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REVIEW ARTICLE

Biosensor for the detection of *Listeria monocytogenes*: emerging trends

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ABSTRACT

The early detection of *Listeria monocytogenes* (*L. monocytogenes*) and understanding the disease burden is of paramount interest. The failure to detect pathogenic bacteria in the food industry may have terrible consequences, and poses deleterious effects on human health. Therefore, integration of methods to detect and trace the route of pathogens along the entire food supply network might facilitate elucidation of the main contamination sources. Recent research interest has been oriented towards the development of rapid and affordable pathogen detection tools/techniques. An innovative and new approach like biosensors has been quite promising in revealing the foodborne pathogens. In spite of the existing knowledge, advanced research is still needed to substantiate the expeditious nature and sensitivity of biosensors for rapid and *in situ* analysis of foodborne pathogens. This review summarizes recent developments in optical, piezoelectric, cell-based, and electrochemical biosensors for *Listeria* sp. detection in clinical diagnostics, food analysis, and environmental monitoring, and also lists their drawbacks and advantages.

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Introduction

Foodborne diseases are delineated by high incidence and low mortality rates. Bacteria such as *Staphylococcus aureus, Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, *Yersinia enterocolitica*, etc. are some of the foodborne pathogens. In case of *L. monocytogenes*, the causative agent of listeriosis, the situation is different with low incidence ranging from 0.1 to 11.3 per 1 00 000, and high hospitalization (90%) and mortality (20–30%) rates (EFSA 2015; Soni, Singh, et al. 2015; WHO SEARO 2017). Recently, it has been adjudged as the life-threatening pathogen, especially in the high-risk population groups such as pregnant women, neonates, elderly, and immunocompromised people leading to abortion, stillbirth, septicemia, meningitis, and meningoencephalitis (Soni and Dubey 2014; WHO 2017).

L. monocytogenes is ubiquitous in the environment due to its ability to survive against a wide range of pH and salt concentrations and low-temperature conditions (Soni, Singh, et al. 2015; Soni et al. 2017). Human population is consistently exposed to *L. monocytogenes* as it frequently contaminates raw produce, and cross-contaminates other food items (Pouillot et al. 2016). Numerous outbreaks of listeriosis have been reported in countries like USA, Japan, New Zealand, Germany, France, England, and other European countries (Warriner and Namvar 2009). In 2014, several countries reported increasing incidence of human listeriosis and the highest annual number of deaths since 2009 (EFSA 2015). The increasing incidences undoubtedly are due to modifications in surveillance programs, enhanced coverage of disease, and changes in the consumption behaviour. The minimal infectious dose for listeriosis is reported to be at least 100 colony-forming units (cfu) per gram of food (Buchanan et al. 2017). However, most countries have a zero-tolerance policy towards the presence of *L. monocytogenes* in ready to eat foods due to the possible hazards caused by the pathogen (Jadhav et al. 2012). Therefore, detection of the pathogen even in low numbers in different environmental as well as food samples using rapid, accurate, and reliable techniques, has become the necessity. Appropriate detection plan will certainly help in environmental monitoring with necessary examination and implementation strategies to prevent potential contamination of the product

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by *L. monocytogenes*. This will not only help to restrict the spread of listeriosis, provide the effective control measures, but also minimize the unnecessary recalls of food products.

The extensive research in recent decades has given insights into the various methodologies adopted for *L. monocytogenes* detection. The gold standard method for identification of *Listeria* sp. is based on colony morphology, sugar fermentation, and haemolytic properties. However, these culture-based methods are lengthy (require up to 5–7 days), and may not be appropriate to test foods with short shelf life (Chen et al. 2005; Yang et al. 2005; Jadhav et al. 2012; Datta et al. 2013). These traditional methods are therefore not appropriate for food industries where a more rapid testing procedures to detect the pathogen from stressed conditions is required (Bhunia 2014). Longer time required for media and plate preparations, colony counting and biochemical characterization of the isolated colonies, prohibit the use of these methods in food industries where time and cost are the issues (Bhunia 2014). Consequently, more rapid procedures based on antibodies such as an enzyme linked immunosorbent assay (ELISA), flow cytometry, matrix-assisted laser desorption/ionization (MALDI), and molecular-based techniques (DNA hybridization, polymerase chain reaction (PCR), and microarray) were developed (Ingianni et al. 2001; Volokhov et al. 2002; Palumbo et al. 2003; Sergeev et al. 2004; Chemburu et al. 2005; Berrada et al. 2006; O'Grady et al. 2008; Soni et al. 2013; Soni et al. 2014; Soni, Nath, et al. 2015). The quantity of sample required for detection, amount of the waste produced, availability of pure antigens, long enrichment time, and the use of expensive chemicals as well as the need for specialized equipments, limit their use in food industries. Further, these approaches are limited to their use in industry owing to major challenges in sample preparation, recovery, and revival of bacteria from stress, biofilm, and other environmental stresses (Bhunia 2014). In-depth reviews of the aforementioned methods for *Listeria* detection are available (Allerberger 2003; Gasanov et al. 2005; Churchill et al. 2006; Liu 2006; Zunabovic et al. 2011; Jadhav et al. 2012; Bhunia 2014). Recent advancements in biosensors for detection of *L. monocytogenes* require a critical assessment of the present state-of-the art.

Biosensors have the potential to provide fast, direct, and on-site analysis/detection of the pathogen that enables rapid monitoring of significant control points during food processing. The high accuracy and sensitivity of biosensing techniques is the key to its increasing applications in environmental monitoring, clinical diagnostics, food quality control, etc. The opportunities for biosensors in bacterial detection are wide open, especially in clinical diagnostics as well as in food industry for the real-time estimation of contamination. Therefore, the aim of the present review is to give a detailed and systematic description of the utility of various available biosensors designed for the accurate and quick *L. monocytogenes* detection. An overview of the current advancements in biosensing tools that use optical, piezoelectric, cell-based, and electrochemical biosensors for the detection of *L. monocytogenes* has been described. Further, a section on future research requirements is included in order for accomplishing the broad laboratory definition of *L. monocytogenes* detection.

Biosensors for *L. monocytogenes* detection

The detection of *L. monocytogenes* is of utmost importance primarily for the food industry, water and environment quality control, and public health. Recently, biosensors are being used for pathogen detection. In this technology, biological receptors are combined with physical or chemical transducers, and thus it represents a unique tool with the greater potential for rapid, online, and real-time detection of biological agents. The basic model for the utility of biosensors involves pathogen detection from food, humans, animals and also, the samples from environmental sources (Figure 1). Biosensor consists of a biomolecule (e.g. tissue, microorganism, cell receptors, organelles, antibodies, nucleic acids, enzymes, etc.), biologically derived molecule (e.g. engineered proteins, recombinant antibodies, aptamers, etc.), or the biomimic (e.g. combinatorial ligands, synthetic catalysts, imprinted polymers, etc.) which interact with the target analyte, and the signal obtained indicates presence of a specific analyte in a given sample. The most frequently used signals include electrochemical, optical (UV, bioluminescence, fluorescence, etc.), thermometric, piezoelectric, magnetic, and micromechanical transducers. For the detection of *L. monocytogenes,* various types of biosensors have been developed till date. Figure 2 shows the schematic representation of the various types of biosensors, classified on the basis of their biological elements and transducers. In the following section, optical, piezoelectric, cell-based, and electrochemical biosensors are reviewed in detail owing to their wide applicability for the detection of *L. monocytogenes*.

Optical biosensors

The optical biosensors are analytical device, able to sense the interaction between optical field and the

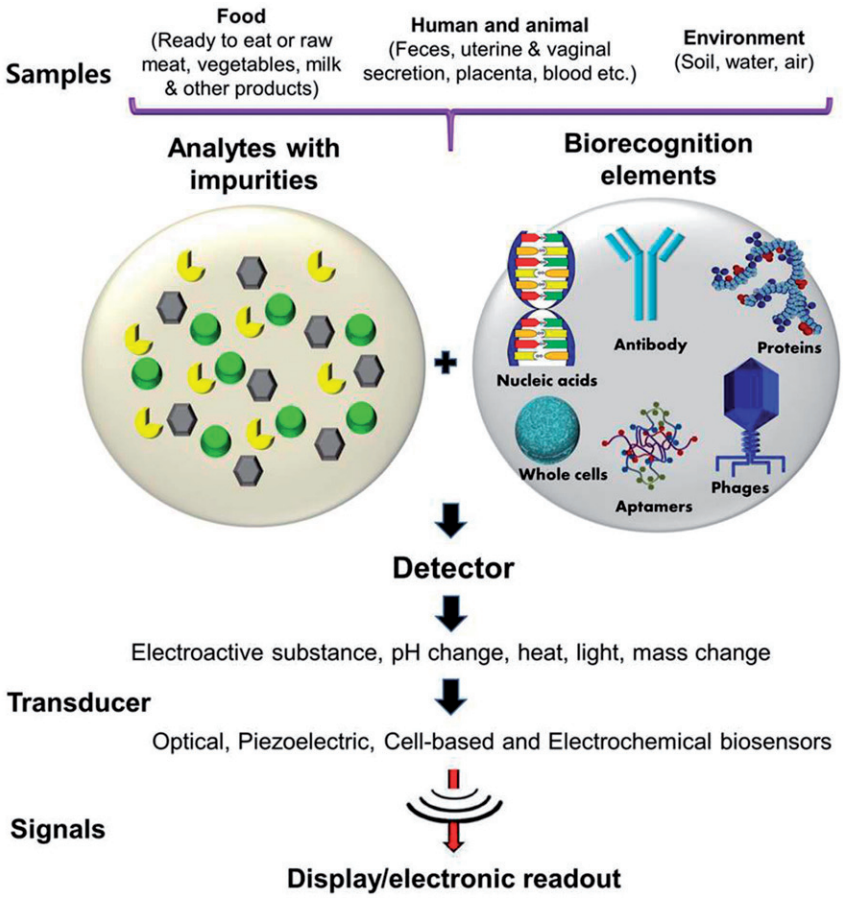

Figure 1. Schematic representation showing common working design of biosensors for pathogen detection in food, human, animal, and environmental samples.

