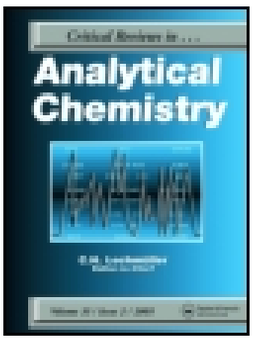

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Carbon Nanomaterial-Based Electrochemical Biosensors for Foodborne Bacterial Detection

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ABSTRACT

The development of easy to use, rapid and sensitive methods for direct detection of foodborne bacterial pathogens has become significantly important due to their impact on human health. In recent years, carbon nanomaterials have been adapted in the fabrication of electrochemical biosensors due to their exceptional combination of intrinsic properties such as high conductivity, stability and biocompatibility that render them as a promising candidate for bio-sensing material. The scope of this review is to provide a brief history of the current methods and different types of electrochemical biosensors used for the detection of bacterial pathogens. We primarily focus on the recent progress and applications of graphene, carbon nanotubes and their derivatives in electrochemical biosensors for foodborne bacterial pathogens detection. Finally, the status and future prospects of carbon-based electrochemical biosensors are also reviewed and discussed.

KEYWORDS

Biosensor; carbon nanomaterials; carbon nanotube; foodborne pathogen; graphene

Introduction

The reliable and rapid detection of foodborne bacterial pathogens is an international priority because accurate diagnosis and treatment can drastically reduce mortality rates caused by foodborne diseases.[1] Foodborne disease remains as a major threat to public health which accounts for more than 250 different types of bacterial foodborne illness and 90% of foodborne disease outbreaks worldwide due to the prevailing poor knowledge of sanitation, improper food handling, inadequate safety standards and weak enforcement of food safety laws.[2,3] Bacterial species such as *Escherichia coli* (especially O157:H7), *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Bacillus cereus* and *Shigella* are the most prevalent foodborne pathogens[4–6] which have led to 18 million Disability Adjusted Life Years (DALYs) including approximately 230,000 deaths of worldwide human population annually.[7] The continuous occurrence of foodborne outbreaks has created a demand for the development of easy to use, cost-effective, highly selective and sensitive electronic devices that enable direct detection and rapid analysis of bacterial contamination in food products.[8]

There are several conventional strategies for bacterial isolation and detection in food samples which are mainly involving (1) homogenization of the contaminated food sample in a buffer, (2) pre-enrichment in appropriate media, (3) selective enrichment in selective media to revive sublethally injured bacterial cells to a detectable level and (4) isolation of bacterial colonies using selective agars.[9] The presumptive positive colonies of bacteria on selective media are further confirmed or identified through biochemical and serological tests. These culture-dependent methods of detection are time-consuming (ca. 4–7 days for non-fastidious bacterial cells) as it involves multi-step approach and the detection limit is higher (10^4–10^6 cfu mL^{-1}) without the pre-enrichment steps.[8] Thus, there is a high demand for highly sensitive and rapid screening methods which can detect the bacterial contamination in food products with high reliability.[10] Biosensor-based technologies have met those requirements and created a breakthrough in the detection of foodborne bacterial pathogens.[11]

The principle of bacterial detection using biosensors involves a chemical or physical translation and transduction of biological information into a digital signal. This biological information is the result of the interactions between recognition elements (antibodies, enzymes, nucleic acids, bacteria and viruses) with their respective biological targets. Typically, recognition elements are immobilized onto a transducer surface via adsorption, entrapment, cross-linking, encapsulation or covalent binding.[12] Depending on the principle of signal transduction, various types of transducers such as mass-based, optical and electrochemical sensors are used to change the biological event into a measurable

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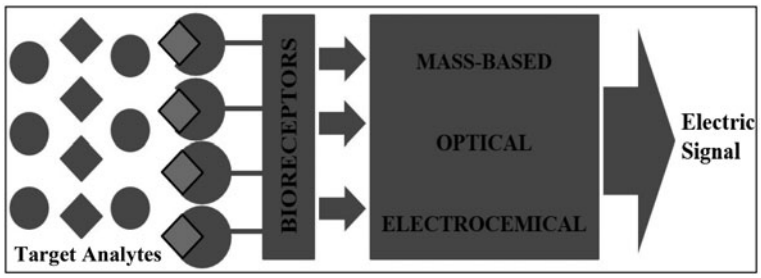

Figure 1. The overall working principle of biosensors.

signal.[13,14] The general working principle of a biosensor is summarized in Figure 1. Biosensors can be categorized into direct (label-free) and indirect (labeled) detection systems. In a direct detection system, physical changes induced by the interaction of target analyte are measured directly in real-time and no label is required whereas, in indirect detection systems, the product of biochemical reactions is detected via a labeled probe using a sensor. Among the many transducer types, electrochemical biosensors are very promising due to their low cost, rapid response, high sensitivity, ease of use, high signal-to-noise ratio, flexibility in employing recognition elements, low interference with food matrices and instrumental simplicity.[15–17] Electrochemical biosensors allow sensitive and selective detection of bacterial cells even at a low concentration in turbid media.

Carbon nanomaterials from zero-dimension (0D) to three-dimension (3D) have been adapted for the development of electrochemical biosensors. Carbon nanomaterials such as carbon nanotubes (CNTs), graphene and its derivatives offer diverse advantages in electrochemical sensing of foodborne bacterial pathogens especially with high performance in terms of field-effect mobility and sensitivity.[18] Carbon nanomaterials with large surface-to-volume ratio possess the ability to acquire and distinguish electrical signals before and after hybridization of biological elements.[19] The high electrical conductivity and other intriguing physics in carbon caused by the delocalized π electron network facilitate rapid electron transfer kinetics from the transducer surface to the electrochemical medium.[20] Electrochemical properties of carbon nanomaterials also enable smooth electron transduction of electrochemical responses.[21,22] Carbon nanomaterials have been found to display biocompatibility and ease interactions between the recognition element and target biomolecules.[23,24] The robust mechanical strength, high stability and ease of modification afforded by carbon nanomaterials also allow facile surface functionalization.[19,25] These unique properties enable the adaptation of carbon nanomaterials for the sensitive detection of biological compounds.[26–28]

The utilization of various carbon nanomaterials in the field of electrochemical biosensors are wide and diverse. In this review, we highlight the history and state-of-the-art in the application of popular carbon nanomaterials such as CNTs and graphene in food safety monitoring. Comprehensive information on these carbon nanomaterials with updated examples in the detection of foodborne bacterial pathogens are also discussed. In addition, the significance and future of carbon-based electrochemical biosensors for the well-being of mankind are reviewed.

From culture-dependent methods to molecular approaches in food safety

Over the years, microbiologists and researchers have devised many molecular methods for bacterial identification and characterization. Immunological and nucleic acid-based methods are some of the examples of the advances in bacterial detection tools used in the early 90s.[29] Molecular methods complemented traditional culture methods which possess limited impact toward outbreak control and decision-making efforts as they are not able to provide rapid results within 24 hours.[30,31]

Immunological or antibody-based assays detect foodborne bacterial pathogens through specific interactions between the antigen–antibody without the need of culturing steps. There are two commonly used immunoassays for bacterial detections which are enzyme-linked immunosorbent assays (ELISAs) and lateral flow immunoassays (LFIs). Both assays are operated by colorimetric or fluorescence-based detection system.[32] Traditional direct ELISA involves immobilization of the antigen directly on the solid support, followed by addition of enzyme labeled primary antibody while indirect ELISA involves addition of enzyme conjugated secondary antibody that interacts with the existing primary antibody.[33,34] Although direct ELISA is favored due to its simplicity and speed as it involves minimal steps, however, it lacks specificity due to non-specific interaction of antigen–antibody.[35] This will increase the background noise, decrease the signal amplification and overall sensitivity of the assay. In contrast, indirect ELISA exhibits higher sensitivity due to the use of secondary antibodies enable them to bind to multiple sites of the primary antibody.[35] The most popularly used ELISA for food products is sandwich ELISA which involves immobilization of the capture antibody on a solid support that captures the low concentration of antigens in a food matrix.[36] The labeled or label-free detection antibody is introduced to the system for a direct or indirect sandwich ELISA detection. Despite the cross-reactivity issue with the capture antibody, this sandwich ELISA offers sensitive bacterial detection.[37] In addition, competitive ELISA is another type of detection involving coating of known antigen onto the solid support followed by addition of unknown antigen. Then, a labeled detection antibody is introduced to the system which initiates the competition between target

antigen in the sample and antigen bound to a solid support. High concentration of antigens in the sample bind abundantly on the primary antibody and blocks its interaction with the plated antigens.[38] Thus, the detection signal arises from this competitive interaction is usually inversely proportional with the concentration of the target antigen. ELISA is not a cost-effective solution as it requires specialized equipment and trained personnel, thus more affordable and simple methods such as LFI was developed for more rapid on-site detection of foodborne bacterial pathogens.[1]

