)
Potentiometric	Recombinant *E. coli*		paraoxon, ethyl parathion, methyl parathion, diazinon			Mulchandani et al. (1998b)
Potentiometric	Recombinant *E. coli*		Paraoxon	0.001–1.0 mM		Rainina et al. (1996)
Optical	*C. vulgaris*	Water	Diuron		1 µg/L	Nguyen-Ngoc and Tran-Minh (2007)
Conductometric	*Arthrospira platensis*	Water	Paraoxon-methyl Parathionmethyl Triazine Diuron		10^{-18} M 10^{-20} M 10^{-20} M 10^{-12} M	Tekaya et al. (2013)
Optical	*E. coli cells* containing periplasmic-expressing OPH	Tap water	Paraoxon	5–320 µM	5 µM	Kim et al. (2013)
EIS	*Arthrospira platensis* cell	Water	Cd^{2+}, Hg^{+}	10^{-20} –10^{-6} M	10^{-20} M	Tekaya et al. (2014)
Potentiometric	*Bacillus badius* cells	Milk	Cd^{2+}	10 µg/L–1 mg/L	1 µg/L	Verma et al. (2011)
Optical	pGL3-pd22–320-luc HeLa cells		$CdCl_2$ $Cd(NO_3)_2$ $NaAsO_2$	10–100 µM 5–100 µM 5–100 µM	10 µM 5 µM 5 µM	Mandon et al. (2005a, 2005b)
Optical	Surface-engineered yeast Cell		Cd	0–1 µM	0.2 µM	Matsuura et al. (2013)
Amperometric	Rec *E. coli*	Water	Cd^{2+}	50–1000 nM	50 nM	Biran et al. (2000)
Optical	*H. polymorpha*		Cd	1–900 µM		Park et al. (2007)
Optical	Rec *E. coli* DH5α	Milk	Cd^{2+}	10–50 µg/L	10 µg/L	Kumar et al. (2017)
Voltammetric	*P. cruentum*	Water	As^{3+}	2.5–20 µg/L	2.5 µg/L	Zaib et al. (2014)

DNP-BSA, Dinitrophenyl-bovine serum albumin; PV, fish allergen parvalbumin; MC3T3-E1, mammalian osteoblastic cell line; EIS, electrochemical impedance spectroscopy; BPAECs, bovine pulmonary artery endothelial cells; MRL, maximum residue limit; CdCl2, Cadmium chloride; Cd(NO3)2, Cadmium nitrate; NaAsO2, Sodium arsenate; OPH, organophosphorus hydrolase; Cd, cadmium; EF enrofloxacin; CF, ciprofloxacin; NF, norfloxacin; DF, danofloxacin; PF, pefloxacin; DFC, difloxacin; OF, ofloxacin; SF, sarafloxacin; MF, marbofloxacin; LF, lomefloxacin; OBF, orbifloxacin; TET, tetracycline; OTC, oxytetracycline; CTC, chlortetracycline; DC, doxycycline; MTC, metacycline; DCC, demeclocycline; MC, minocycline; VCM, vancomycin; GLP, glycopeptides; D-CLS, D-cycloserine; BTC, bacitracin; PCS, Penicillins; CPNS, cephalosporins; CPL, Chloramphenicol; Lux bacterial luciferase; *gfp*, green fluorescent protein; *lac*, galactosidase; MMC, mitomycin; NA, nalidixic acid; OFL, ofloxacin.

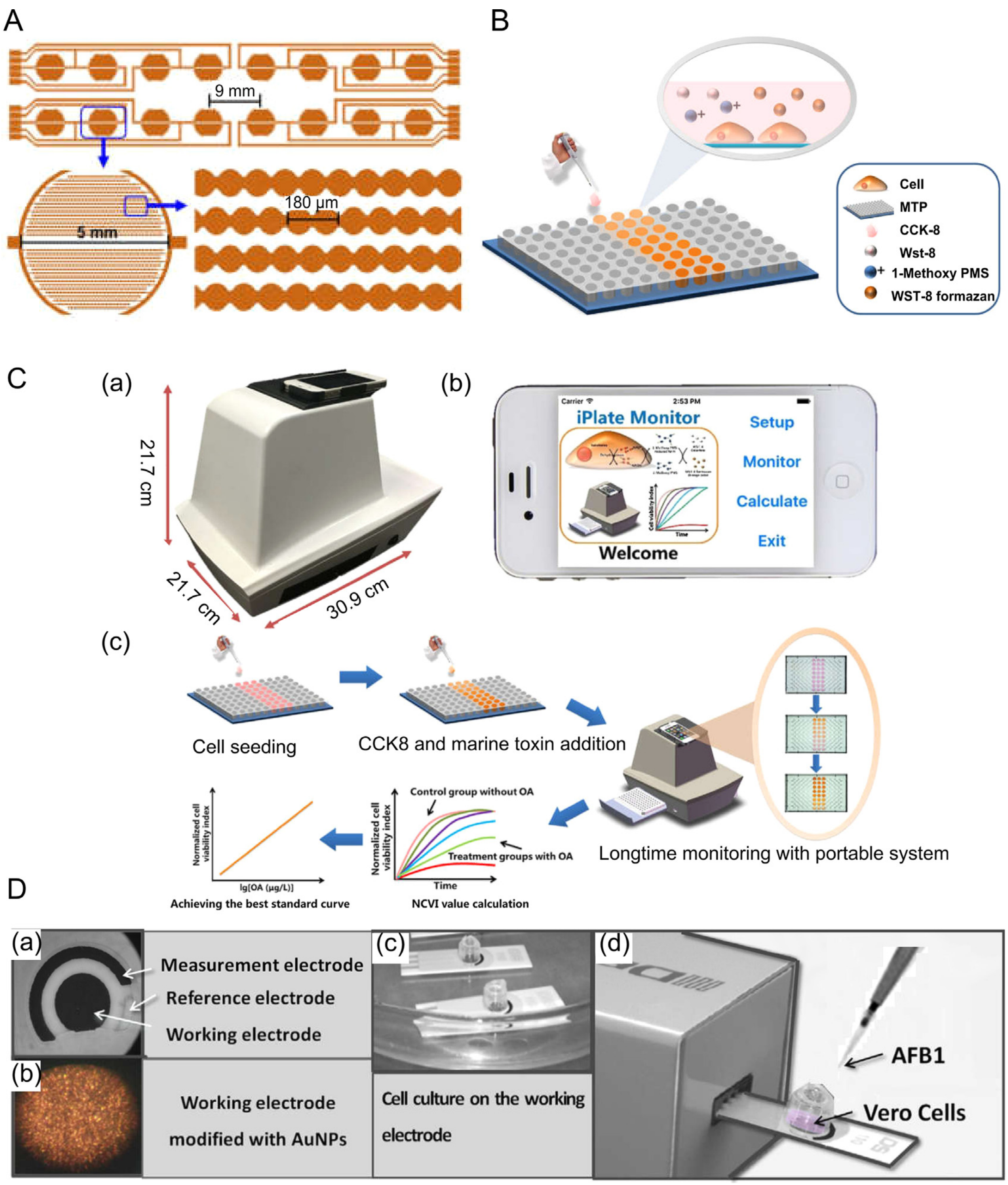

Fig. 4. A. The electrode structures of sensor chip (Zou et al., 2016). B. Construction of CVBS (Su et al., 2017). C. (a) The picture of Bionic e-Eye, (b) the main interface of iPlate Monitor and (c) the workflow schematic of OA measurement by using CVB and Bionic e-Eye (Su et al., 2018). D. CBB assembly: the three electrode structure (a) and the AuNPS on the working electrode (b), vero cells culture (c) and measurement process (d) (Mavrikou et al., 2017).