biorecognition elements. The optical transducers enable direct (label-free) detection of bacteria. These sensors can detect even the small alterations in the refractive index or thickness that results from binding of cells to receptors immobilized on the transducer surface. Optical biosensors are sub-categorized based on reflection, refraction, resonance, dispersion, phosphorescence, infrared absorption, Raman scattering, fluorescence, and chemiluminescence. Surface plasmon resonance (SPR), fluorescence, microfluidic, and electrochemiluminescence (ECL) based optical biosensors are used for the detection of *L. monocytogenes*. Table 1 summarizes various studies on optical biosensors for the detection of *L. monocytogenes*.

Surface plasmon resonance (SPR)-based biosensors

SPR-based optical technique produces signals for the altered refractive index in close proximity of the sensor surface due to bindings between analyte and ligand (Figure 3). Koubová et al. (2001) reported successful detection of *L. monocytogenes* at concentrations of 10^6/mL using the SPR-based optical sensor on the conventional Kretschmann prism arrangement. A BIA3000 detector based on SPR method was used to detect *L. monocytogenes* cells in solution by Leonard et al. (2004). In this technique, *L. monocytogenes* cells were incubated (30 min) with anti-*L. monocytogenes* polyclonal rabbit antibody, followed by the removal of dead cells and the bound antibodies using centrifugation to permit rapid detection of 1×10^5 *L. monocytogenes* cells/mL. Taylor et al. (2006) reported a multichannel SPR sensor for the quantitative and simultaneous detection of four different bacteria. The limit of detection (LOD) of the reported biosensor was 3.5×10^3 cfu/mL for *L. monocytogenes*. Whole cells of *L. monocytogenes* were detected by SPR based sensor by Nanduri et al. (2007). They obtained a LOD of 2×10^6 cfu/mL. In the study conducted by Poltronieri et al. (2009), SPR immunosensors with a gold electrode bound to anti-bacterial antibodies were used. They found that the biosensor could detect 10^2–10^6 cfu/mL of *L. monocytogenes* cells on introducing a gold-labelled secondary antibody. With this, the suitability of SPR biosensors in pathogen detection from water, vegetable, and environmental samples was established

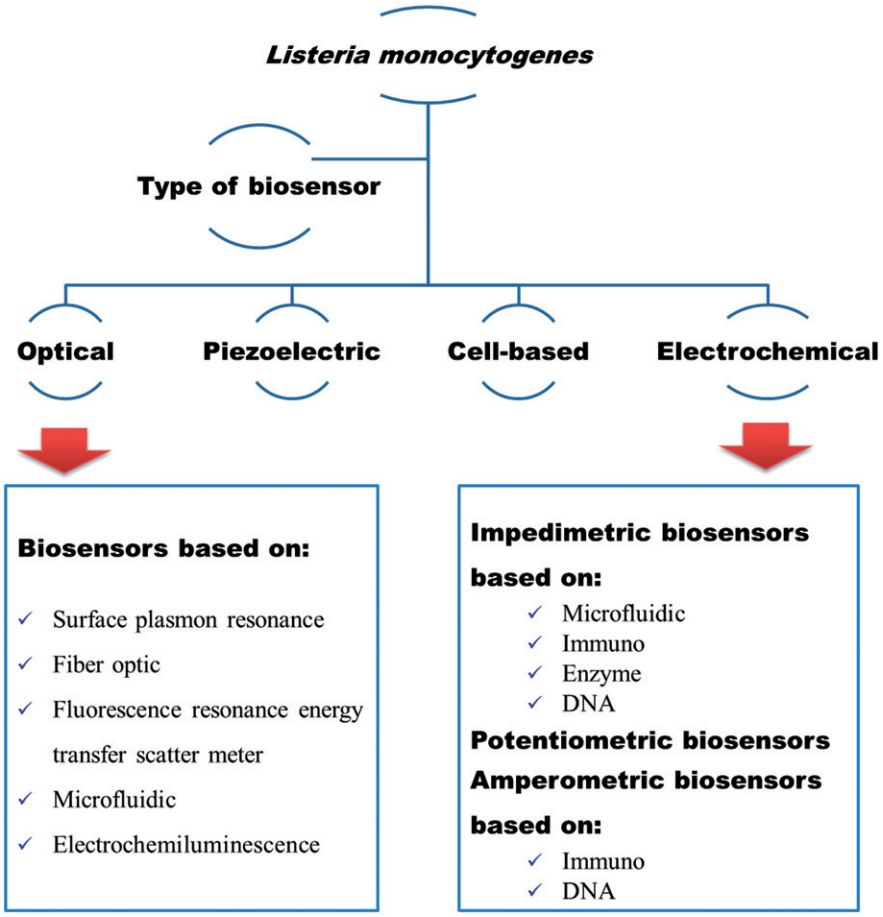

Figure 2. Types of biosensors used for *Listeria monocytogenes* detection.

(Leonard et al. 2004; Poltronieri et al. 2009). Schmelcher et al. (2010) used differently labelled reporter CBDs of *Listeria* phage endolysins for detection and differential staining of different *Listeria* strains. The principle involved isolation of *Listeria* cells from milk and soft cheese using endolysin magnetic concentration and separation steps. This was followed by plating of the cells on an oxford agar (incubated overnight), after which colonies were differentiated with fluorescently labelled endolysins and their subsequent detection by fluorescence microscopy in less than 15 min. Recently, Marusov et al. (2012) utilized grating-coupled SPR imaging to detect the microbes. In this method, a gold-coated sensor chip has been used to couple collimated incident light with surface plasmons that permits the use of disposable biosensor chips. The compact microarray sensor chips utilize antibodies or other specific capture molecules to detect multiple analytes. Especially, a LOD of 10^6 cfu/mL was obtained for *Listeria*. The SPR biosensor is limited by the penetration depth (approximately 100–500 nm) of evanescent field and also the change in refractive index and bacterial cell size.

Using *L. monocytogenes* reactive monoclonal antibody (C11E9), Lathrop et al. (2003) fabricated a resonant mirror biosensor to detect its all the serotypes. However, at higher concentration (10^8 cells/mL) the biosensor could not able to detect *L. monocytogenes* cells. With the help of *L. monocytogenes* serotype 1/2a cells as the immonogen, Hearty et al. (2006) produced a panel of hybridomas, which was enhanced by preparing cells in *Listeria* enrichment broth. An ELISA-based format and SPR biosensor were used to evaluate the monoclonal antibody. This method could identify *L. monocytogenes* at concentrations more than or equal to 1.0×10^7 cells/mL. In spite of such developments, future studies seem imperative to improve the sensor's sensitivity through newer immobilization processes, and development of portable systems.

Fibre optic based biosensors

Fibre optic biosensors use a tapered fibre of either silica or polystyrene to transmit and receive light signals. These biosensors utilize a sandwich immunoassay system where the captured antibody is attached to the

Table 1. Performance of various biosensors used for *Listeria monocytogenes* detection.