LFI utilizes a simple dipstick which is called as lateral flow test strip coated with colored particle labeled antibodies or antigens. This strip is divided into four different parts such as (1) glass fiber or cellulose application pad, (2) a conjugate release pad with antibody-labeling tag conjugates that interact with the bacterial targets in applied liquid sample, (3) a nitrocellulose membrane contains a test and control lines containing high affinity receptors that capture the analyte and (4) an absorption pad that ensure proper flow of the liquid sample.[39] Once the filtrate of food homogenate is introduced into the system, it travels via capillary action and mixes with the conjugate resulting in visualization of colors approximately between 2 and 10 minutes at the test line. The pathogen-labeled probe conjugates react with the immobilized capture ligands in the nitrocellulose membrane and generate visible line or spot. The colloidal biotinylated labeled sample and a streptavidin as capture ligand are often used which results in high affinity interactions.[40] Two different formats such as competitive assay and sandwich assay are used in lateral flow test strip which poses both advantage and disadvantage. Sandwich assay usually used for pathogens with more than two different epitopes for a single antibody pair (labeled probe and capture ligand). When a sample applied to the system, the targeted pathogen will interact with the conjugates and flows into the nitrocellulose membrane that contains the capture antibody and forms a colored line. The excess samples that not captured in the membrane will flow to the control zone which composed of the secondary antibody and creates another colored line. Thus, the positive sample will create two colored line while negative samples creates a single line.[41] The intensity of the color is dependent on the quantity of the targets. The analyte with single epitope that binds only to one antibody and cannot bind to two antibodies simultaneously can be detected using competitive assay. In contrast to sandwich assay, positive sample will not form a colored line in the nitrocellulose membrane, but the control line will still form in a competitive assay. This technique allows simple, cost-effective and rapid detection of foodborne bacterial pathogens using simple or no instrumentation.[42] The most challenging issue in the analysis of food samples via the immunoassay approach is the separation of target organisms from the debris of the food homogenates.[43] Moreover, all immunological assays still suffer from lack of specificity due to the common epitopes present on the cell surface of bacterial species despite the introduction of many monoclonal, polyclonal and recombinant antibodies to overcome these limitations.[44]

Nucleic acid-based techniques, most notably PCR-based detection is highly specific in pathogen detections.[45,46] The invention of PCR in 1985 by Kary Mullis has revolutionized the implementation of molecular methods in the foodborne bacterial detection. The general principle of PCR involves in vitro enzymatic synthesis and amplification of DNA sequences with thermo-resistant DNA polymerases. The invention of the automated duplication of a target DNA sequence in a short amount of time made PCR as a widely used method in the field of microbiology. There are many variations of PCR but only multiplex and real-time PCR (quantitative-PCR) have been adopted in food safety monitoring with an improved detection limit of 10^2–10^4 cfu mL^{-1}.[32] Multiplex PCR is capable of amplifying more than one target genes of the same or different pathogens simultaneously, whereas real-time PCR enables concurrent detection and quantification of the target sequence in real time with the aid of fluorescence technology. Although, these methods are far more advantageous in terms of cost and time than a simple PCR method, an improvement on detection of samples from food matrices is still needed due to the presence of food-inhibitors that affect the PCR reaction by degrading the target nucleic acid or inhibiting the polymerase enzymes activity.[47,48] However, the problem involving removal of inhibitory substances from food matrices is often rectified by coupling PCR-based techniques with immunomagnetic separation (IMS). In IMS, super-paramagnetic beads coated with specific ligand complementary to the target bacteria are utilized to capture viable bacterial targets up to 10^4 cfu mL^{-1} within 5 hours from the heterogeneous suspension and concentrate bacterial cells from complex food matrix.[49] IMS coupled with PCR capable to speed up the detection process and offers a useful sample for PCR with little or no nonspecific DNA or interfering factors. Loop-mediated isothermal amplification (LAMP) is another novel nucleic acid amplification method which provides a rapid, sensitive and specific detection of foodborne bacterial pathogens.[50,51] LAMP involves auto-cycling strand displacement DNA synthesis carried out under isothermal conditions. This method utilizes two inner primers and two outer primers to amplify six specific regions of target DNA which will result in a final product of cauliflower-like DNA structures. LAMP capable to produce a huge amount of amplified products within 60 minutes assay time which is usually 10^3-fold or higher as compared to simple PCR and those amplifies amplicons can be detected by agarose gel electrophoresis or SYBR Green I dye.[1] The development of real-time LAMP also favors the real-time monitoring of turbidity or fluorescence of the reaction mixture which eliminates the need for staining and gel electrophoresis.

The nuclei acid-based technique is highly specific as compared to the immunological assay due to the usage of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) probe that specifically targets and hybridize to a complementary genetic sequence of the target pathogen.[1] The specificity increases as the DNA/RNA bases only pair with their complementary bases (adenine: thymine, A: T and cytosine: guanine, C: G base pairing). Moreover, the overall detection

Table 1. Summary of current analytical methods used for bacterial detection.

Method	Foodborne bacteria	LOD	Assay time	Ref
ELISA	*E. coli* O157:H7	6.8×10^2 cfu mL^{-1}	3 h	[52]
	Salmonella spp.	10^6–10^7 cfu mL^{-1}	4 h	[53]
	Vibrio parahaemolyticus	10^3 cfu mL^{-1}	16 h[a]	[54]
LFI	*Vibrio cholerae*	5.0×10^5 to 10^6 cfu mL^{-1}	15 min	[55]
		10^2 to 10^1 cfu mL^{-1}	12 h[a]	
	Staphylococcus aureus	3.0×10^0 cfu mL^{-1}	2 h	[56]
Multiplex-PCR	*Salmonella* spp., *Salmonella* Enteritidis	10^3 cfu mL^{-1}	24 h	[57]
	E. coli O157:H7, S. aureus, Y. enterocolitica, Salmonella	10^3 cfu mL^{-1}	Not stated	[58]
Real-time PCR	*L. monocytogenes* and *E. coli* O157:H7	1.0×10^1 cfu g^{-1}	4 h[a]	[59]
	L. monocytogenes, *Salmonella* spp.	2.0×10^2 cfu mL^{-1}	24 h[a]	[60]
	V. parahaemolyticus	1.0×10^2 cfu mL^{-1}	6 h[a]	[61]
	L. monocytogenes, E. coli O157, Salmonella spp.	1.8×10^1 cfu/10 g	24 h	[62]
LAMP	*Salmonella* spp. and *Shigella* spp.	1.0×10^0 cfu mL^{-1}	<20 h	[63]
	Vibrio vulnificus	2.5×10^3 cfu mL^{-1}	8 h	[64]

[a]With pre-enrichment.

process takes a shorter time as compared to the immunological assay, and the use of multiple primers in PCR enable accurate differentiation between the bacterial strains. Despite high sensitivity, nuclei-acid method is a promising alternative to tedious and time-consuming detection techniques used in food safety.[45] However, the advancement and modification of PCR technology has increased the overall cost of the detection system. The examples of some currently used foodborne bacterial pathogen detection techniques were discussed in Table 1.