phospholipase C, and obtained a LOD of 10–40 ng in 2 h. Based on the antagonistic effect of paralytic shellfish poisoning (PSP) toxins on cells, Zou et al. (2015) designed a neuroblastoma cell-based impedance biosensor to detect PSP toxins (Fig. 4A). The LOD was 0.03 ng/mL, showing high sensitivity and good specificity for detecting saxitoxins.

A portable smartphone-based system using a HepG2 cell viability biosensor was developed to detect okadaic acid (OA) by Su et al. (2017) (Fig. 4B). This system synchronously detects OA in 96 channels, with a LOD of 33.9532 g/L and a wide linear working range of 10–800 g/L to achieve real-time image acquisition that is easy-to-use. Subsequently, they optimized and improved this system for OA spot fast detection (Fig. 4C) (Su et al., 2018). OA was detected at concentrations as low as 3.41 g/L and 13.45 g/L using HepG2 and THP-1 cells, respectively. The normalized cell viability index caused by OA were about 17.08 ± 1.93 and 20.19 ± 2.57 at 100 g/L and 300 g/L. Mavrikou et al. (2017) proposed a cellular-potentiometric biosensor based on Vero cells that were membrane engineered with anti-aflatoxin B_1 (AFB_1) antibody as recognition element reacting with AFB_1 molecules on modified screen printed electrodes (Fig. 4D). The peak current was increased (15–27 μA approximately) with the AFB_1 concentrations of 0–5 ng/mL, and a good linear range of 0.15–30 ng/mL with a LOD of 0.5 ng/mL was displayed.

Individual and combined toxicity of deoxynivalenol (DON) and ZEN were studied by electrochemical impedance spectroscopy (EIS) measurements with an impedance biosensor utilizing BEL-7402 cells (Fig. 5A) (Gu et al., 2015). The impedance values (R_{et}) decreased (9700 Ω to 8000 Ω, 9600 Ω to 7750 Ω) in the treatment times of 0–30 h for DON (5 μg/mL) and ZEN (10 μg/mL) with LOD of 0.03 and 0.05 μg/mL, respectively. A synergistic effect was found for a binary mixture of these two mycotoxins. This group also (Xia et al., 2017) developed a HepG2 cells-based electrochemical biosensor to assess the concomitant

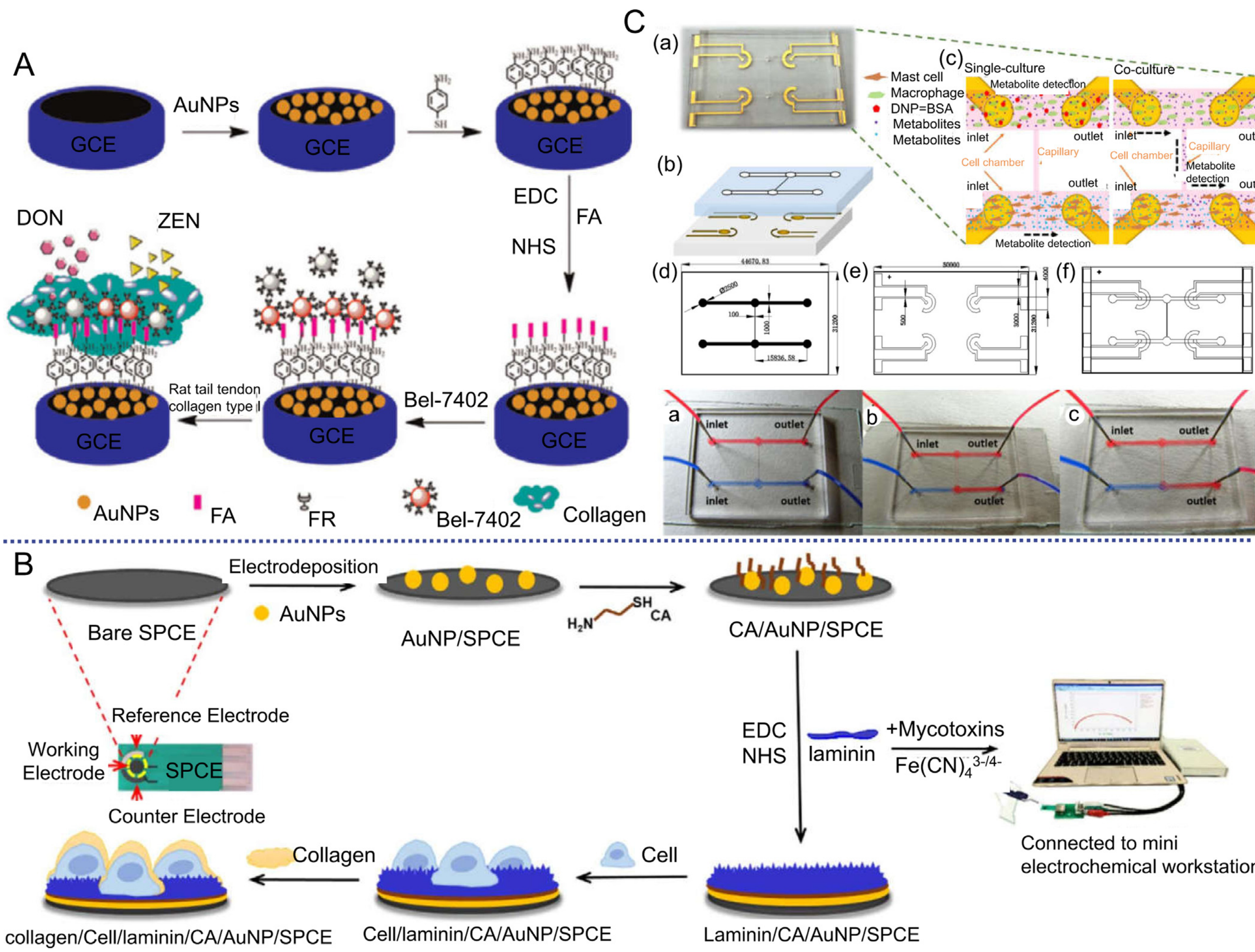

Fig. 5. A. and B. Schematic illustration of the preparation of modified electrode (Gu et al., 2015) and cell-based electrochemical sensor (Xia et al., 2017). C. Assembly and modification of a two-layer cell co-culture microfluidic chip (Jiang et al., 2016).