Detection technique	Bio-recognition event	Sample type	Analysis time	Detection range/limit	References
Optical biosensors					
SPR	Antigen-antibody	Culture	–	10^6 cells/mL	Koubová et al. (2001)
Fibre-optic	Antigen-antibody	Culture	<20 h	10 cfu/mL	Tims et al. (2001)
FRET	Antigen-antibody	Culture	–	2.0 μg/mL	Ko and Grant (2003)
SPR	Antigen-antibody	Culture	<30 min	1×10^5 cells/mL	Leonard et al. (2004)
Fibre-optic	Antigen-antibody	Hot dog or bologna	<24 h	10–1000 cfu/g	Geng et al. (2004)
SPR	Antigen-antibody	Culture	–	3.5×10^3 cfu/mL	Taylor et al. (2006)
Scatterometer	Phenotypic characters	Culture	5–10 min	–	Banada et al. (2007)
Epifluorescence microscope	DNA	Culture	90 min	10^4–10^6 cfu/mL	Bhattacharya et al. (2008)
SPR	Antigen-antibody	Culture	–	10^2–10^6 cfu/mL	Poltronieri et al. (2009)
SPR	Bacteriophage endolysin	Mixed culture	<15 min	–	Schmelcher et al. (2010)
Fibre-optic	Antibody-aptamer	Ready-to-eat meat	–	~10^3 cfu/mL	Ohk et al. (2010)
Electrochemiluminescence	DNA	Culture	5 h	~0.0002 ng/μL	Long et al. (2011)
Piezoelectric biosensors					
QCM	Antigen-antibody	Culture	–	1×10^7 cells/mL	Vaughan et al. (2001)
Cantilever	Antigen-antibody	Milk	<1 h	10^2 cfu/mL	Sharma and Mutharasan (2013)
Cell-based biosensor					
Rat basophilic leukemia mast cell	Antigen-antibody	Culture	–	1×10^{-7} cells/mL	Curtis et al. (2008)
Ped – 2E9 cell	Cytotoxicity	Culture	3–6 h	24–98%	Banerjee et al. (2008)
Ped – 2E9 cell	Cytotoxicity	Food and beverage	4–6 h	10^2–10^4 cfu/g	Banerjee and Bhunia (2010)
Amperometric biosensors					
Immuno	Liposome	Culture	–	–	Kim et al. (1995)
Immuno	Nanowire-antibody-bacteria	Culture	–	1000 cells	Susmel et al. (2003)
DNA	DNA-daunomycin	Culture	–	–	Ligaj et al. (2003)
DNA	DNA-biotin	Synthetic oligonucleotide	–	~1.4 fM/7 μL	Kavanagh and Leech (2006)
DNA	DNA-biotin	Culture	<1 h	5 nM	Farabullini et al. (2007)
DNA	DNA-toluidine blue	Synthetic oligonucleotide	–	1.0×10^{-7} to 8.0×10^{-5} M	Gao et al. (2010)
DNA	DNA-$[Co(phen)_3](ClO_4)_3$	Food	–	–	Wu et al. (2010)
DNA	DNA-biotin	Synthetic oligonucleotide	–	0.2 nM/500 μL	Hajdukiewicz et al. (2010)
DNA	DNA-methylene blue	Deteriorated fish	–	2.9×10^{-13} M/L	Sun et al. (2012)
Immuno	Antigen-antibody	Food	1 h	2 log cfu/g	Davis et al. (2013)
Immuno	Antigen-antibody	Milk	–	10^2 to 10^6 cfu/mL	Cheng et al. (2014)
Impedimetric biosensors					
Microfludic channel	Microbial metabolism	Culture	1 h	–	Gomez-Sjoberg et al. (2005)
Immuno	Antigen-antibody	Culture	–	4.1 pg/mL	Tully et al. (2008)
Immuno	Nanowire-antibody-bacteria	Culture	1 h	10^2 cfu/mL	Wang et al. (2008)
Enzyme	Bacteriophage endolysin	Culture and milk	–	1.1×10^4 and 10^5 cfu/mL	Tolba et al. (2012)
Immuno	Antigen-antibody	Filtered tomato extract	–	4 cfu/mL	Radhakrishnan et al. (2013)
Immuno	Magnetic nanoparticles-antibody-urease	Spiked lettuce	–	300 cells	Chen et al. (2015)
DNA	DNA-platinum nanomaterials-chitosan matrix	Milk	–	1×10^{-12} M to 1×10^{-4} M	Kashish, Gupta, et al. (2015)
DNA	DNA-poly-5-carboxy indole	Clinical	–	10^{-13} M	Kashish, Prakash, et al. (2015)
Microfludic channel	Modified magnetic nanoparticles-antibody-urease	Spiked lettuce	1 h	1.6×10^2 cfu/mL	Chen et al. (2016)
Immuno	Immunomagnetic nanoparticles-urease-screen-printed electrode	Spiked lettuce	<3 h	1.6×10^3 cfu/mL	Wang et al. (2017)

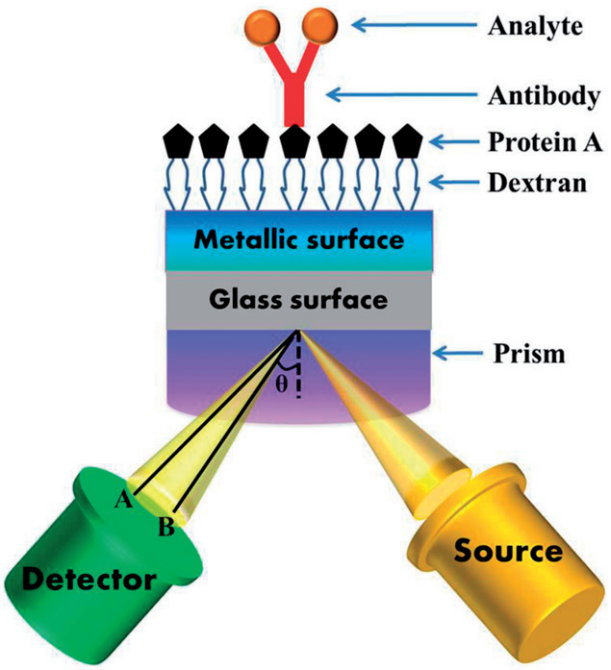

Figure 3. Schematic representation of the geometry and signal transduction mechanism of SPR optical biosensors.

fibre by avidin–biotin interaction or simple adsorption. Tims et al. (2001) reported detection of *L. monocytogenes* in low concentrations (≤10 cfu/mL) within 20 h using evanescent wave, and concluded that the enrichment methods allowed higher antigen expression, and thus increased sensitivity. After enrichment, the detection of *L. monocytogenes* took only 20–45 min. Further study was carried out to detect *L. monocytogenes* in both naturally and artificially contaminated (with 10–1000 cfu/g) food stuffs like hot dog or bologna. For this purpose, to capture *Listeria* cells on the fibre, Geng et al. (2004) used polystyrene fibre wave guides *via* a biotin–streptavidin reaction. The pathogen was detected in less than 24 h. An antibody-aptamer functionalized fibre-optic biosensor using Aptamer-A8, specific for internalin A (an invasin protein of *L. monocytogenes*) in combination with the antibody was developed by Ohk et al. (2010) for specific detection of *L. monocytogenes* in food products. This method selectively detected pathogenic *Listeria* at concentrations approximately 10^3 cfu/mL both in pure cultures as well as in groups of bacteria, and has the potential to detect *L. monocytogenes* cells from ready-to-eat meat products such as sliced beef, chicken, and turkey using an initial inoculation of 10^2 cfu/25 g following 18 h of enrichment. However, several factors such as the nature of antigen, quality of antibody, food component, resident microflora, sample enrichment, fibre variations, background liquid media, and the non-specific binding to non-target analytes can affect the overall signal.

Fluorescence resonance energy transfer (FRET)-based biosensor

FRET-based biosensor is a device involving the radiation-less energy transfer from a "donor" to the "acceptor" fluorophore. FRET technology enables quantitative analysis of biomolecular dynamics in terms of interactions between protein–DNA, protein–protein, including protein conformational changes. The utilization of FRET-based biosensors has been extended to monitoring the cellular dynamics not only in heterogeneous cell populations, but also at the single-cell level. Ko and Grant (2003) developed a FRET-based method to detect *Listeria*. The antibodies and proteins A and G were labelled with a FRET fluorophore pair, and then, the presence of specific antigens was detected in solutions utilizing spectrofluorometry. The results indicated LOD to be 2.0 μg/mL for *Listeria* antigens. Despite their diverse applications, FRET-based sensors face challenges pertaining to the increased need for higher fluorescence resolutions and improved specificity of FRET biosensors. The FRET-based technologies have significant utility in clinical diagnosis; however, the FRET reagents are expensive.

Scatterometer-based biosensors

The non-invasive optical forward-scattering system called "scatterometer" is based on differences in refractive indices and size, relative to the arrangement of bacterial cells in colonies on semi-solid agar surface as it generates different forward-scattering patterns. Banada et al. (2007) exploited the known differences in their phenotypic characters for the identification of *L. monocytogenes* from other *Listeria* species. They described a model of scattering patterns and elucidated radial spokes and rings seen in the scattering images of *L. monocytogenes*. They achieved detection and differentiation of *L. monocytogenes* and other *Listeria* colonies grown on agar within 5–10 min with 91–100% accuracy. In another study, Banada et al. (2009) used a similar method for label-free detection of multiple bacterial pathogens with an accuracy of 90–99% in samples derived from food or infected animals. They detected *L. monocytogenes* at levels as low as 1 cfu/25 g in different samples, that is, hotdog, tomato, and chicken. Huang et al. (2015) reported an ultrasensitive detection of *L. monocytogenes* using light scattering immunoassay. They utilized homogeneous gold nanoparticles which bind to the antigen epitopes present on *L. monocytogenes* surface. Under optimized conditions, they reported low LOD (3.5×10^1 cfu/mL) in 0.01 M phosphate-buffered saline. However, the major challenge to

use optical scattering technology for bacterial detection in suspension is that the liquid culture may also carry other microorganisms. For instance, culture may not be pure, and the arrangement of cells may vary (in chains or clusters). The orientation and distances between cells changes with time. Therefore, an averaging approach to account the relative orientation and movement is needed.