For the aforementioned detection techniques above, low sensitivity (without pre-enrichments) is the major limitation as the techniques are not sensitive enough to detect pathogenic microorganisms in food samples without further processing steps. Typically, the concentration of foodborne bacterial pathogens in food samples is less than 10^2 cfu mL^{-1}.[65] Consequently, these methods are unfavorable for direct detection of the pathogen in food matrices and also requires time-consuming pre-enrichments of bacterial cultures prior to analysis to achieve low detection limit or high sensitivity.[66]

Electrochemical biosensors for foodborne bacterial pathogen detections

Electrochemical biosensors are one of the recent approaches that have been adapted for the foodborne bacterial detection. Electrochemical biosensors have some advantages over other analytical systems such as the ability to work with turbid samples, low fabrication cost and instrumental sensitivity. Technological advancements have allowed for the miniaturization of devices including biosensors as an ideal tool for identifying and quantifying foodborne bacteria present in various food matrices with high sensitivity (low detection limit up to 10° cfu mL^{-1}).[67,68] Electrochemical biosensors are categorized according to the parameters measured by the device, namely amperometric (current), potentiometric (potential or charge accumulation), impedimetric (impedance) and conductometric (conductivity).[69] Amperometric biosensors measures the changes is potential or current in the electrochemical system that arise from the oxidation or reduction of the electrochemically active analyte.[70] Both differential pulse voltammetry (DPV) and cyclic voltammetry (CV) are the common analytical techniques used to measure the changes in current or potential, although square wave voltammetry has also been considered for a sensitive detection of the targeted analyte.[69] Potentiometric biosensors measure the changes in pH or ion concentration occurring during the interaction of an analyte with the working electrode (an ion selective electrode or ISE).[71] One of the advantages of potentiometric sensors is that redox probes are not required to measure the interaction between the target analyte and biorecognition elements. However, the lack of overall sensitivity and interference from the sample matrix are the main disadvantages of this system.[72] Impedimetric and conductometric biosensors measures the conductivity of the solution/medium or the rate of electron transfer at the electrode–electrolyte interface.

Biosensor platforms usually coupled with biorecognition elements such as DNA, aptamers, antibodies and enzymes to recognize a targeted foodborne bacteria and results in transduction of an electronic signal. The effective miniaturization of these devices especially compact screen-printed sensing chips allow point-of-care detection to directly detect the presence of foodborne bacteria in food sample of various matrices.[73] This will ensure safety of food products. Generally, all types of electrochemical biosensor use immunosensor and nuclei-acid based sensor as the mode of detection in the field of food safety.[74]

Immunosensors is the affinity ligand type solid-state biosensor which has a similar concept as traditional ELISA. The immunochemical reactions occur at electrode interface (transducer) generates an electrochemical signal. The immunosensors can be either labeled or label-free. Label-based electrochemical immunosensors utilize a label (marker) tagged to an antigen or antibody which act as an electroactive or generate an electroactive product for an efficient electron transfer.[75] However, the use of markers can cause interference and influence the efficiency of the binding event.[76] For bacterial detection, a sandwich format assay which captures targets between two specific antibodies are often used. Whereas, label-free immunosensors use redox probe to monitor the biorecognition event in the system in which the formation of immunocomplexes with targeted antigen will create a steric hindrance that prevents the electron from reaching the electrode's surface.[77] Simplified

preparation, low cost and exemption of use of a secondary antibody of label-free electrochemical immunosensor has attracted tremendous attention.[78,79] The detection of foodborne bacteria involves immobilization of the detection antibodies on the electrode surface and complementary bacterial targets probed on the surface of antibody through hydrophobic, ionic and hydrogen bonds resulting the changes in the current.[80] Both labeled and label-free immunosensors for detection of foodborne bacterial pathogen directly in food matrix was established in many studies.[81,82] Although, electrochemical immunosensor is an easy-to-operate and cost-effective solution for foodborne bacterial detection as compared to ELISA, interference from the food matrix decreases the specificity and sensitivity of the sensor.[83] Besides that, the shared homologs of surface-presented proteins by more than one pathogen may lead to inaccurate detection but this can be overcome by generating antibodies that target species-specific constitutively expressed antigen.[84]

Nucleic acid-based electrochemical biosensors are based on the use of DNA/RNA as a probe that targets complementary specific gene extracted from the bacterial cell. This mode of detection enables rapid, simple and economical testing of the bacterial targets with a less complex instruments.[85] The nucleic acid probe usually immobilized on the transducer surface through sorption or most importantly through a covalent modification that promotes orientation control for more stable detection.[86] Typically in the presence of bacterial target gene (usually the species-specific 16S rRNA), it hybridizes with the single-stranded oligonucleotide capture immobilized on the surface of the electrode through hydrogen bonds with complementary nucleic acid sequences at regular intervals.[87] In immunosensors, this interaction only happens at a few specific sites of the epitopes which may decrease the stability and reproducibility of the immunosensor.[88] The interaction between the capture and probe DNA induce changes in electrochemical parameters (e.g. conductivity or capacitance) depending on the type of transducers (amperometric, potentiometric, conductometric or impedimetric) which can be quantified either by label-free or labeled types of detection.[89] In label-free detection, the bio-recognition event will be directly translated into readable signal using kinetic measures, whereas labeling approach involves the use of markers (enzymes or fluorescent tags) that binds to the duplex formation to form a three-component "sandwich" complex on the sensor surface and the redox reaction upon the addition of the redox substrate will create current signal exponential to the concentration of the target-probe complexes.[90] The labeling process allows sensitive and selective detection but increases the complexity, cost and assay time.[90] The main advantage of the DNA-based sensor is the high stability of the biorecognition using nucleic acid which can increase the shelf-life and reproducibility of the system as compared to immunosensors.[91] In contrast, this method requires preliminary amplification of the highly conserved target gene by PCR which adds to the cost and time. Some of the constraints of DNA-based method were overcome by the introduction of aptasensors which still uses the nucleic acid sequence but, it is purely synthesized with high reproducibility through in vitro combinatorial biochemistry method (SELEX) which is a single step and PCR-free approaches and capable to bind to whole-cell that allows direct detection of foodborne bacteria from food matrix.[92,93]

Despite many advancements made in the field of electrochemical biosensing, the technical problems such as interference of complex "real samples" compounds decrease the desired sensitivity and selectivity of the sensors and reliability of the system which is important to produce low maintenance functioning instruments for a longer period. Moreover, for some sensors, researchers face difficulties in miniaturizing the detection system by keeping the overall cost minimal. The summary of advantages and disadvantages of different types of electrochemical detection techniques were discussed in Table 2.

The role of biorecognition elements in electrochemical biosensing

Biosensor provides analytical information (quantitative or semi-quantitative) based on the interaction between target analytes with bioreceptors (antigen/antibody, enzymes, DNA, bacteriophages, cell structure or cells and biomimetic bases) immobilized on an electrochemical transducer (Figure 2).[98] The electrical signal produced during the analyte-bioreceptor interaction is proportional to its concentration. Specificity is a key element of consideration in the process of designing a biosensor. The bioreceptor serves as a binding site for the target analyte to produce a change in signal. Hence, the selection of the biorecognition element is an important step contributing toward the specificity of a biosensor. Enzymes, antibodies and nucleic acids are the most commonly used bioreceptors in biosensor applications. Antibodies are made up of hundreds of individual amino acids arranged in a highly ordered sequence and constitute the main class of bioreceptor used in immunosensors. There are three categories of antibodies based on their selectivity and production techniques: polyclonal, monoclonal and recombinant. Antibodies can be immobilized on the electrode using self-assembled monolayer (SAM), physical adsorption and biotin-streptavidin system. In the presence of antigens; the antigen interacts with its unique antibody in a highly specific manner like the lock-and-key mechanism.[99,100] This three-dimensional interaction serves as a powerful analytical tool that can distinguish molecular structures even at minute concentrations with high specificity and sensitivity. This property enables the tailoring of antibodies as a recognition element to bind to particular chemicals, biomolecules and microorganisms.[101]

Enzyme-based biosensors have also received immense attention due to their high sensitivity, selectivity, stability and shorter assay time.[102] Enzymes possess specific binding to the substrate and high catalytic property that provides a sufficient rate of electron transfer to the working electrode.[99] Most enzymes are proteins except some which may be categorized as ribonucleoprotein. Some commonly used enzymes for bacterial detection are alkaline phosphatase, horseradish peroxidase (HRP) and beta-galactosidase.

Table 2. The advantages and disadvantages of different types of electrochemical biosensors.