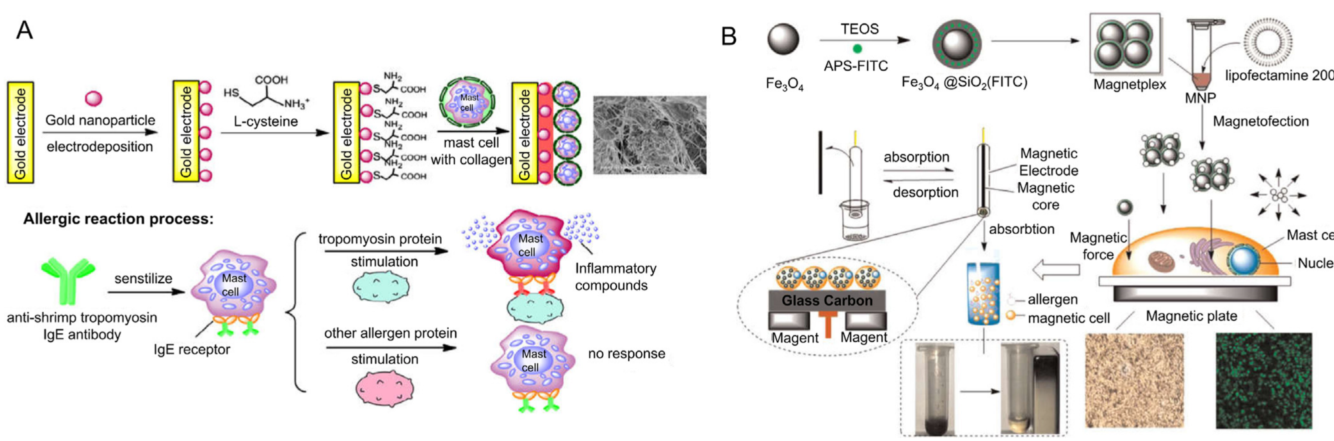

Fig. 6. A. Schematic illustration of the preparation of modified electrode (above) and principle of allergen reaction process t (below). Inset: for the SEM images cell-collagen 3D structure (Jiang et al., 2013). B. Schematic illustration of the preparation of magnetic fluorescent RBL-2H3 mast cell sensor and process of allergen detection (Jiang et al., 2015).

action of three mycotoxins (DON, ZEN, and AFB_1) (Fig. 5B). The extremely decreased R_{et} of AFB_1 (0.1–3.5 µg/mL) was range from 14,450 Ω to 3000 Ω, and different combined toxins showed a synergistic or an antagonistic effect. These mycotoxin cytotoxicity evaluation methods provide a new way for combined toxicity evaluations of various toxins in food.

3.3. Foodborne allergens analysis

Food allergies are a type I hypersensitivity immune response that can be life threatening (Wang and Sampson, 2012; Tammineedi and Choudhary, 2014). Patients face a high risk of accidental allergen exposure from adulterated products, undeclared substances, and crosscontamination, even with the best precautions (Cucu et al., 2013). As a result, there is an immediate and definitive need to develop and apply strategies to assess existing and new food allergens. More recent innovations combine biosensors with genosensors and cell assays (Table 2).

CBBs designed for detecting food allergens are commonly electrochemical and microfluidic sensors (Bahadır and Sezgintürk, 2015a, 2015b). In a study, a cell-based electrochemical biosensor was constructed for quantify of the shrimp allergen Pen a 1(tropomyosin) and assess its immunoglobulin E (IgE)-mediated hypersensitivity (Jiang et al., 2013). Rat basophilic leukemia (RBL-2H3) mast cells, encapsulated in type I collagen were immobilized on a self-assembled modified gold electrode to monitor IgE-mediated mast cell sensitization and activation (Fig. 6A). The impedance value increased linearly (approximately from 2400 to 8200 Ω) with the concentration of purified shrimp allergen Pen a 1 (tropomyosin) in the range of 0.5–2.5 μg/mL with a LOD of 0.15 μg/mL. Similarly, an electrochemical RBL-2H3 cell biosensor integrated with fluorescent magnetic beads was designed to detect and evaluate different allergens in foodstuffs by this group (Jiang et al., 2015). Fluorescein isothiocyanate was fused inside the SiO_2 layer of SiO_2 shell-coated Fe_3O_4 nanoparticles, and the as-synthesized fluorescent magnetic beads were encapsulated with lipidosomes to form cationic magnetic fluorescent nanoparticles for mast cell magnetofection (Fig. 6B). The R_{et} decreased as shrimp allergen Pen a 1 (tropomyosin) (4500–2400 Ω) and fish allergen parvalbumin (4600–2250 Ω) concentration increased ranging of 0.5–4.5 ng/mL and 0.1–1.5 μg/mL, respectively, and showed high accuracy for these two targets in real sample detection.

Microfluidics are increasingly popular in food safety monitoring methodologies due to their many advantages, including smaller reagent volumes, shorter reaction times, and higher sensitivity (Weng et al., 2016). An efficient food allergen system has been developed using the cell-mediated technique for microfluidic chips, including two cell cultivation microfluidic channels and four groups of gold electrodes, combined with culture of macrophages and a mast cell co-culture system (Fig. 5C) (Jiang et al., 2016). Microfluidic cell culture, food allergen-induced cell morphological changes, and cell metabolic measurements can be performed simultaneously using this device. Impedance signals were detected by electrochemical sensors when dinitrophenylated bovine serum albumin was stimulated. The results show that this cell-based electrochemical microfluidic biosensor is sensitive, with a detection range of 10^{-3}–10 ng/mL and a LOD of 10 ng/mL.

3.4. Antibiotics residue analysis

Most antibiotics are used to treat bacterial diseases in humans and animals. Excessive use of antibiotics promotes the development of bacterial resistance leading to decreased efficiency in treating human or animal diseases (McDermott et al., 2002; Nina et al., 2008). Monitoring antibiotics is very important for the quality and safety of food products and is necessary to ensure consumer safety (Wang et al., 2014; Mishra, Sharma and Bhand, 2014). The development of biosensors for screening antibiotic residues has been increasing since 1980s (Gaudin, 2017). Electrochemical and optical (fluorescent, bioluminescent, and colorimetric) CBBs have been developed for multianalyte detection of one or more classes of antibiotics (Su et al., 2011), such as tetracyclines (Korpela et al., 1998; Virolainen et al., 2008; Bahl et al., 2005), β-lactams (Ferrini et al., 2008; Smolander et al., 2009), chloramphenicol, ofloxacin (Shapiro and Baneyx, 2007), and quinolones (Ben-Yoav et al., 2009) (Table 2). CBBs that detect antibiotic residues have allowed for the simultaneous detection of multiple analytes within 30 min to 3 h.