Microfluidic-based biosensors

Microfluidic-based biosensors are gaining momentum in this field of research as they provide an integrated platform to regulate the fluidic transport *via* micron-sized channels. In conventional microfluidic systems, an aqueous solution is used for immobilizing target species and concentrating the sample solution. The concentrated solution improves sensitivity of the probe, so also the LOD. To concentrate samples in a microfluidic channel, various methods like physical entrapment based on microstructure or microfilters, dielectrophoresis (DEP), and magnetic or polymeric beads are frequently employed. Bhattacharya et al. (2008) designed a microfluidic system to utilize dielectrophoretic force in combination with the flow of fluid to diverge all the cells into a small sensing channel for on-chip PCR using interdigitated microelectrodes. The results demonstrated a threefold rise in the signal through the preconcentration step. The sensor detected as few as 60 cells of *L. monocytogenes* V7 in less than 90 min even in the presence of other bacterial strains, and the application of DEP enhanced detection from 10^6 to 10^4 cfu/mL (Bhattacharya et al. 2008). However, this technique has certain limitations, that is, the higher cost and complexities in the fabrication of glass and silicon micro devices, which are not reusable and even if re-usable, difficult to maintain them sterile. Taitt et al. (2004) developed a portable multi-array biosensor for different analyte in complex samples. They used a fluidic cube module for the initial sample loading that allowed rapid detection of multiple unrelated targets without significant sample preparation. During *L. monocytogenes* detection, LOD of 2.4×10^4 cfu/mL obtained falls within the favourable detection range of any sensor.

Electrochemiluminescence (ECL)-based biosensors

ECL is also referred to as electrogenerated chemiluminescence based on the electron-transfer reaction on the electrode surface with suitable luminophore and co-reactant. This method is versatile due to its wide range of applications. ECL in combination with the magnetic beads can be considered as the important and powerful tool for clinical applications, food, and water testing, and the detection of biological threats. Long et al. (2011) proposed a method using HRCA (hyper branching rolling circle amplification) combined with the magnetic bead-based ECL for the detection of *hly* gene in *L. monocytogenes*. Through this approach, as low as 10 aM synthetic *hly* gene targets and ~0.0002 ng/μL of genomic DNA from *L. monocytogenes* could be detected. Zhu et al. (2015) constructed Fe_3O_4/Vancomycin (Van)/PEG magnetic nano-carrier for efficient sample enrichment and *in situ* nucleic acid preparation of pathogenic *L. monocytogenes* for subsequent gene sensing (Figure 4). The results showed better dispersibility and biocompatibility for PEG modified Van Fe_3O_4 nanocarrier than PEG-free nanocarrier. Using designed nano-carrier, 90% *L. monocytogenes* were efficiently captured from the complex sample matrices within 30 min. The ECL-based highly sensitive gene-sensing assay showed 10 cfu/mL LOD. In another recent report, Yang et al. (2017) also utilized magnetic beads to detect *L. monocytogenes* using ECL detection method. However, to overcome the problem of lower amounts of pathogens in the complex natural environments, they enriched the bacteria using Van modified magnetic nanoprobes (MNPs) (Figure 5(A)). In brief, they utilized direct modified Van-MNPs and brush-like MNPs. The brush-like MNPs were constructed using poly-L-lysine (PLL) and amino-modified magnetic beads, followed by coupling of PEG (amine-PEG5000-COOH) to the amine sites of PLL and Van conjugation to the carboxyl of PEG (Figure 5(B)). The integrated enriched Van-PEG-PLL-MNP nanoplatform with ECL resulted in the high enrichment (>94%) efficiency, rapid detection time (within 20 min), accurate detection of *L. monocytogenes* at levels as low as 10 cfu/mL, and satisfactory specificity (Figure 5(C–E)). This approach could detect even a low bacterial concentration level (10^2 cfu/mL), and hence, it holds a great potential for pathogen detection in clinical samples with low bacterial population.

Piezoelectric (PZ) biosensors

PZ biosensors are very attractive, and the principle involved enables their utilization in direct label-free detection of bacteria. This technology offers a real-time output simple to use and quite cost-effective. The general practice is coating the surface of PZ sensor with the selective binding substance (e.g. antibodies), which then is placed in a solution containing bacteria. The bacteria bind to the antibodies thus increasing the crystal mass while proportionately decreasing the resonance oscillation frequency. PZ immunosensor was developed for *L. monocytogenes* detection (Jacobs et al. 1995).

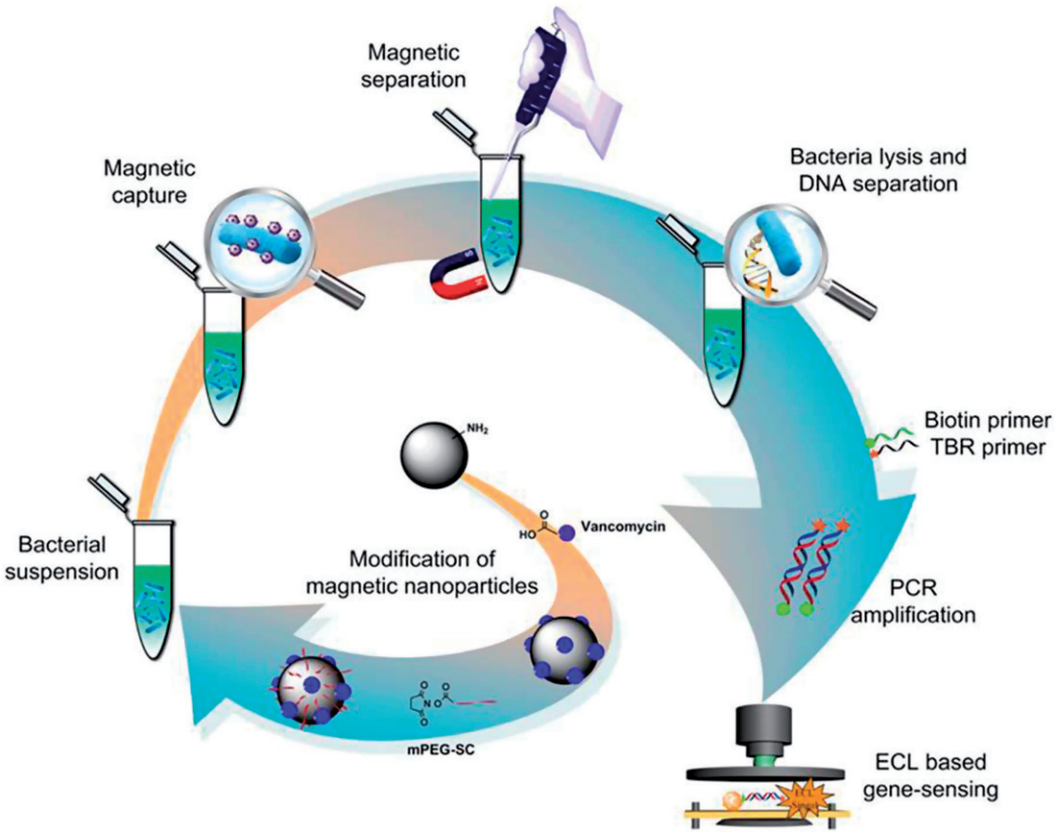

Figure 4. Schematic representation of the integrated bacterial enrichment/gene-sensing system with Fe_3O_4/Van/PEG nanocarrier (Zhu et al. 2015). Reproduced with permission by American Chemical Society.

Vaughan et al. (2001) reported a quartz crystal microbalance immunosensor using self-assembled monolayer (SAM) for the detection of *L. monocytogenes*. In solution, it detected 1×10^7 cells/mL. The sensor could be reused 10 times without any detection loss, and also, cuts down the cost. This is an important aspect regarding sensors since PZ crystals are not cheap enough to justify their disposal following a single assay. Additionally, this would sometimes save the expensive reagents. Sharma and Mutharasan (2013) detected *L. monocytogenes* in milk samples using asymmetrically anchored PZ cantilever sensor and a commercially available antibody. Detection of *L. monocytogenes* (10^2/mL) was achieved by incorporating a third antibody-binding step. However, the main drawback of PZ biosensor is the immobilization of antibodies that bind *via* amine groups (likely to their Fab sites), and thus inhibit antigen binding. Table 1 summarizes studies by various research groups for the detection of *L. monocytogenes* using PZ biosensors.