Electrochemical biosensor	Device configuration and working principle	Immobilization technique	Advantages	Disadvantages	Ref
Amperometric	Three electrode system-measures the current produced between by electrochemical oxidation or reduction	Cross-linking, entrapment and layer-by-layer assembly	• Exhibits highly sensitivity detection • Low fabrication cost	• Signal reduction from fouling agents and interferents present in the sample matrix.	[94]
Potentiometric	Three electrode system-measures the changes in ionic concentration between an ion-selective electrode and reference electrode under the conditions of no current flow	Entrapment and physical adsorption	• Provide direct current changes without the need of redox mediators. • Simple detection technique	• The dynamic response is slower	[95]
Impedimetric	Three electrode system-measures the interfacial electrical arise from biorecognition events in the electrode systems	Oriented immobilization, physical adsorption and covalent interaction	• Non-destructive technique • Directly monitor binding events which overcomes the issues with interference • High signal to noise ration	• Accuracy of measurement depends on the instrumental technical precision and the operating procedures • Slow response	[96]
Conductometric	Three electrode system-measures the changes in conductance of the system in the presence of the analyte	Oriented immobilization, physical adsorption and covalent interaction	• Does not use a reference electrode and able to operate at low-amplitude alternating voltage • Enable easy miniaturization of the device	• Low signal/noise ratio • Low specificity	[97]

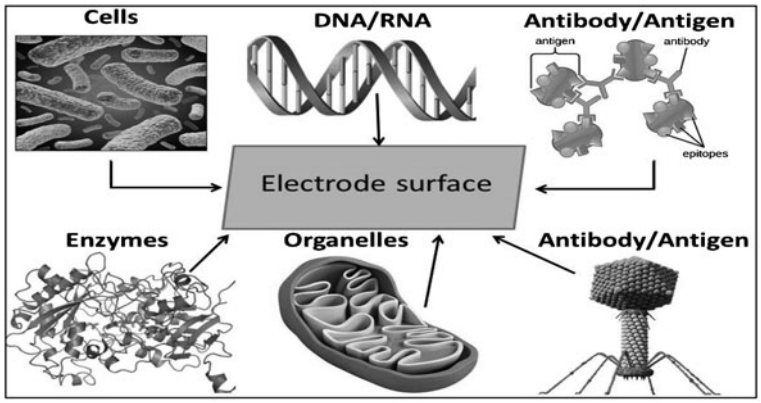

Figure 2. Examples of different types of bioreceptors used in electrochemical biosensing.

For more accurate detection of pathogen present in food samples, most of the studies have also proposed the use of enzymes as labels for immunosensors.[4]

Many researchers have also focused on phage-based sensors for pathogen detection. Phages are composed of a capsid that stores genetic material and a tail containing spiked protein, which enables the phage to bind to the receptors of bacterial cells and transfer genetic material to the host cell for reproduction.[103,104] Typically, the size of a phage varies from 20 to 200 nm. These bacteriophages can be combined with various analytical tools to detect pathogens present in food samples such as milk and meats directly.[105] This recognition process is highly specific and stable, which allows phages to be adapted as bioreceptors.

The biorecognition specificity can be further improved using nucleic acid/DNA-based bioreceptors. The working principle of these bioreceptors is based on base pairing (adenine : thymine or uracil and guanine : cytosine) between the DNA or RNA molecules of target organism.[106] The oligonucleotides can be immobilized on the surface of a sensor through covalent bonding or by terminal modifications with functional groups such as an amine (-NH_2) and thiol (-SH) which aids self-assembly to the sensor material surface.[107,108] The hybridization of the probe sequence with targeted bacterial sequence produces a measurable signal. DNA-based detection methods are highly specific, sensitive at low concentrations, simple, rapid and cost-effective.[109] This type of receptors can be readily synthesized and regenerated unlike antibody- and enzyme-based receptors. Recent advances in nucleic acid recognition have led to the development of aptamers which can be tailored to bind to various biological targets such as small molecules, peptides, proteins, nucleic acids or whole cells.[110] Aptamers offer many advantages over other types of recognition element mainly,

high stability and can be created by simple in vitro chemical synthesis.[110]

Microbial biosensors that utilize cell structure or cell-based bioreceptors are also promising as it creates a specific binding to sense functional and biological activity of the targeted analyte.[111] The biorecognition mechanism either uses whole-cell bacteria/microorganism or specific cellular component. In cell-based biosensors, whole cells or cellular structures act as the molecular recognition element. For the detection process, two transduction phases are involved, namely primary and secondary transducers which are responsible to convert the analyte detection into a cellular response and an electronic signal, respectively. Living cells are used as recognition elements due to several reasons such as (a) cells capability to recognize and respond to biochemical stimuli with high sensitivity, (b) the role of cells for the functional analysis of biochemical agents and (c) the detection limit is very low due to its ability to amplify the detection signal.[109,112] Besides that, mammalian cells are also exploited as a biorecognition element for the detection of foodborne bacteria due to their potential to differentiate between viable and non-viable bacterial cells in food samples which is a critical issue in food safety.[113] The live mammalian cells release chemical signaling molecules when it is infected by foodborne bacterial pathogens present in the system and the signal reading can be measured using optical such as luminescence or fluorescence that evaluates toxigenic exposures.[114] Only viable bacterial cells are able to exert cytotoxicity to the sensing mammalian cells, thus the signal will not be produced by non-viable cells.

Carbon nanomaterial electrochemical biosensors as a promising analytical tool for bacterial detection

Electrochemical biosensor is one of the popular approaches that has been adapted for the foodborne bacterial detection. Electrochemical biosensors have some advantages over other analytical systems such as the ability to work with turbid samples, low fabrication cost, instrumental sensitivity and offers easy miniaturization. Electrochemical biosensors are categorized according to the parameters measured by the device, namely amperometric (current), potentiometric (potential or charge accumulation), impedimetric (impedance) and conductometric (conductivity).

A great effort has been channeled into the development of highly sensitive and selective biosensors worldwide over the past decade. Advancements in the field of nanotechnology have made a profound influence on biosensor development and application due to its fascinating properties such as high surface area, mechanical properties and high thermal and electric conductivity.[115–117] At the nanoscale, unique physicochemical changes occur to nanomaterials due to surface effect and quantum effect.[118] As the surface to volume ratio increases, the interface for recognition element increases and results in overall improvement of biosensors sensitivity. Most importantly, the quantum confinement phenomenon in nanomaterials leads to the rise in the band-gap energy with decreasing size. This causes the electrical and optical properties of nanomaterials to become size and shape dependent and this size tunable property is a crucial point for biosensing devices.[119] The high sensitivity of detection can be achieved in nanosized materials due to its high reactivity and enhanced physical properties which includes electrochemical, electrical and optical properties.[120] Owing to their intriguing high electrical conductivity, chemical stability, biocompatibility and promising mechanical strength accompanied by the capacity to hybridize into sp, sp^2 and sp^3 configurations with narrow gaps between 2s and 2p electron shells of carbon-based nanomaterial leads to the sensitive detection of biological compounds.[121–124]

Most carbon nanomaterials, especially the planar and tubular geometries of graphene and CNTs, respectively, possess a comparable Debye length, λD to the dimensions of these nanostructures. When an electrode is immersed into an electrolyte solution, an electric double layer is formed, consisting of opposing-charged molecules adsorbed on the electrode surface (inner Helmholtz plane) and a diffuse layer with decreasing concentration of the molecules with respect to distance from the electrode.[125] The surface charge of graphene and CNTs is able to influence the thickness of the diffuse layer (i.e. Debye length, λD) at the electrode surface, which in turn will modify the electrostatic screening effect. Thus, any biorecognition event with the target analyte that happens within the Debye layer increases the ion concentration due to charge screening by counter-ions which in turn decreases the Debye length.[126] Higher sensitivities and low detection limits caused by modifying the electric double layer will permit label-free detection of analytes,[127] even at high ionic strength and allows label-free detection due to the close proximity of the charged species.[128,129] These superior properties of graphene and CNT allow a wide range of applications in microelectronics through its incorporation in biosensor device fabrication processes. Moreover, the usage of graphene/CNTs in the biosensing field has been greatly extended by modifying the physicochemical properties through surface modification.