Korpela (1998) constructed a CBB based on a bioluminescent *E. coli* K-12 strain to specifically detect tetracycline antibiotics. The CBB contained five genes from the *P. luminescens* bacterial luciferase operon. The optimal induction time of light emission was 90 min for this biosensor. When without existed Mg^{2+} ions, the maximal induction level was 100-fold over uninduced levels by using 20 ng of tetracycline, and picomole sensitivities were determined for seven different tetracyclines. Another study described a CBB to detect new producers of known macrolides or producers of macrolides, which is based on coupling of the *Vibrio fischeri* structural luciferase gene to the regulatory control mechanism of a bacterial erythromycin resistance operon (Mohrle et al., 2007). Moreover, a potentiometric microbial biosensor based on a pH electrode modified by permeabilized *P. aeruginosa* was developed to selectively detect cephalosporin antibiotics (Kumar et al., 2008). Hydrolysis of cephalosporin was accompanied by the production of protons near the pH electrode, then resulting in a change in the electric potential difference between the working and the reference electrodes.

3.5. Pesticides residue analysis

Although most pesticides are used within recommended limits, the bioaccumulation effect and continuous exposure can increase safety risks to human health (Bird et al., 2008). In addition, some new types of pesticides with highly effective activity, whose toxic mechanisms are not clearly understood, are being continuously commercialized (Nsibande and Forbes, 2016). Therefore, analyses of pesticide residues is needed to ensure food quality and safety and safeguard the ecosystem and protect human health from possible hazards. CBB technologies are useful to detect pesticide residues in food for their low cost, highly sensitive, rapidly responsive, and easy to operate (Zhang et al., 2014). Some of the CBBs applications in detecting pesticides in food are summarized in Table 2.

A whole cell array biosensor for detecting organophosphates (OPs) was developed by immobilizing recombinant *E. coli* cells containing periplasmic expressing organophosphorus hydrolase onto the surface of a 96-well microplate using mussel adhesive protein as a microbial cell-immobilizing linker (Kim et al., 2013) (Fig. 7A). The absorbance intensity increased (OD_{410} = 0.09–0.25) linearly with increasing paraoxon concentration in the range of 5–320 μM with a LOD of 5 μM, and the CBB showed good long-term stability (28 days with 80% retained activity) and a reusability (20 times). A portable automated bench-top mammalian CBB that incorporates enclosed fluidic biochips containing endothelial cells monitored by ECIS technology to detect lindane has been reported (Fig. 7B) (Curtis et al., 2009). The transducer cells can be maintained in the toxicity sensor device for at least 9 days using an automated media delivery system and survive for up to 4 months on the fluidic biochips and remain responsive to a model toxicant, longer-term storage for cells has become possible. Similarly, Rodriguez et al. (2015) used *Streptomyces* strain M7 as the sensing element for a biosensor to detect lindane based on the impedance changes produced in cells. The R_{et} drastically dropped orders of magnitude (from 1×10^{13} to 1×10^{6} Ω), during the 48 h of lindane consumption, and the LOD was 120 μg/L in < 2 days without sample pretreatment using this impedimetric biosensor.

Genetic modification clearly enhances specificity of a microbial biosensor. A microbial biosensor using recombinant *E. coli* cells showed an enhanced detection limit (2 mM) for all 3 OPs (paraoxon, methyl parathion and diazinon), and an improved response time (2 min) and stability for more than 2 months (Mulchandani et al., 1998a, 1998b). In addition, Tang et al. (2014) immobilized the same recombinant microbial cells on the surface of ordered mesopore carbons. The differential pulse voltammograms showed peak current (0–3.25 μA) was increase linearly with the increase of paraoxon (0.05–25 μM). LOD values of parathion, paraoxon, and methyl parathion were 10 nM, 9 nM, 15 nM, respectively.

3.6. Assess of heavy metals contamination

Heavy metal contamination is a critical threat to human health because these substances are nonbiodegradable. Some CBBs have been developed to detect heavy metals using different recombinant

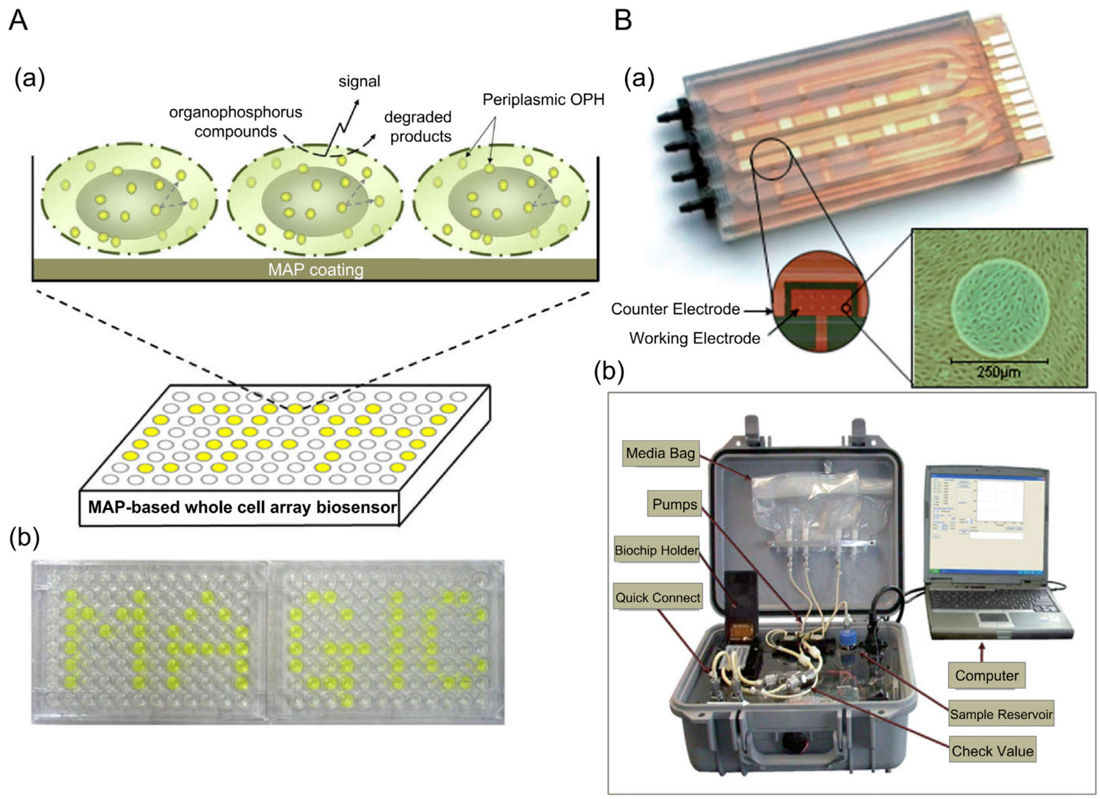

Fig. 7. A. Schematic representation of the whole cell array biosensor for the detection of Ops (Kim et al., 2013). B. The assembled fluidic biochip and portable CCB equipment (Curtis et al., 2009).

microbes. The biosensors used to detect heavy metals in food are summarized in Table 2.