Cell-based biosensors

Cells and their components have been adopted in the evolution of biosensors and biochips. Cell organelles are utilized as bioreceptors, which represent the unique system to be exploited in future. Among the many biosensing elements, mammalian tissue slices or *in vitro* cultured mammalian cells are widely employed as bioreceptors. Curtis et al. (2008) developed a mast cell-based biosensor to detect *L. monocytogenes*. They used Rat Basophilic Leukemia mast cells and created an IgE chimeric protein by fusing the Fc region of the IgE antibody to CD14 that acted as the receptor for lipopolysaccharides. The designed chimeric protein binds with *L. monocytogenes* and IgE receptors on the mast cells, which then detects the presence of bacteria. A mammalian cell-based biosensor was developed, wherein, the B-lymphocyte Ped-2E9 cell line embedded in a collagen/gel matrix served as the sensing element. This was tested for the detection of *L. monocytogenes* and its toxin (listeriolysin O) from the artificially inoculated food samples (Banerjee et al. 2008; Banerjee and Bhunia 2010). Such a unique sensor differentiated between pathogens and non-pathogens based on their cytotoxicity. In artificially inoculated food and beverages, it yielded a detection limit of 10^2–10^4 cfu/g. Also, the biosensor could detect the toxin even in very small quantity (10–40 ng). However, the problems faced with such devices, are analytical strategies, batch-to-batch

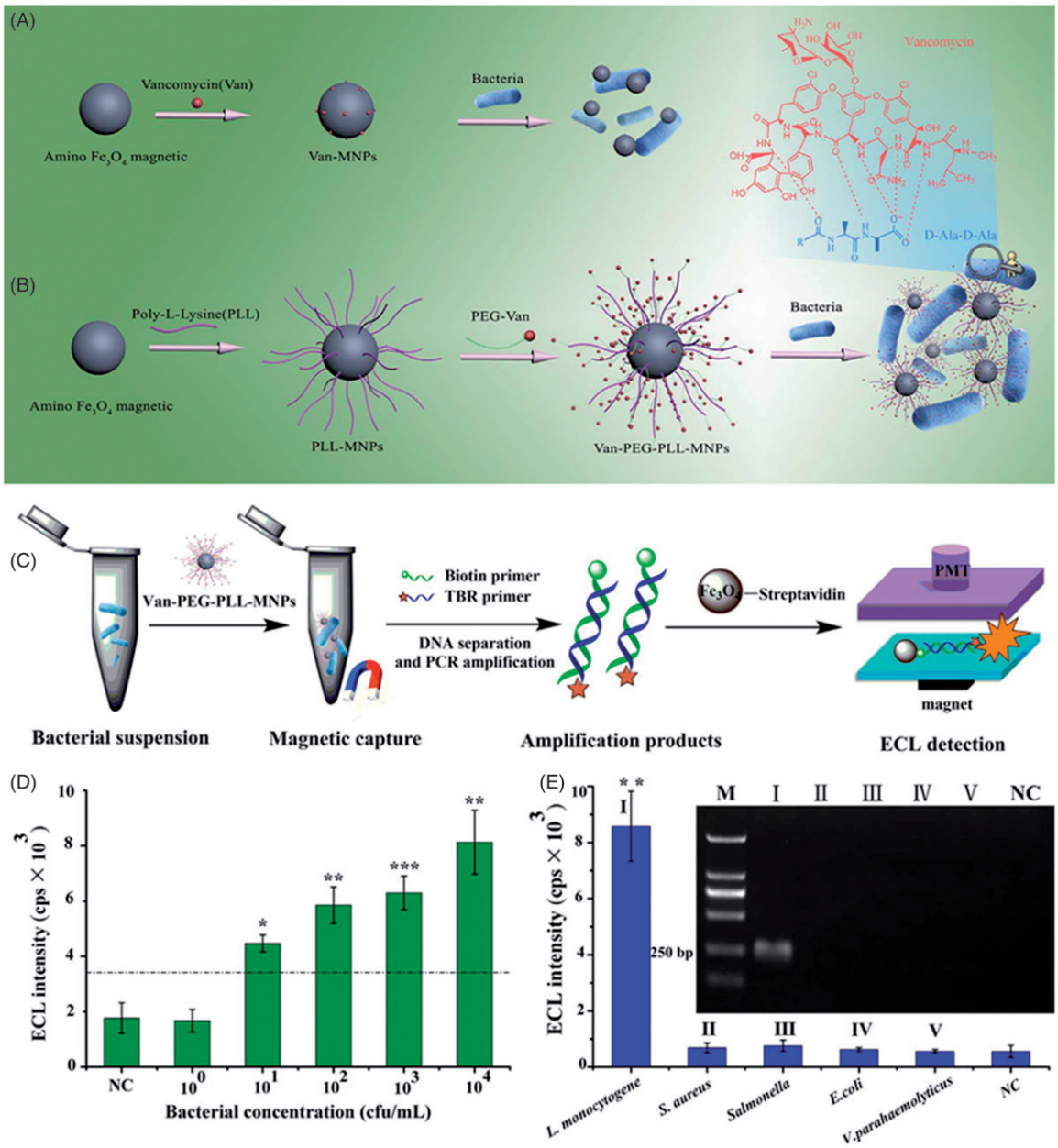

Figure 5. Schematic representation of the bacteria enrichment using: (A) direct Van-MNPs, (B) multivalent brush-like magnetic nano-platform, (C) gene-sensing of enriched bacteria using ECL, (D) ECL signals for PCR products, and (E) specificity statistical results (Yang et al. 2017). Reproduce with permission from Elsevier.

reproducibility, and the lack of selective placement of biological cells on the sensing sites. A summary of studies on cell-based biosensors for the detection of *L. monocytogenes* is shown in Table 1.

Electrochemical biosensors

Electrochemical biosensors use a bio-electrode to convert the biological events to measurable electronic signals. Due to the presence of specific recognition elements in the bio-recognition layer, such biosensor have advantages over the optical-based ones as the former could be operated in turbid media, offer comparable sensitivity, and are more amenable to miniaturization. Modern electro-analytical techniques have shown very low LODs (typically 10^{-9} M) achievable in small sample volumes (1–20 mL). Furthermore, on-line control is feasible due to the continuous response of the electrode system with the added advantage of simple and cheaper equipment required for electrochemical analysis contrary to other analytical techniques. A schematic outline of the typical electrochemical biosensor cell is shown in Figure 6. It consists of reference, counter, and working electrodes, which are dipped into

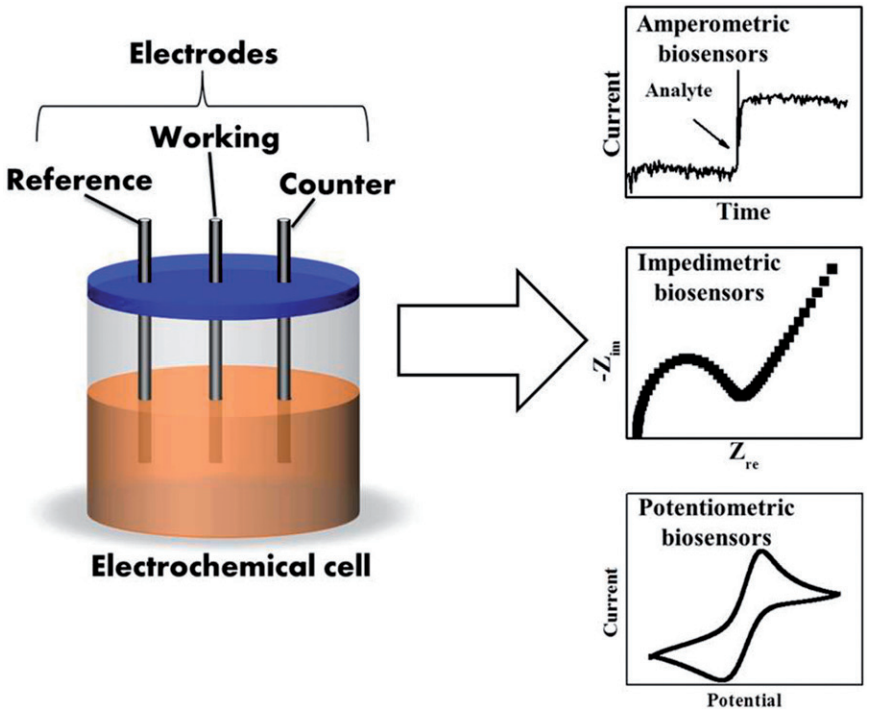

Figure 6. Schematic of the typical electrochemical biosensor cell. Analyte detection using three types of transducer (amperometric, potentiometric, and impedimetric).

buffer containing the analyte. Three methods (i.e. amperometric, potentiometric, and impedimetric) are used for the analyte detection using electrochemical biosensors.

Amperometric-based biosensors

Amperometric biosensors are used to investigate the electrochemical reactions at a constant potential while measuring the current change. The analyte concentration in solution is proportional to the current response of biosensors. Amperometric biosensors have the advantage of being highly sensitive, rapid, and inexpensive. Such systems have a linear concentration dependence compared to the logarithmic relationship in potentiometric systems. In recent years, a number of amperometric biosensors have been developed for the detection of *L. monocytogenes*. These are classified on basis of the difference in the use of biological elements. Table 1 shows the list of various amperometric biosensors used for the detection of *L. monocytogenes*.