Graphene-based electrochemical biosensors

To date, the use of graphene nanomaterials in the development of biosensors have received great attention. Graphene is a 2D planar sheet that exists in a honeycomb lattice structure with zero-band-gap semiconductor and serves as a basic building block for graphite and graphitic nanomaterials of all dimensions.[130] Graphene can be stacked together to form 3D graphite, rolled-up to 1D CNTs or wrapped into 0D fullerenes as shown in Figure 3. The fullerene carbon allotropes is a fundamental building block of crystalline phase with sixty carbon atoms completely bonded in an icosahedral symmetry that forms a spherical structure.[131] In a 1D structure, carbon atoms are arranged in tubular-like structures as CNTs whereas in 2D, carbon atoms form a monolayer packed into honeycomb lattice called graphene.[132,133] Graphite which exists in 3D is another form of carbon atoms that arranged as stacks of graphene layers coupled by van der Waals forces of attraction.[134]

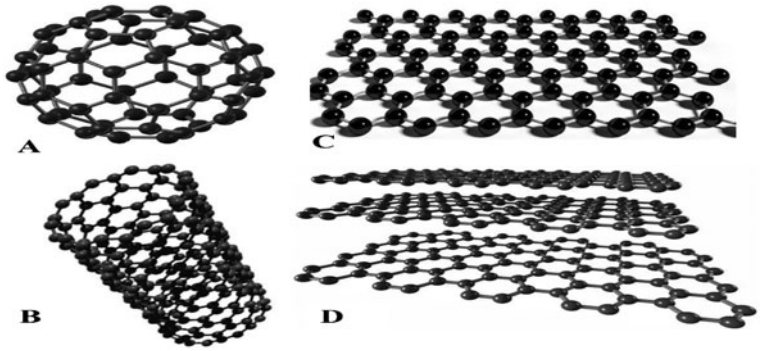

Figure 3. The different dimension of carbon nanomaterials which consist of (A) 0D fullerenes, (B) 1D CNT, (C) 2D graphene and (D) 3D graphite.

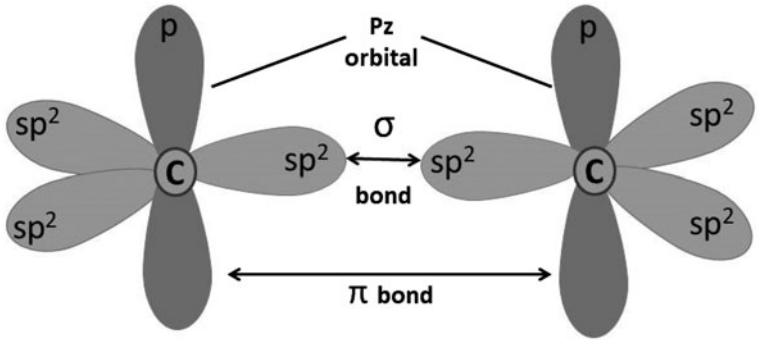

Figure 4. Schematic diagram of carbon atom hybridization in graphene.

The carbon atoms in graphene are sp^2 hybridized which consist of one s-orbital and two p-orbitals that hybridize together to form a honeycomb lattice structure.[133] Whereas, the p_z (π-orbitals) regulates the conductivity or charge mobility of graphene through the formation of delocalized π systems. In detail, the s, p_x and p_y orbitals of carbon forms a σ-bond between neighboring carbon atoms leaving the p_z orbital unhybridized as shown in Figure 4. This p_z orbital on each carbon atom overlaps with neighboring carbon atoms to form a π-bond (valence energy band), causing the delocalization of excess π-orbitals to form a conduction energy band.[135] This atomic structure of graphene largely contributes to its exceptional mechanical strength (130 GPa), excellent electrical conductivity (6000 S cm^{-1}) due to the fast electron transportation.[136,137]

Besides that, graphene also possesses several extraordinary properties including large surface area (2630 m^2 g^{-1}), superior thermal conductivity (~3000 W/mK) and most importantly good biocompatibility.[130,138,139] Thus, graphene nanomaterials have great potential for the development of highly sensitive and selective biosensors.[140] Besides these physicochemical properties, biocompatibility is an important selection criterion. The high surface area of graphene enables the smooth interaction with biological molecules such as enzyme, nucleic acid, aptamer, protein, antibody, virus and bacterial cell.[141,142] The honeycomb lattice structures also favor non-covalent forces, such as hydrogen bonding, π–π stacking, electrostatic forces, van der Waals forces and hydrophobic interactions with biomolecules.[141,143]

Graphene can be exfoliated and oxidized to form graphene oxide (GO) to produce stable dispersion in water due to the presence of the hydroxyl, carboxyl and epoxide functional groups.[144] After reduction, GO is transformed into reduced graphene oxide (rGO) with some residual oxygen and structural defects yielding high thermal conductivity.[145] The enriched oxygen functional groups of GO endow it with the ability to immobilize the biomolecules through covalent binding which is greatly influenced by the C/O ratio. Usually, high C/O ratios promote stronger binding to biomolecules than low C/O ratio.[146] Besides the interaction with biomolecules, these functional groups also facilitate the production of graphene-based composites. There are many physical and chemical methods adapted for the production of graphene, graphene-derivatives and graphene-based composites such as mechanical exfoliation, chemical vapor deposition (CVD), chemical and thermal exfoliation.[147] Mostly, chemical and thermal exfoliation are used in many research due to the ease of operation and mass production of graphene.[118] All the production methods produce graphene with surface defects and oxygen-bearing groups but, these defects favor further surface modification with chemical or biomolecules.[145] The chemical modification of graphene is important to prevent aggregation in water and after pre-functionalization its surface can be easily modified.

Graphene has been successfully used as a transducer nanomaterial in impedimetric, amperometric and conductometric sensors for the detection of pathogenic bacteria. A summary of the working principles of each electrochemical detection method is described earlier in this review and in Xu et al.[148] Graphene has many interesting properties that make it a valuable nanomaterial in biosensing. For instance,

the fluorescence quenching property of GO was used in a PDMS/paper/glass hybrid microfluidic biochip for the simultaneous detection of *S. aureus* and *S. enterica*.[149] Graphene has also been found to be an excellent host for ssDNA, aptamers and antibodies owing to the surface functional groups being able to participate in the covalent immobilization of the biorecognition elements. The electroconductive properties of graphene are affected by factors such as the number of layers, substrate, adsorbed impurities, flatness, defects, size of the sheet, edge types and functionalization.[150] The tunable properties of graphene are widely exploited to cater to the specifications of each designed sensor. A summary of the available biosensor graphene-based platforms is presented in Table 3.

Impedimetric and conductometric graphene-based biosensors

In recent years, graphene has been applied widely in impedimetric biosensors as a transducer nanomaterial. Graphene has been found to provide added values: (1) provides efficient electron-transfer properties, (2) serves as an alternative flexible solid substrate, (3) reinforces mechanical strength, (4) provides functionalized surface for ready immobilization of biorecognition element and (5) increases the binding affinity between metal nanoparticles and solid substrate.[161,162]

Bhardwaj and coworkers immobilized bacteriophages onto graphene screen-printed electrodes for the impedimetric quantitative analysis of *Staphylococcus arlettae*.[163] The graphene screen-printed electrodes were functionalized with –COOH groups by electrochemical oxidation in a solution of 0.1 M HCl. Graphene surfaces which have been functionalized with –COOH group can be used as a platform for facile covalent immobilization of biorecognition elements. The bacteriophages were then easily immobilized onto the graphene surface using 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) to form amide bonds. The authors reported a detection limit of $2.0 \times 10^{\circ}$ cfu mL^{-1} of *S. arlettae* for the proposed cell assay, using spiked samples of river water and apple juice.

The number of graphene layers affects the performance and cost-effectiveness, and the balance between these two factors is necessary to enable the commercialization of biosensors. For instance, an impedimetric immunosensor was prepared by immobilizing anti-*E. coli* antibody on MG and GNP using PASE in methanol solution and the sensitivity was evaluated for both MG and GNP immunosensor. In this study, the charge carrier mobility was compared between pristine, defect-free monolayered-graphene (MG) and less-expensive few-layered graphene nanoplatelets (GNPs). A minimum sensitivity of 10^1 cfu mL^{-1} and 10^2 cfu mL^{-1} was achieved for MG and GNP immunosensors, respectively.[153] This observation is reasonable as pristine MG has a reduced number of defects compared to GNPs, hence better electron mobility.