A cell bioassay has been developed for total toxicity testing of liquid samples (Mandon et al., 2005a, 2005b). The assay is based on the induction of bioluminescent activity in genetically manipulated mammalian cells. A DNA sensing element was designed to direct the expression of a reporting gene (firefly luciferase) through activation of the *Drosophila melanogaster* hsp22 promoter, which responds to heavy metals, and was transfected in HeLa cells. However, this approach is not suitable to specifically detect cadmium ion because of the genotoxic and endocrine disruption activities needed to induce the *luc* gene expression. Kumar et al. (2017) used a specific recombinant *E. coli* DH5α (pNV12) biosensor to detect the highly toxic metal ion cadmium (Cd^{2+}) based on expression of *gfp* gene under control of the cad promoter and the cadC gene of *S. aureus* plasmid pI258. Fluorescence count has upturn (intensity: 310–342) with increasing Cd^{2+} concentration (10–50 μg/L), and showed a good linear relationship (R^2 = 0.9946). The LOD was 10 μg/L after 15 min, while the incubation time was increased to 30 min the LOD was 5 μg/L. Moreover, Tekaya et al. (2014) designed a bienzymatic biosensor by immobilizing *Arthrospira platensis* cells on gold interdigitated transducers (Fig. 8A). A widely detection linear for Cd^{2+} and Hg^{2+} range from 10^{-20} M to 10^{-6} M with a similar detection limit of 10^{-20} M, and there was no synergistic effect of the pollutant.

4. Applications of CBBs in food quality control

4.1. Quantification and functional evaluation of active compounds

Traditional detection of active compounds usually employs standard methods of analytical chemistry, such as thin layer chromatography (Wuthold et al., 2004) and high performance liquid chromatography (Kenstaviciene et al., 2008), which are superior for precise quantification but are not suitable for primary detection as complicated and time-consuming operation process and expensive detection system. CBBs for detecting active compounds and for functional evaluations have attracted attention due to numerous advantages of the assay. The CBBs present specific cellular responses to various chemicals and provide a cell-level evaluation of active factors, such as quantification and function. Moreover, the detection process is usually quick, and the operation is simple and convenient.

The generation of reactive oxygen species (ROS) in cells can be used to indirectly assess changes in intercellular oxidative stress by detecting variations in electrochemical signals, and evaluating the antioxidant effect of active compounds. A Caco-2cell-based electrochemical biosensor based on platinized gold electrode modified with silver nanowires was developed to determine the antioxidant activity of *Asp-Leu-Glu-Glu* (DLEE) isolated from dry-cured Chinese Xuanwei ham (Fig. 8B) (Xing et al., 2018). The H_2O_2 level was detected using redox signaling, the peak values for DLEE and GSH treatment ranged from 53.53 to 63.77 μA and 52.78–62.09 μA respectively at the concentrations of 0.5, 1.0 and 1.5 mg/mL. The LOD of this biosensor system was 0.12 μM, with a linear relationship from 0.2 to 2 μM for peptides, which showed relatively outstanding catalytic effects towards the reduction of H_2O_2. The antioxidant effect of phloretin (Ph) has also been researched by a CBB based on an A549 cells-modified working electrode (Fig. 10F) (Ye et al., 2018). Under optimized conditions, the response impedance of the biosensor was linear to Ph concentrations from 20 μmol/L to 100 μmol/L (R_{et} was decreased from 2211.225 Ω to 1795.967 Ω) with a LOD of 1.96 μmol/L. A significant correlation was also observed between the ROS levels and the impedance values following the concentrations of Ph.

Another group successfully constructed an electrochemical microbial biosensor for detecting phenolic compounds by directly adsorbing engineered *E. coli* MB275 cells expressing surface-immobilized laccase on a glassy carbon electrode (Z. Zhang et al., 2017). Under optimized

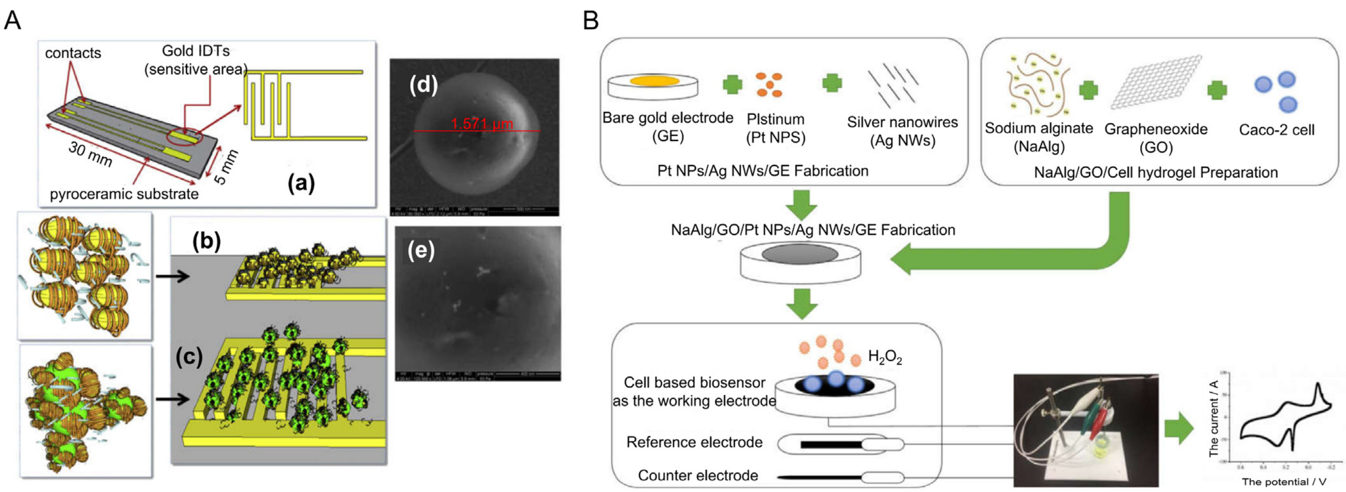

Fig. 8. A. Sensor design for heavy ion detection (Tekaya et al., 2014). B. The working process for the detection of H2O2 in Caco-2 cell-based Pt NPs/Ag NWs/GE biosensor (Xing et al., 2018).