Amperometric-based immuno biosensors

Quantitative detection of analyte in solution can be performed using the amperometric based immuno biosensors which utilize antigens and antibodies to form an immunocomplex on the blocking layer coated electrode surface. A liposome-based amperometric biosensor was described by Kim et al. (1995) for the detection of haemolytic microorganisms. The potential of this approach was illustrated for the detection of various strains (*L. monocytogenes*, *L. welshimeri*, and *E. coli*) with the bacterial concentrations in the range of 4.7×10^6–2.4×10^9 cfu/mL. Susmel et al. (2003) demonstrated the feasibility of one-step, specific, label-less, quantitative detection of *L. monocytogenes* based on the measurement of the diffusion of a redox probe using screen-printed gold electrodes and thiol-based SAM for immobilization of antibodies on the electrode surface. Nanomaterials/nanowires have unique semiconductive properties, and are ultrasensitive for single molecule sensing which extends their importance in impedimetric biosensing technologies. An optimized amperometric immune-biosensing strip utilizing gold nanoparticles-modified carbon electrodes devised by Davis et al. (2013) could detect *L. monocytogenes* contamination in food samples. The sensor had a LOD of 2 log cfu/mL (or cfu/g), while the screen-printed carbon electrode (SPCE) strip could detect as little as one bacterial cell, with in the approximate time of 1 h for the accurate detection of any contaminations. Cheng et al. (2014) reported detection of *L. monocytogenes* in concentrations ranging between 10^2 and 10^6 cfu/mL in milk samples by SAM-modified gold electrodes in a direct sandwich format with horseradish peroxidase (HRP). In another sandwich flow through immunoassay, Chemburu et al. (2005) fabricated biosensors using

highly dispersed carbon particles that could detect pathogenic cells by antibodies immobilized onto the activated carbon particles labelled with HRP conjugated antibodies. They detected *L. monocytogenes* in milk and chicken extract samples with LOD of 10 cells/mL.

Amperometric-based DNA biosensors

Amperometric DNA biosensors detect the current change in DNA hybridization between the target analyte and the probe on the electrode surface. Ligaj et al. (2003) described an electrochemical genosensor using carbon paste electrode and daunomycin for the amperometric detection of listeriolysin (*hlyA*) gene of *L. monocytogenes* by square-wave voltammetry. It was shown that the indicators applied could be very useful in the detection of hybridization. Farabullini et al. (2007) described detection of *L. monocytogenes* by the disposable electrochemical and low density genosensor array. A screen-printed array of gold electrode used was modified using thiol-tethered oligonucleotide probes (*inlA*). They could detect *L. monocytogenes* at nanomolar levels in less than 1 h without any cross-interference. Gao et al. (2010) described the electrochemical indicator toluidine blue for DNA electrochemical biosensors with different redox behaviour on ssDNA and dsDNA modified electrodes. The electrochemical DNA biosensor was effectively applied for the detection of specific sequences of *actA* gene from *L. monocytogenes* with the dynamic range of 1.0×10^{-7} to 8.0×10^{-5} M. Wu et al. (2010) reported a hybridization biosensor for electrochemical detection of PCR amplification products of LLO toxin gene in food products and the organism without labelling the target DNA. The hybridization reaction on the electrode surface was evidenced by cyclic voltammetric analysis using $[Co(phen)_3](ClO_4)_3$ as the indicator. There was a significant increase in the peak current in cyclic voltammeter following hybridization of the immobilized ssDNA with the PCR amplification products in the solution. Sun et al. (2012) described electrochemical DNA biosensors by electrodeposition methods for the preparation of GR and nano-gold to fabricate Au/GR nanocomposite film-modified CILE. Under optimal conditions, specific *L. monocytogenes hly* ssDNA sequences could be detected by measuring the differential pulse voltammetric response of methylene blue molecules accumulated on dsDNA. The linear concentration range achieved was 1.0×10^{-12} to 1.0×10^{-6} mol/L with the LOD of 2.9×10^{-13} mol/L. The PCR product of *hly* gene samples extracted from the deteriorated fish was also detected satisfactorily.

The development of enzyme based biosensors has been of particular interest due to the commercial availability of enzymes, and the large signal amplifications resulting from the substrate turnover. Some of the commonly used enzymes include alkaline phosphatase, HRP, glucose oxidase, glucose dehydrogenase, soyabean peroxidase, and bilirubin oxidase. The process involved chemical coupling of enzymes to the target or reporter DNA strand or *via* avidin (or streptavidin, neutravidin)-biotin binding. A simple capture-based assay involving gold electrode modified with a SAM of cysteamine, cross-linked with redox polymer $[Os(2,2'\text{-bipyridine})_2(\text{polyvinylimidazole})_{10}Cl]^{+}/2^{+}$ as the recognition surface and the probe sequence of DNA, was used to detect the specific target sequence of DNA (designed from *ssrA* gene of *L. monocytogenes*) (Kavanagh and Leech 2006). The results were displayed as the linear increase in the current response with respect to the concentration of target DNA in the range of 10^{-9} to 10^{-6} M with a LOD of ~1.4 fM in a 7 μL droplet (corresponding to 0.2 nM of target DNA). An enzyme-amplified amperometric DNA hybridization assay was proposed by Hajdukiewicz et al. (2010), wherein all the immobilization and detection steps were carried out in the solution phase applying the detection platform in a microfluidic flow system. In this assay, an amine-terminated ssDNA (designed for binding of the *ssrA* gene sequence of *L. monocytogenes*) bound within carboxymethylated dextran (CMD) films coupled to graphite surfaces possessing grafted arylamine and solution-phase ferrocene methanol (which mediates oxidase for glucose oxidation) was used. The signal produced was measured by cyclic voltammetry and constant potential amperometry, which scales the biotin-complementary DNA concentration (2.5×10^{-6} M to 3×10^{-7} M) with a LOD of 0.2 nM in 500 μL sample. Liébana et al. (2016) reported utilization of silica-based magnetic particles for electrochemical magneto-genosensing of *L. monocytogenes*. This method was based for the detection of the tagged amplified DNA obtained using single-tagging PCR with a set of specific primer *prfA* (217 bp) tagged with biotin. For the first time, Liébana et al. (2016) combined PCR amplification with electrochemical magneto-genosensing using silica-MPs as the platform. The DNA immobilization and PCR assay within 3 h, could detect 0.13 ng/μL *L. monocytogenes*. The amperometric methods are limited by the potential interference in the response that generated false current values.

Potentiometric biosensors

Potentiometric biosensors sense the change in ionic concentrations in ion-selective electrodes sensitive to the analyte. As potentiometric assays rely on recording

the potential/pH variations, such biosensors are more suitable for food, clinical, or environmental samples analysis. The analytical signal obtained indicates concentration variations of an ionic species. Potentiometric biosensors were developed by combining a biorecognition element (essentially an enzyme) with a transducer that senses variations in the number of protons (or other ions). The analytical signal then is logarithmically correlated with the analyte concentration. Ding et al. (2014) reported a label-free potentiometric aptasensor based on the polycation-sensitive electrode for the detection of *L. monocytogenes* in an aquatic environment with the LOD of 10 cfu/mL. In this technique, an aptamer binds specifically to internalin A, and target-binding event prevents the aptamer from electrostatically interacting with the protamine.

Impedimetric biosensors

Electrochemical impedance spectroscopy (EIS) is the powerful tool for characterization of electrochemical systems. The fundamental principle involved in any of the impedance methods is the application of a small amplitude sinusoidal excitation signal to the system before measuring the response. To detect *L. monocytogenes*, several researchers used impedimetric biosensors, which can be classified further on the basis of the biological elements used. The sub-categories of this biosensor have been described in detail below. Table 1 lists a summary of published work on the use of impedimetric biosensors in *Listeria* detection.

Impedimetric-based microfluidic biosensors

Impedimetric-based microfluidic method involves measurement of changes in the electrical impedance induced by bacterial growth in a culture medium or a reaction solution. The viable cells could be differentiated from the dead ones by the presence of ionic metabolites reflected as the change in impedance. This method is rapid, and has the potential to detect bacterial growth within 24 h. A modified version of the technique known as "Impedance microbiologyon-a-chip" was demonstrated by Gomez-Sjoberg et al. (2005) for the detection of microbial metabolism. In this innovative approach, a technique called "DEP" was incorporated into the chip so that the bacterial cells in diluted samples were concentrated to a very small volume. The LOD achieved was 1 h in the sample with the starting concentration of 10^4 cfu/mL, while the major challenge was the confinement or capture of cells into a small volume. Koo et al. (2009) utilized heat shock protein 60 (Hsp60) as the novel molecular recognition element on streptavidin-coated silicon dioxide surface to fabricate a microfluidic chip for selective detection of pathogenic *Listeria*. Compared to other monoclonal antibody (i.e. C11E9 and E-cadherin), Hsp60 could detect pathogenic *Listeria* with high specificity. A newer approach for the detection of *Listeria* species was developed by Chen et al. (2016) integrating electrochemical impedance with urease catalysis with the microfluidics in spiked lettuce sample. A LOD of 1.6×10^2 cfu/mL in $\sim$1 h was obtained using this method.