The selection of the biorecognition element is another strategy to achieve high sensitivity, specificity and ease of biosensor fabrication. Aptamers which target outer cell membranes have gained increasing attention in recent years.

Table 3. Overview of graphene-based electrochemical biosensors for foodborne bacterial pathogen detections.

Transducer material	Sensor type/ Detection mode	Analyte	Biorecognition element	Immobilization technique	Assay time	Detection limit	Ref.
CeO_2-rGO-PANi	Voltammetry/Aptasensor	*A. hydrophila*	Aerolysin toxin gene fragment	Non-covalent binding	3 h	1.0×10^{-16} M	[151]
Graphene oxide-gold nanoparticles	Impedimetry/ Immunosensor	*E. sakazakii*	HRP-anti-*E. sakazakii*	Non-covalent binding	2 h	1.2×10^{2} cfu mL^{-1}	[152]
MG and GNPs	Impedimetry/ Immunosensor	*E. coli* O157:H7	Anti-*E. coli* O157:H7	PASE in methanol	1 h	1.0×10^{1} cfu mL^{-1}	[153]
GOx-Thi-Au@SiO_2	Amperometry/ Genosensor	*E. coli* O157:H7	DNA/G-quadruplex	Non-covalent binding	Not stated	0.01 nM	[154]
Au nanoparticle modified graphene paper	Impedimetry/ Immunosensor	*E. coli* O157:H7	Biotinylated anti-*E. coli* O157:H7	Streptavidin-biotin interaction	Not stated	1.5×10^{2} cfu mL^{-1}	[155]
rGO-Cu	Impedimetry/ Immunosensor	*S. aureus*	Anti-*S. aureus*	EDC-NHS	Not stated	4.4×10^{0} cfu mL^{-1}	[156]
rGO and gold nanoparticles	Impedimetry/ Aptasensor	*S. aureus*	*S. aureus* binding aptamer	Non-covalent binding	1 h	1.0×10^{1} cfu mL^{-1}	[157]
GCE-modified with GO and gold nanoparticles	Impedimetry/Aptasensor	*Salmonella*	Thiolated ssDNA aptamer	Non-covalent binding	Not stated	3.0×10^{0} cfu mL^{-1}	[158]
GO-chitosan nanocomposite	Amperometry/ Genosensor	*Salmonella* Typhi	5′-amine labeled probe	Glutaraldehyde	1 min	10 fM	[159]
rGO-MWCNTs	Impedimetry/ Aptasensor	*Salmonella* ATCC 50761	Amino-modified aptamer	EDC-NHS	1 h	2.5×10^{1} cfu mL^{-1}	[160]

A *Salmonella* aptasensor was prepared on a glassy carbon electrode modified with GO and gold nanoparticles by non-covalent immobilization of thiolated ssDNA specific to *Salmonella* outer membrane proteins.[158] The ssDNA sequence used in the reported study was previously reported in Ref.[164] In this study, the sensor quantified *Salmonella* based on the resistance between the electrode and electrolyte. The impedance increases with increasing bacterial concentration as more bacterial cells bound to the electrode surface. This detection system was reported to produce a detection limit of $3.0 \times 10^{\circ}$ cfu mL^{-1} and the low detection limit was attributed to the binding specificity of the aptamer and the electron-transfer ability of the GO and gold nanoparticle composite.

Parallel to the increasing understanding of the factors that affect the detection of bacteria, the sensitivity of biosensors and multiple amplification strategies are often combined into new biosensor designs. Hu et al. proposed a combined study of various amplification strategies to enhance the specificity and sensitivity for the immunosensing of *E. sakazakii*.[152] In their study, horseradish peroxidase (HRP)-labeled antibody specific to *E. sakazakii* was immobilized on a graphene and gold nanoparticle (ERGO-AuNP) composite. This immunosensor was able to detect *E. sakazakii* with a detection limit of 1.2×10^{2} cfu mL^{-1}. The enhanced sensitivity was attributed to (1) the presence of HRP which preserved the enzymatic activity of the anti-*E. sakazakii* antibody and (2) the electroconductive ERGO-AuNP composite provided efficient electron-transfer at the electrode-electrolyte interface. An impedimetric *S. aureus* immunosensor with a detection limit of $4.4 \times 10^{\circ}$ cfu mL^{-1} was reported using a reduced GO-Cu cysteine hierarchical structure.[156] The addition of cysteine enhanced the immobilization of anti-*S. aureus* antibody on the substrate, whereas the addition of Cu enhanced the chemical interaction between cysteine and GO. Overall, the sensor showed an increased impedance with increasing bacterial concentration, caused by the formation of a capacitive dual layer between the electrode–electrolyte interfaces. In another study, a gold nanoparticle and reduced graphene oxide (Au-NP/rGO) nanocomposite were utilized as an electroconductive immobilization matrix for the immobilization of thiolated aptamers specific to *S. aureus*.[157] The Au-NP/rGO/glassy carbon sensor was utilized to quantify *S. aureus* in the concentration range from 10° to 10^{6} cfu mL^{-1} and gave a detection limit of 10^{1} cfu mL^{-1}. The authors attributed the increase in sensitivity and stability of the Au-NP/rGO/glassy carbon biosensor to the binding affinity between GCE and rGO, in comparison to direct deposition of Au-NP on the bare glassy carbon electrode. A free-standing graphene paper electrode was recently proposed as an alternative solid substrate to glassy carbon, indium tin oxide (ITO) glass and screen-printed electrodes. Gold nanoparticles were grown on the graphene paper and anti-*E. coli* was immobilized on the paper electrode via biotin-streptavidin system.[155] The graphene paper immunosensor exhibited a detection limit of 1.5×10^{2} cfu mL^{-1} for *E. coli*. This immunosensor also provided an added advantage of flexibility.

An indirect approach to detect bacterial contamination is by measuring the number of metabolites or extracellular molecules associated with the bacterial activity of interest using the designed biosensors. For instance, a graphene conductometric sensor was used to detect changes in pH as an indicator of *E. coli* concentration.[162] In that study, anti-*E. coli* antibody was immobilized on CVD-grown graphene using 1-pyrenebutanoic acid succinimidyl ester linker molecule. When the sensor was immersed in a suspension of *E. coli* cells, cells bound to the graphene electrode. The bacterial metabolism of glucose altered the local pH and led to a change in graphene conductance. The graphene immunosensor showed a detection limit of 10 cfu mL^{-1}. In another study, an alginate/graphene oxide (NaAlg/GO) hydrogel matrix was used to immobilize rat basophilic leukemia (RBL-2H3) mast cells onto a gold electrode for the detection of N-3-oxo-dodecanoyl-L-homoserinelactone ($3OC_{12}$-HSL).[165] $3OC_{12}$-HSL is an N-acylated homoserine lactone (AHL) and is one of the bacterial quorum signaling molecules produced by *Pseudomonas aeruginosa*. AHLs have been found to interact with eukaryotic cells to reduce immune responses and facilitate infection. Hence, that study evaluated the toxicity of food-borne pathogenic bacteria by quantifying $3OC_{12}$-HSL present in a food sample. An electrochemical signal was produced when the RBL-2H3 mast cells interacted with $3OC_{12}$-HSL, and the change in resistance at the electrode-electrolyte interface was detected by EIS. The detection limit for $3OC_{12}$-HSL was 0.034 µM with a linear range of 0.1 to 1 µM. The authors highlighted that the wrapping of MWCNTs with reduced GO improved the electrochemical performance of the composite due to synergistic effects between MWCNT and reduced GO.