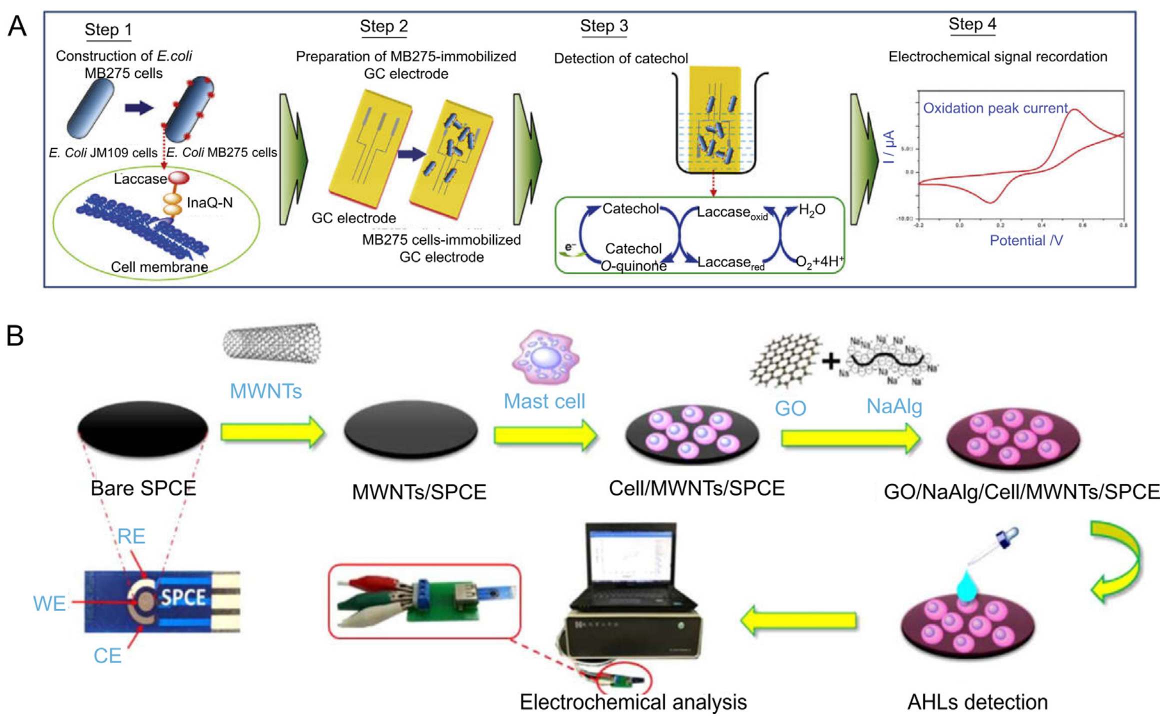

Fig. 9. Schematic illustration for the modification of cell sensors and process of A. catechol detection (Zhang et al., 2018) and B. AHLs detection (Jiang et al., 2018).

pH conditions, the electrochemical response of the biosensor was linear within a concentration range of 5.0–500.0 μM for five phenolic compounds (catechol, caffeic acid, dopamine, gallic acid, and 2-amino phenol) with a LOD of 1.0–5.0 μM. They attempted to improve sensitivity of the CBB (Fig. 9A) (Zhang et al., 2018). As a result, the LOD was as low as 0.1 μM with a linear relationship from 0.5 μM to 300.0 μM (corresponding current value was about of 0.50–65.02 μA) for catechol under the optimized conditions. This system exhibited good stability, reproducibility, and offered a considerable level of accuracy for determining catechol in red wine and tea samples.

4.2. Food processing and storage control

As quality control is important in the food industry, rapid, affordable, and specific analytical tools are needed to assure the quality of food products, and processing controls are needed. CBBs, particularly microbial biosensors, have filled the gaps to ensure the requirements imposed by customers and governments (Pacheco et al., 2017). Table 3 shows some examples of microbial biosensors used to control food industrial bioprocesses.

An increasing number of microbial biosensors have been developed to monitor food fermentation processes (Dai and Choi, 2013). Isolated bacteria, such as *Candida tropicalis* (Akyilmaz and Dinckaya, 2005), *Gluconobacter oxydans* (Valach et al., 2009), and *Methylobacterium organophilium* (Wen et al., 2012) have been used to determine the ethanol generated during fermentation. Several strategies were used to immobilize the bacteria on the transducers to improve stability and operability, such as glutaraldehyde, gelatine (biopolymer), O-ring rubber, dialysis membrane, and other polymers (chitosan and nanomaterials) (Akyilmaz and Dinckaya, 2005; Valach et al., 2009; Tuncagil et al., 2009; Sefcovicova et al., 2015). Phenyl acetic acid (PA) is used as a flavor in the food industry, and is produced biotransformation of 2-phenylethanol (2-PE) (Molinari et al., 1999). A microbial biosensor for detecting 2-PE was developed using *G. oxydans* as a biorecognition

Table 3
Application of CBBs in food quality control.

Transducer Type	Cell line	Food	Target Molecule	Linear Range	Limit of Detection	Refers
CV, DPV, and EIS	Caco-2 cell	Dry-cured Xuanwei ham	Peptide Asp-Leu-Glu-Glu (DLEE)	0.2–2 µM	0.12 µM	Xing et al. (2018)
Impedence	Bioengineered HEK-293 cell		Salicin		0.055 mM	Hu et al. (2017)
Optical	*E. coli*		Shikimic acid	0–20 mM		Li et al. (2017)
CV	Engineered *E. coli* MB275 cells	Red wine, tea	Catechol, caffeic acid, dopamine, gallic acid, and 2-amino phenol	5.0–500.0 µM	1.0–5.0 µM	Zhang et al. (2017a, 2017b)
CV	Engineered *E. coli* MB275 cells	Red wine, tea samples	Catechol	0.5–300.0 µM	0.1 µM	Zhang et al. (2018)
EIS	RBL mast cells	Fish	$3OC_{12}$-HSL	0.1–1 µg/mL	0.094 µg/mL	Jiang et al. (2018)
Amperometric	*Candida tropicalis*	Liqueur, beer, and wine	Ethanol	0.5–7.5 mM		Akyilmaz and Dinckaya (2005)
Amperometric	*Methylobacterium organophilium*	Alcoholic drinks	Ethanol	0.050–7.5 mM	0.025 mM	Wen et al. (2012)
Amperometric	*G. oxydans*	Monitoring of alcoholic fermentation	Ethanol	10 µM–1.0 mM	5 µM	Sefcovicova et al. (2012)
FIA-amperometric	*G. oxydans*	Monitoring of alcoholic fermentation	Ethanol	10 µM–1.5 mM	3.3 µM	Valach et al. (2009)
Amperometric	*G. oxydans*		Glycerol	0.02–2 mM	20 µM	Tkáč et al. (2000)
Voltammetric	*E. coli* BL21	Beer, peach juice, and product of lignocellulose degradation samples	Glucose and xylose	0.25–6 mM	0.1 mM	Li et al. (2013)
Amperometric	*P. alcaligenes MTCC 5264*	Instant coffee and tea	Caffeine	0.1–1 mg/mL		Babu et al. (2007)
FIA-amperometric	*G. oxydans*		Glycerol	5–100 mM	103 µM	Ergön-Can et al. (2015)
Amperometric	*G. oxydans*	Monitoring of 2-phenylethanol biooxidation process	2-Phenylethanol	0.02–0.70 mM	0.1 µM	Schenkmayerova et al. (2015)
EIS	NCI-H716 cells expressed with sweet taste receptors (T1R1/T1R3)		Sucrose, HCl, NaCl, and $MgSO_4$, sucrose solutions	0.017–0.2 M		Wang et al. (2010)
LAPS	Taste bud cells from adult female SD rats		Mixture composed of NaCl, sucrose, glumate, etc.			Zhang et al. (2008)
LAPS	Taste bud cells from Sprague Dawley rat		acidic			Chen et al. (2009a, 2009b)
LAPS	Primary taste cells from SD rat		$MgSO_4$, denatonium, D-(-)-salicin			Wu et al. (2012a)
EIS	Taste receptor cells (NCI-H716 and STC-1 cells)		13 sweetener mixtures, 7 tastant mixtures (sucrose,			Hui et al. (2014)
MEA	HEK-293 cells with PKD channel transfection		Hydrochloric acid $MgSO_4$, monosodium glutamate, sucrose			Wu et al. (2012b)
Electrochemical	*M. vulgaris*	Biscuit, Beer, Soup, Vinegar	Sulfte	0.2–1.8 µM	0.2 µM	Sezgintürk and Dinçkaya (2005)
Optical	*Pseudomonas* sp. P2		Delor 103	0.5–5 mg/L	0.5 mg/L	Gavlasova et al. (2008)
Optical	*Pseudomonas* sp. P2		2,4,4'-trichlorobiphenyl	0.5–5 mg/L	0.2 mg/L	Gavlasova et al. (2008)