Impedimetric-based immuno biosensors

In impedimetric immuno biosensors, the electron transfer resistance change is monitored as the antigens and antibodies form immunocomplex on the blocking layer coated electrode surface. The first discovery of immuno biosensors involved development of label-less immunosensors for the detection of a cell-surface protein (InlB) on *L. monocytogenes* by Tully et al. (2008). The methodology was to fabricate sensors with PANI (polyaniline) by electropolymerization of planar SPCE to synthesize a conductive substrate. Using site-specific immobilization, the polyclonal anti-InlB antibody was subsequently incorporated into the PANI layer using the biotin–avidin system. A LOD of 4.1 pg/mL was achieved for InlB. Previous studies reported exploitation of SAMs to enhance the biosensor performance. SAMs are structure-oriented layers containing the functional groups well ordered at the monolayer liquid interface. These functional groups control the orientation of SAMs during their binding with the biological components. Later, a TiO_2 nanowire bundle microelectrode-based impedimetric immunosensor was developed by Wang et al. (2008) for the rapid and sensitive detection of *L. monocytogenes*. The bacterial count was displayed as the change in impedance caused by the nanowire-antibody-bacteria complex with a LOD of 10^2 cfu/mL for *L. monocytogenes* within 1 h. Radhakrishnan et al. (2013) used mouse monoclonal antibody immobilized onto gold electrode for the detection of *Listeria* in ideal solutions and filtered tomato extracts. A sensitivity of 0.825 kWcm^2/(cfu/mL) and 1.129 kWcm^2/(cfu/mL) and the LOD of 5 cfu/mL and 4 cfu/mL, respectively were obtained using this method. Chai et al. (2014) fabricated impedimetric-based immunosensor on an aluminium oxide layer insulated aluminium surface after immobilizing anti-*L. monocytogenes* antibody. The resistance of impedance decreased linearly with the increasing concentration of *L. monocytogenes* in the range of 1.3 to 4.3 log cfu/mL. Antimicrobial peptides were utilized as the selective probes by Etayash et al. (2014) for label-free and real-time detection of foodborne pathogens,

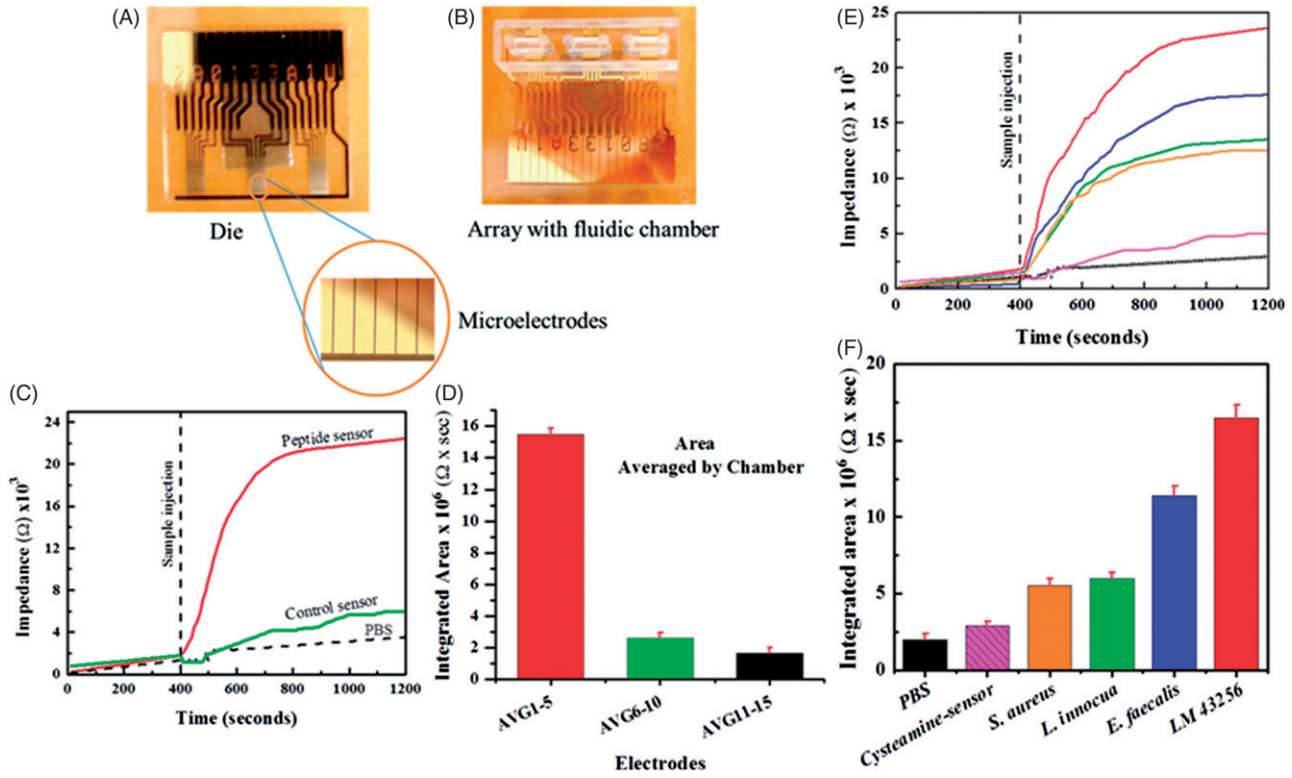

Figure 7. Photograph of the impedance arrays with (A) interdigitated gold microelectrodes (B) attached to a microfluidic chamber, (C) binding curves showing the normalized impedance signals from specific binding of *Listeria monocytogenes* (10^3 cfu/mL) and (D) the corresponding binding curve parameter, (E) real-time impedimetric response of the peptide sensor to various bacterial species (10^3 cfu/mL at 100 Hz) along with (F) the binding curve parameter for impedance sensor responses to the corresponding strains (Etayash et al. 2014). Reproduced with permission by American Chemical Society.

that is, *L. monocytogenes*, *L. innocua*, *Enterococcus faecalis*, and *S. aureus*. They used antilisterial, antimicrobial peptides from class IIa bacteriocins for selectivity, and the new generation of impedance array analyzer operating at very low frequencies. The fabricated LeuA functionalized impedance microelectrodes with each cell having five microelectrodes (Figure 7(A)) were attached with the microfluidic chamber with three separate cells (Figure 7(B)). The impedance microelectrode showed remarkable response to bacterial samples compared to the blank and the control sensors, as shown (Figure 7(C)), and calibrated the binding curve parameter (Figure 7(D)). This biosensor platform detected the closely related bacterial strains, especially *L. monocytogenes* at very low concentrations (10^3 cfu/mL). Further, Chen et al. (2015) combined immunomagnetic separation and urease catalysis to develop impedance biosensor for detection of *L. monocytogenes* using an antibody immobilization-free interdigitated array microelectrode. The technique was reported to detect as low as 300 cells of *Listeria* in both, the pure cultures and the spiked lettuce samples. In another study, Wang et al. (2017) used immunomagnetic nanoparticles, urease, and screen-printed interdigitated electrodes for pathogen detection in spiked lettuce sample. They reported a LOD of 1.6×10^3 cfu/mL within 3 h with the mean recovery of 98.2%. The major advantage of the technique was that it could detect the presence of *L. monocytogenes* without any significant interference from other foodborne pathogens.

Impedimetric-based enzyme biosensors

The impedimetric method is mainly for enzyme-based impedimetric biosensors, which analyze the enzyme-mediated reactions. Bacteriophages recognize and bind to specific receptors on the host bacteria using their tail proteins. Endolysins from the bacteriophage infecting *Listeria* are peptidoglycan hydrolases that mediate bacterial lysis at the end of phage multiplication cycle. The CBDs (cell wall binding domains) from *L. monocytogenes* phage endolysins Ply118 and Ply500 specifically recognize and bind to the *Listeria* cell wall. A biosensor was developed using the CBD of bacteriophage-encoded peptidoglycan hydrolases (endolysin) immobilized on a gold SPE (screen printed electrode), and the subsequent EIS analysis for the rapid and specific detection of *Listeria* cells (Tolba et al. 2012). The electrode system

was used to capture and detect *Listeria* from pure cultures and 2% artificially contaminated milk as well as with the LOD of 1.1×10^4 and 10^5 cfu/mL. Reyes-De-Corcuera et al. (2005) fabricated amperometric exogenous time–temperature integrator-based biosensor using glucose oxidase. They detected *L. monocytogenes* through pasteurization. This method is advantageous, that is, simple to prepare, rapid in response, inexpensive, and can detect different microorganisms by changing the enzyme and substrate. However, there are some drawbacks of this method such as larger volume of time–temperature integrator, difficult to recover for rapid assay, and requires long preparations for analysis.