Hybrid carbon nanomaterials have also been considered as an alternative sensing platform. The hybridization of carbon nanomaterials for instance, CNTs and graphene are believed to enhance the detection performance by combining the desirable qualities of both nanomaterials. For instance, an amino-modified aptamer specific to *Salmonella* was attached to a reduced GO and MWCNT nanocomposite using EDC-NHS chemistry.[160] This *Salmonella* aptasensor demonstrated a detection limit of 2.5×10^{1} cfu mL^{-1}. The authors attributed the wrapping of MWCNTs with reduced GO improved the electrochemical performance of the composite due to synergistic effects between MWCNT and reduced GO. Moreover, the highest sensitivity in electrochemical bacterial sensing bio-device was demonstrated by Mohanty and Berry with a single bacterium resolution. In that study, chemically modified graphene functionalized with biomolecules was used to build a biomolecular transistor. The conductivity of bio-transistor device was found to increase with the attachment of single *B. cereus* bacterium on the graphene surface.[166]

Amperometric graphene-based biosensors

Amperometric detection has been used in the detection of DNA (genosensors) as well as for whole-cell bacteria. For instance, an amperometric electrochemical genosensor was prepared using a GO-chitosan nanocomposite. The

biorecognition element used was 5'-amine labeled ssDNA probe from Vi capsular antigen gene region of *S. enterica* serovar Typhi and was immobilized on the GO-chitosan nanocomposite using glutaraldehyde.[159] Using CV and DPV techniques, the proposed genosensor was able to detect DNA up to a detection limit of 10 fM. Li et al.[154] prepared a sensitive electrochemical *E. coli* O157:H7 DNA biosensor using a graphene oxide-thionine-gold nanoparticle-coated SiO_2 (GOx-Thi-Au@SiO_2) nanocomposite as the conducting substrate. A DNAzyme consisting of hemin-intercalated G-quadruplex was immobilized onto the GOx-Thi-Au@SiO_2 nanocomposite to mimic the signal amplification activity of horseradish peroxidase (HRP). GO provided a high surface platform and structural support for the transducer and biorecognition element components.

Moreover, an alternative methodology for the immobilization of DNA on electrode surfaces was proposed by Jafari et al. using reduced GO decorated cerium oxide nanoparticles on polyaniline/glassy carbon electrode (CeO_2NPs-RGO/PANI/GCE). Single-stranded DNA (ssDNA) identified as the aerolysin toxin gene fragment was used as the biorecognition element for the detection of *A. hydrophila* and was fixed on the CeO_2NPs-RGO/PANI/GCE electrode *via* non-covalent binding.[151] The combination of CeO_2 and graphene prevented the agglomeration of CeO_2 nanoparticles as well as restacking of the graphene layers, thus, providing a high surface area platform for the immobilization of aerolysin toxin gene fragment as the biorecognition element. The interaction between immobilized ssDNA on the electrode surface was found to be strong and was attributed to the high catalytic activity of CeO_2 as well as the ability of CeO_2 to adsorb DNA through electrostatic attraction. A low detection limit of 10 fM was achieved due to the use of Fourier square wave voltammetry which was able to filter the background signal.

Besides decorating graphene with metal oxide nanoparticles, the functionalization of graphene with electrochemically active dyes may also increase the sensitivity of the sensor. Muniandy et al. recently reported a whole-cell *Salmonella* aptasensor using a reduced GO-azophloxine nanocomposite as the sensing platform.[167] ssDNA specifically targeting the outer membrane of *S.* Typhimurium was immobilized onto the reduced GO-azophloxine surface via non-covalent binding. The aptasensor was evaluated in comparison to a conventional PCR amplification assay and an enhanced detection limit of 10^1 cfu mL^{-1} was observed for the reduced GO-azophloxine aptasensor as compared to a 10^2 cfu mL^{-1} detection limit for the PCR assay. The electroactive azophloxine molecule was able to enrich the free charge-carrier density of reduced GO. In another study, the detection of *S.* Typhimurium was carried out with an aptasensor prepared using reduced GO-chitosan (rGO-CHI) composite as the conductive substrate.[168] The aptamer was functionalized with a thiol group and immobilized on rGO-CHI using glutaraldehyde as the crosslinker. This aptasensor demonstrated a detection limit of 10^1 cfu mL^{-1} in raw chicken samples spiked with *S.* Typhimurium. In the selectivity test, the aptasensor was found to specifically detect *S.* Typhimurium and did not give any response to non-*Salmonella* bacteria (*S. aureus, K. pneumoniae* and *E. coli*).

CNT-based electrochemical biosensors

Rolled up nanoscale tubes of graphene sheets are known as CNTs. These nanotubes are made of sp^2 hybridized carbon atom arranged in a cylindrical shape at nanosize and can be classified into two groups such as single-walled carbon nanotubes (SWCNTs) and double or multiwalled carbon nanotubes (DWCNT or MWCNTs). It can be mass produced using CVD, carbon arc methods or laser evaporation. Their unique combinations of structural, chemical and electronic properties have a profound impact on the fabrication of electrochemical biosensors as compared to other nanomaterials.[169–171] SWNTs are made up of the rolled-up graphene sheet, whereas MWCNTs are composed of multiple graphene sheets stacked up the cylindrical shape with a diameter ranging to 100 nm. SWCNT can be categorized into armchair, zigzag and chiral models (Figure 5)

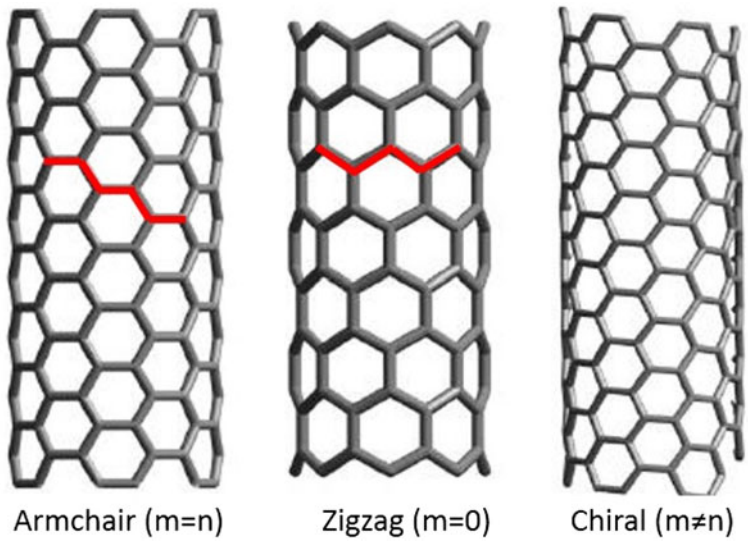

Figure 5. Types and chirality of carbon nanotubes.

depending on the chirality represented by a pair of indices (n,m) called the chiral vector.[172,173] The armchair configurations often behaves as metallic and the others behave as semi-conductors.[174] There are another two structural models for MWCNTs, namely the Russian doll and Parchment model. In the Russian doll model, the carbon tubes contain another nanotube with smaller diameter wrapped interior to it whereas in the Parchment model, the single graphene sheet wrapped around itself many times.[175]

The electronic properties of carbon nanotubes are similar to graphene with three $2sp^2$ electrons and one 2p electron in each carbon atom. The three $2sp^2$ electrons are responsible to form three bonds in the plane of graphene sheet leaving an unsaturated $P_{z.}$ orbital.[176] This $P_{z.}$ orbitals are perpendicular to the graphene sheet and cause the formation of a delocalized π network on the surface of nanotube when the sheet is rolled. This delocalized network is responsible for its electronic properties.[177] However, the electronic properties of CNTs solely depend on the geometrical structure or chirality which gives them metallic and semi-conducting electrical behavior.[178]

CNTs-based electrochemical biosensors are advantageous due to some captivating factors. The most important factor are its excellent conductivity and electrochemical properties such as fast electron transfer capacity associated with a good electrocatalytic activity involving the transfer of an electron between the electroactive species and electrode.[179–181] The larger surface area and surface chemistry of the graphitic wall of CNT also enhance the interactions of different biomolecules and increase the overall biocompatibility of the nanomaterial.[182] The desired biomolecules can be either covalently or non-covalently linked to the carbon nanomaterial surface. The covalent binding involves surface modification with a chemical functional group, whereas non-covalent bonding uses π- π stacking interactions which involve physical adsorption of chemical groups on CNT's surface.