FIA: flow-injection analysis, GA, glutaraldehyde; GCE, glassy carbon electrode; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes; LAPS, light-addressable potentiometric sensor; SD, Sprague Dawley; MEA, micro electrode array; 3OC12-HSL, N-3-oxododecanoyl homoserine lactone.

element. They immobilized the bacteria onto a modified Clark-oxygen electrode and successfully off-line monitored the entire course of 2-PE bio-oxidation process to produce PA with sensitivity of 864 nA/mM (Schenkmayerová et al., 2015). The change in oxygen concentration depended on the 2-PE concentration, and a linear range from 0.02 to 0.70 mM was obtained with a LOD of 1 μM.

Control of food freshness is of growing interest for consumers and the food industry. Jiang et al. (2018) constructed an electrochemical biosensor based on RBL-2H3 mast cells to detect the N-acylhomoserine lactones, which are bacterial quorum signaling molecules that often used as indicators to evaluate freshness (Fig. 9B). RBL-2H3 mast cells encapsulated in alginate/graphene oxide hydrogel were immobilized on a multi-walled carbon nanotubes/screen-printed carbon electrode (SPCE) to serve as a recognition element. A linear response of impedance (decreased from 3700 Ω to 2250 Ω) to N-3-oxododecanoyl homoserine lactone was obtained in the range from 0.1 to 1 μM, and the LOD was 0.094 μM, which provided a new avenue for real-time monitoring of spoilage bacteria during freshwater fish production.

5. Other applications in food analysis

Sour, sweet, bitter, and salty tastants elicit action potentials in taste receptor cells (TRCs). An analysis of these action potentials indicated that the firing patterns correlate with taste modality coding (Jung et al., 2004). Taste CBBs are based on electronic tongue research, in which culture taste living cells are placed on the surface of chips (Wang et al., 2007). At present, various sensors have been utilized for taste cell- and receptor-based chemical sensing, including field effect transistor sensors, light-addressable potentiometric sensors (Zhang et al., 2008; Chen et al., 2009b, 2011), extracellular recording micro electrode array (Wu et al., 2012a), electrochemical sensors (Wang et al., 2010; Hui et al., 2012), and mass sensitive quartz crystal microbalance devices (Wu et al., 2013).

A biosensor system has been developed using human sweet taste-receptor enteroendocrine NCI-H716 cells to distinguish four basic tastants and sucrose solutions of seven concentrations (Fig. 10A) (Wang et al., 2010). The four basic tastants were distinguished by their maximum signal-to-noise ratio and relevant noise intensity, while the SPCE with the mammalian cell line without gustducin, TIR2, and T1R3 expression lacked the ability to detect the tastants. The authors proposed that the variation of EIS contributed to the cellular Ca^{2+} resonance. Different taste cells or receptors are better suited with different sensor types and designs. Chen et al. (2009a) designed a CBB that integrated acid-sensing taste receptor cells with LAPS to investigate electrophysiological signals of taste receptor cells and realize the sour sensation in a biomimetic manner [more information about the theory of LAPS showed by Wang et al. (2007)] (Fig. 10B). Results showed that the magnitude of ΔR (Spikes/$Spikes_{total}$) was proportional to the firing rate, while the power of the characteristic frequency in response to sour stimuli was 28.9 ± 0.97. This group also used LAPs combined with TRCs to test the taste cell response to taste stimuli of NaCl, HCl, $MgSO_4$, sucrose and glutamate (Fig. 10C) (Zhang et al., 2008).

Furthermore, a TRCs-LAPS hybrid biosensor that mimics nature's gustatory system to detect bitter taste was developed, which may have great potentials to be applied for food safety (Fig. 10D) (Wu et al., 2012b). The results of detecting bitter substances demonstrate that this TRC-LAPS hybrid biosensor successfully discriminated three different bitter substances tested in this study. Hui et al. (2014) investigated several sweeteners, bitter tastants, and mixtures of tastants discriminated from complex chemical mixtures using NCI-H716 CBB or enteroendocrine STC-1 CBB (Fig. 10E). Results showed that the tastant perception abilities of the STC-1 cell-based sensor are much better than that of the NCI-H716 cell-based sensor.

Moreover, CBBs are also useful to detect food additives, such as L-glutamate, and sweeteners (Song et al., 2015). Nafion and multiwalled carbon nanotubes have been used to incorporate glucose oxidase and xylose dehydrogenase displayed on a whole cell from *E. coli* BL21 cells onto a glass carbon electrode (Li et al., 2013). This electrochemical microbial biosensor was developed to identify two major sweeteners (D-glucose and D-xylose) used in food processing. Linear concentrations of D-glucose (reduction peak around 0.5 V) and D-xylose (oxidation peak current at 0.55 V) were in the range of 0.25–6 mM and 0.25–4 mM with a low LOD for both 0.1 mM.

6. Conclusions and future perspectives

As summarized above, CBBs have mainly been focused on analyses of food security (pathogens, toxins, allergens, pesticide residue, etc.), food composition, and processing control. They play a vital role as powerful analytical devices in the food industry by providing fast, cost-effective, highly sensitive, and specific measurements. Moreover, integrating nanomaterial and gene recombination technologies improve some analytical features and allows the possibility of new features, such as new platforms for cells immobilization, continuous monitoring, and higher sensitivity.