Impedimetric-based DNA biosensors

A DNA sensor based on EIS detection is the device that transduces changes in the interfacial properties between the electrode and the electrolyte to an electrical signal. These changes are mainly induced by DNA hybridization, conformational changes, or any damage to the DNA. Most of the DNA sensors comprise a target DNA labelled with a fluorophore, magnetic beads, or an enzyme for its detection. On the contrary, the EIS detection-based DNA sensors are label-free and, therefore, have the advantage of low cost, simplicity, and the ease of miniaturization. A recent study reported a genosensor based on *hlyA* gene probe and glassy carbon electrode modified with platinum nanomaterials dispersed in a chitosan matrix for the detection of *L. monocytogenes* in milk samples with a wide detection range from 1×10^{-12} M to 1×10^{-4} M (Kashish, Soni, et al. 2015). Furthermore, in another study, a label-free genosensor based on the *hlyA* gene probe and the conducting polymer poly-5-carboxy indole with the LOD of 10^{-13} M was established for the detection of *L. monocytogenes* (Kashish, Gupta, et al. 2015). These investigators suggested the utility of this technique in early diagnosis of listeriosis both in clinical and environmental samples. In a recent report, Sharma et al. (2017) developed a protocol based on the hybridization of natural DNA, and explored the hybridization events involving genomic DNA extracted from *L. monocytogenes* through a compact magnetic tunnelling junction (MTJ)-based biosensing apparatus. In this study, they designed the portable biosensor setup based on MTJ platform to detect *L. monocytogenes* (Figure 8(A–C)). The SEM image of MTJ based biosensing device comprised 12 sensor arrays with a common ground contact at low and high resolutions (Figure 8(D)). The optical images of the sensors taken before and after the experiments, showed that the beads were immobilized on top of the sensors (Figure 8(E)). The magnetoresistive response curve of a sensor measured along the sensing direction (magnetic field applied parallel to the shorter side of the junction area) showed a 45% MR ratio and a low-field sensitivity of 13.6%/mT (Figure 8(F)). They detected *Listeria* using *Listeria* target DNA at concentrations ranging from 1 μM to 1 mM (Figure 8(H and I)). The experimental results showed that MTJ-based platform could detect concentrations of DNA extracted from *Listeria* with high sensitivity (below the nanomolar range).

Conclusions and future prospects

Conventional enrichment and identification methods of *L. monocytogenes* are proven to be highly sensitive. However, due to the multi-step processing of samples, they are slow for practical applications. For that reason, biosensor-based methods are needed for the accurate and quick detection. Among the several biosensors used for the detection of *L. monocytogenes*, optical methods conceivably provide better sensitivity over the electrochemical ones, but the method is limited by its cost and the complexity of performance. The impedimetric biosensors find their wide applications in bacterial detection as they integrate biological recognition technology with the impedance. Moreover, the technique has several other advantages in being label-free thus simplifying the assembly process is cost-effective and rapid with a detection time of usually less than 30 min. In addition, they reach the lower LODs than SPR and ELISA. There lies a wider scope for commercialization of impedimetric biosensors for the detection of pathogenic bacteria, and can be achieved by improved stability, reduced volume, and increased sensitivity at low cost.

Despite all these efforts, there is a need for substantial improvement in the development of biosensors to overcome the inherent technical shortcomings, reduce the number of false positive results as well as to channelize their performance on a wider scale. Commercialization of biosensors is a matter of debate since routine applications of the technique need to be checked for their reliability, robustness, and reproducibility in bacterial detection. The potential of biosensors could be increased particularly if these could be designed with specific characteristics such as specificity to distinguish the target bacteria in a multi-organism matrix, the sensitivity for direct on-line bacterial detection without pre-enrichment, adaptability for multiple analyte detections, and the rapidity to give real-time results. One other most important aspect is the presence of relatively simple and inexpensive configurations of a biosensor. It is believed that in the near future, fully automated biosensors with analytical systems based on

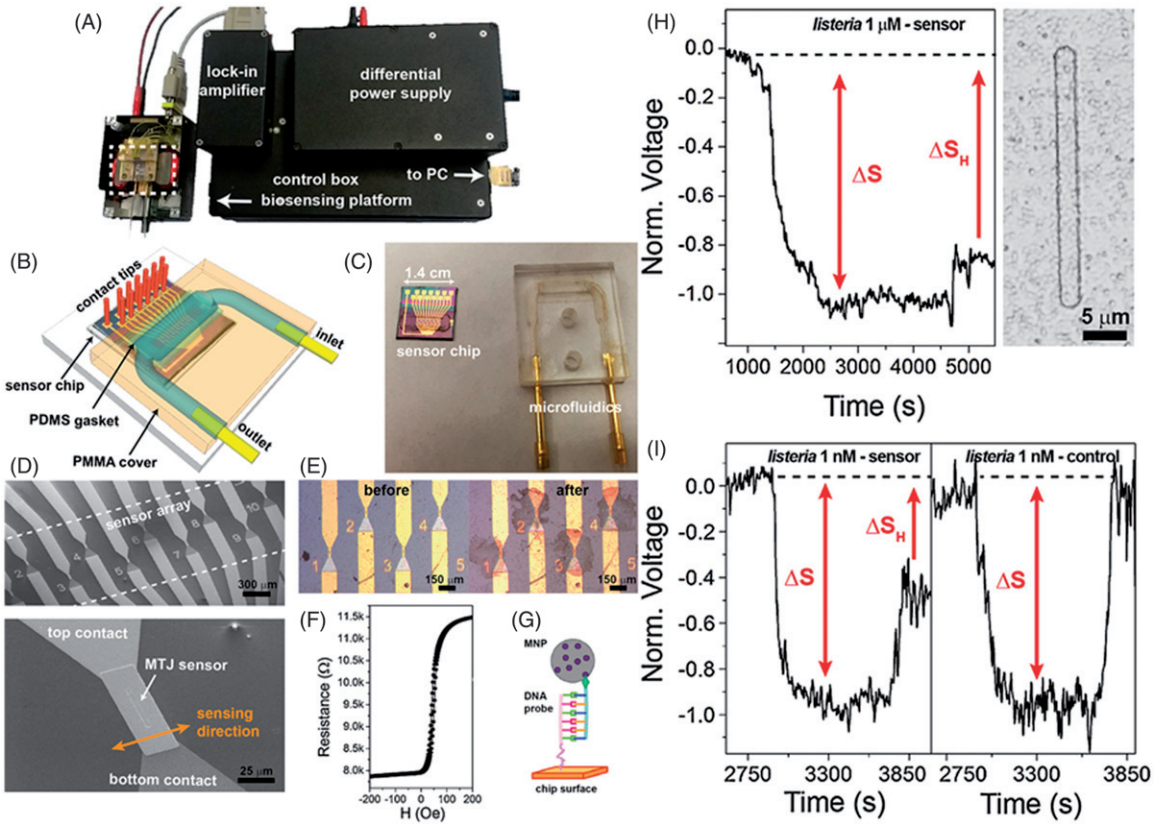

Figure 8. (A) Optical image of the biosensing setup, (B) schematic of the fabricated sensor array integrated within the microfluidic apparatus, (C) optical image showing sensor chip and microfluidic cell, (D) SEM images of the sensor array, (E) optical image of MTJ sensor before and after the detection of 100 nM target *Listeria* DNA, (F) magnetoresistive response curve of a sensor, (G) detection schematic with magnetic nanoparticles, (H) sensor response from a sensor functionalized with *Listeria* probe with optical image of the sensor area after bead immobilization (in right panel), and (I) normalized signal from a sensor functionalized with *Listeria* probe (hybridized with 1 nM *Listeria* target DNA) (Sharma et al. 2017). Reproduced with permission from Elsevier.

the combination of a multi-sensor technology with the artificial neural network or with other analytical and discriminative mathematical methods could be designed. This advanced technique then is expected to have certain advantages such as quick results, simple to operate, modest equipment requirements, high specificity and sensitivity, and cost and energy efficiency. This, in turn, would facilitate environmental monitoring and clinical diagnosis of the disease and also to control infection at an early stage.

As discussed before, the biosensing techniques effectively detect pathogens in a variety of samples. However, these do not offer the insight for complexities of the pathogen at the molecular level. Many omics approaches have been used for the detection of pathogenic and non-pathogenic *L. monocytogenes* (Singh et al. 2011; Beale et al. 2014; Tatituri et al. 2015; Jadhav et al. 2015; Ojima-Kato et al. 2016; Martinović et al. 2016; Cheng et al. 2016). These observations provide information related to pathogen's ability to overcome a diverse range of environmental regimes (e.g. salt concentrations, pH, temperature, etc.), close association between the pathogens, and also the molecular level complexities (Fratamico 2008; Stasiewicz et al. 2015; Donaldson et al. 2015). With the advancement of omics-based research (metabolomics, lipidomics, and proteomics), the prospects of advanced biosensor design are numerous and potentially ground breaking in terms of innovation and technology development. Yet, the identification of novel biomarkers or the understanding of the *Listeria* metabolome, lipidome, or proteome is least explored. However, few investigators have used proteome-based approach for the detection of *L. monocytogenes* in diverse environment (Mazzeo et al. 2006; Jadhav et al. 2014). These are the tools that can thrust biosensor development further, and constitute a significant proportion of the future prospects. The information generated by such innovative high throughput technologies would in turn, solve the questions on reducing microbial food safety hazards. It is imperative to facilitate the use of such advanced tools by food industries as the information generated hold promise for the development of novel strategies to combat food infections and their prevention. It is hoped that future

investigations will open newer opportunities to discriminate bacteria in environmental monitoring, clinical and diagnostic laboratories, and also food-related industries. It is, therefore, expected that miniaturization and introduction of newer high throughput technologies such as lower detection limits, reduced sample and solvent amounts, and shortened analysis time would certainly be a boon to safeguard the human health against microbial pathogens.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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