It cannot afford to ignore that the practical applicability of CBBs and their commercialization are greatly influenced by their storage stability. The stability properties of CBBs are considerable attributed to the biosensor type, cell line and modification methods. For mammalian cells, the storage time is relatively short, and the requirements for operation and storage environment are strict, including temperature, sterility, storage method, etc. Recently, several studies have shown excellent detection and long-term storage stability of CBBs. The stable trend of electrochemical signal changes of the fish juice samples based on RBL cells biosensor within 10 days verified the CBB can be effectively for stable detection by Jiang et al. (2018). Another report manifested that, endothelial cells survive for > 4 months on the fluidic biochips and remain responsive to a model toxicant by using an automated media delivery system (Curtis et al., 2009). Moreover, microorganisms show longer lifetime and higher activity as the sensing element, and microbial CBBs exhibit good long-term storage and operational stability (Tekaya et al., 2014; Tang et al., 2014; Mulchandani et al., 1998). A very low RSD (1.91%) was obtained for 12 days measurement using a microbial CBB, and this CBB had excellent storage stability for 28 day with a retention of 80% activity (stored at 4 °C) (Kim et al., 2013). In another research, the initial biosensor sensitivity after 57 days was even maintained 94% and still 55% after 127 days of storage (also stored at 4 °C).

Despite all this, CBBs still need improvement:

- Only limited information can be obtained about the biomolecular nature of the observed responses, which is still the main shortcoming.
- The culture and maintenance of selected cells with functional phenotypes is one of the critical hurdles in CBBs systems.
- The insufficient functionality and cytotoxicity of some existing modified components, novel biomaterial substitutes are urgently required.
- Some deficiencies for recombined cells, such as their narrow detection range, unstable expression of fluorescence reporter genes, poor reproducibility and stability, which are all greatly affected by external conditions.
- How to keep the cells in a healthy state, turn traditional cells into portable devices, develop new immobilization technologies, and utilize cell fusion technology to develop efficient, stable, and toxicity tolerant whole-cell sensors are the main challenges of CBBs development.

Further improvements of CBBs in the food field can be addressed by the following aspects: (1) new reporting genes with faster and stronger expression need to be discovered and recombined with cells, and the feasibility of using dual reporting genes to expand the detection scope

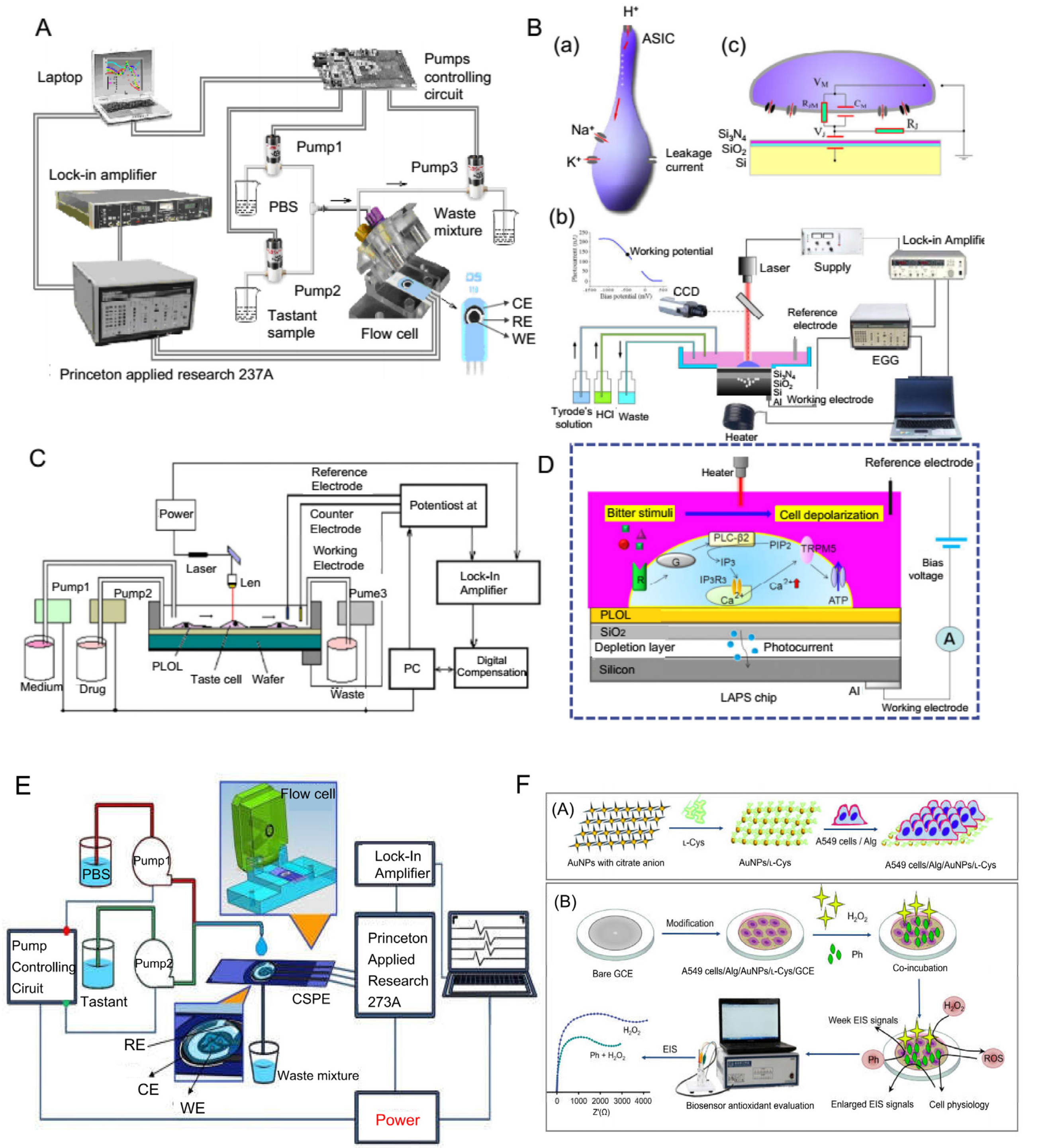

Fig. 10. Schematic diagram of biosensor system for taste detection, reproduce from published papers: A. Wang et al. (2010). B. Chen et al., 2009a, 2009b. C. Wu et al., 2012a, 2012b. D. Wu et al. (2012b). E. Hui et al. (2014). F. Schematic illustration for the preparation of A549 cell-based sensor (A) and process of antioxidant evaluation (B) (Ye et al., 2018).

should be investigated, (2) multi-channel technology needs to be developed for detecting multiple compounds based on different excitation wavelengths of different fluorescence proteins and bioluminescence resonance energy transfer, (3) CBB arrays for high throughput detection need to be developed, (4) development of CBBs for the continuous monitoring of the physiological and biochemical changes of cells under sample loading.

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