Surface functionalization helps to improve the overall solubility of hydrophobic buckytubes of CNT and increases its biocompatibility.[183,184] CNT can be functionalized with hydroxyl, carboxyl or amino groups with combinations of other nanomaterials such as metal nanoparticles, polymers or ionic liquid.[185] Functionalized CNT exhibits increased solubility, catalytic activity and surface area of both basal and edge plane.[186] Modified CNTs with functional groups also pave a way for the effective adsorption of biological recognition of molecules (enzymes, DNA, antigen/antibody, etc.) for further biosensing applications.[187]

CNT has been recognized as a promising nanomaterial for integration into electrochemical biosensors due to its unprecedented electrical and electrochemical properties. CNT-based biosensors are also popularly used because of their intrinsic advantages such as high sensitivity, fast response time, easy operation, cost-effective and favorable portability. However, there are a few challenges in using CNT in the field of electrochemical biosensing such as, expensive commercial production of defect-free and pure nanomaterial and aggregation of the tubes in solvents.[122] These limitations hinder the practical applications of CNT. CNT-based electrochemical biosensors can be categorized into biocatalytic sensors and bioaffinity sensors. The biocatalytic event involves sensing the electroactive species produced resulting from the use of the biological recognition element (e.g. enzyme), whereas bioaffinity sensors monitor the interaction between the biological recognition element and the analyte.[17,69] A summary of the available biosensor CNT-based platforms is presented in Table 4.

Amperometric CNT-based biosensors

The unique properties of CNT have led to numerous studies that investigate the interactions between biomolecules and nanomaterials. CNT biosensors have been applied widely for the detection of foodborne bacteria due to their ability to monitor and measure even a small change at CNT's interface from adsorption of biomolecules.[197]

Punbusayakul et al. have developed an amperometric biosensor exploiting the first usage of as-grown DWCNT bundles for fabricating a covalently immobilized label-free immunosensor for the detection of *S.* Typhimurium using anti-*Salmonella* monoclonal antibody.[198] The DWCNT used in the study was previously reported to have a faster rate of electron transfer with good redox activity and biocompatibility for various species.[199] This immunosensor was electrochemically characterized by CV and chronoamperometry to study the interaction of the sensor with *S.* Typhimurium cells. This immunosensor exhibited detection ranged from 10^2 to 10^7 cfu mL^{-1} with a detection limit of $9.0 \times 10^{\circ}$ cfu mL^{-1}. In addition, Liu et al. proposed a novel portable amperometric biosensor modified with MWCNT-chitosan-peroxidase for the detection of *S.* Pullorum in chicken samples using anti-*Salmonella* polyclonal antibodies.[200] In the study, amperometric detection of reaction product formed from catalytic activity between catalase enzyme (biomarker of *S.* Pullorum) and hydrogen peroxide (substrate) was measured. This amperometric biosensor can detect viable cells of *S.* Pullorum up to 10^2 cfu mL^{-1} within 30 minutes of assay time. The combination of the sensing system with pre-enrichment step of analyzed food sample relatively improved the detection limit up to 6.0×10^1 cfu mL^{-1} within 1.5 hours of assay time. However, the proposed methodology in that experiment could lead to false positive results due to the interaction of the polyclonal antibodies with other non-target bacteria. Thus, inclusivity and exclusivity tests are needed for more accurate detection.

Some studies proposed layer-by-layer or electrophoretic deposition process for the assembly of CNT on the electrode surface for pathogen detection. It precisely controls the spatial arrangement and distribution of the nanoparticles onto the substrate which will ease the automation process in industrial scale. For instance, the detection of pathogenic *E. coli* in food samples using an amperometric immunosensor was reported by Li et al.[201] They successfully made a self-assembled gold surface with immobilized gold nanoparticles and chitosan-MWCNT-SiO2-thionine through the layer-by-layer deposition process. *E. coli* O157:H7 antibody (anti-*E. coli* O157:H7) was covalently bound to the GNP monolayer and its bioactivity was measured with an amperemeter. This

Table 4. Overview of CNTs-based electrochemical biosensors for foodborne bacterial pathogen detections.

Transducer material	Sensor type/ Detection mode	Analyte	Biorecognition element	Immobilization technique	Assay time	Detection limit	Ref.
MWCNTs	Amperometry/Aptasensor	*S.* Typhimurium *S.* Enteritidis	Amino-modified aptamer	Covalent bonding	30 min	5.5×10^1 cfu mL^{-1} and 6.7×10^1 cfu mL^{-1}	[188]
Pyrrole/gold-MWCNTs/chitosan	Amperometry/ Immunosensor	*E. coli* O157:H7	Monoclonal anti-*E. coli*	Non-covalent binding	Not stated	~3.0×10^1 cfu mL^{-1}	[189]
CNTs modified screen-printed electrodes	Impedimetry/ phage-sensor	*E. coli* ATCC 11303	T2-bacteriophage	Covalent bonding and electrostatic interaction	1 h	5.0×10^1 cfu mL^{-1}	[190]
Gold-polyamine-MWCNTs chitosan	Impedimetry/ Immunosensor	*Salmonella*	Anti-*Salmonella*	Covalent bonding	Not stated	5.0×10^2 cfu mL^{-1} in milk	[191]
SWCNTs	Voltammetry/ Immunosensor	*S. aureus*	Anti-*S. aureus*	Covalent bonding	30 min	4×10^0 cfu mL^{-1}	[192]
SWCNTs	Impedimetry/ Immunosensor	*Salmonella*	Anti-*Salmonella* monoclonal antibodies	Diimide activated amidation coupling	Not stated	1.6×10^4 cfu mL^{-1}	[193]
MWCNTs-polyallylamine	Conductometry/ Immunosensor	*E. coli* O157:H7, *Campylobacter* and *Salmonella*	Anti-*E. coli*, anti-*Campylobacter* and anti-*Salmonella*	Nanocrystal bioconjugates	3 h	8×10^2 cfu mL^{-1}, 4.0×10^2 cfu mL^{-1} and 4.0×10^2 cfu mL^{-1}, respectively	[194]
MWCNTs	Conductometry	*E. coli* O157:H7	Not stated	Non-covalent binding	Not stated	2.0×10^2 cfu mL^{-1}	[195]
SWCNTs	Potentiometry/ Aptasensor	*E. coli*	Aptamer	Covalent binding	1 h	6.0×10^0 cfu mL^{-1} in milk and 2.6×10^1 cfu mL^{-1} in apple juice	[196]

sensor allowed the detection of bacterial cells ranging from 10^2 to 10^5 cfu mL^{-1} within 45 minutes of assay time. Similarly, Bhardwaj and colleagues have fabricated an electrophoretically deposited MWCNTs genosensor for the detection of *E. coli*.[202] The MWCNT was functionalized with –COOH groups to increase its overall solubility and biocompatibility. Then, a DNA probe derived from 16S rRNA region of the *E. coli* was covalently linked onto the CNT surface to allow label-free electrochemical genosensor for sensitive and selective detection ranging from 10^{-7} to 10^{-12} M and detection limit of 1×10^{-12} M. This electrophoretic nanomaterial deposition strategy has opened new avenues for the designing of electrochemical nucleic acid sensors.

The solubility of MWCNTs in most common solvents creates a barrier for the development of well-defined modified electrodes. To solve this problem, Zhao et al. designed a disposable electrochemical immunosensor based on MWCNT/sodium alginate (SA) composite electrode for the detection of for *Shigella flexneri*.[203] SA binds non-covalently to MWCNTs to effectively improve its solubility and synergistically improves the biocompatible and electronic properties of the composite. A horseradish peroxidase-labeled antibody to *S. flexneri* was immobilized on the MWCNT/sodium alginate composite by physical adsorption. This proposed immunosensor was a sensitive, simple, stable and reproducible analytical tool for the detection of *S. flexneri* with a detection range of 10^4 to 10^{11} cfu mL^{-1} and detection limit of 3.1×10^3 cfu mL^{-1} in saline. Another disposable enzyme-labeled MWCNT amperometric immunosensor was developed for the detection of *L. monocytogenes* using HRP-labeled antibody.[204] This immunosensor showed a linear relationship between *L. monocytogenes* concentration ranging from 10^2 to 10^5 cfu mL^{-1} with LOD of 1.1×10^2 cfu mL^{-1}. The developed immunosensor was also tested with milk sample spiked with a cocktail bacterial species. The results obtained showed a detection of 1.5×10^3 cfu mL^{-1} with good reproducibility, specificity and stability.

Impedimetric and conductometric CNT-based biosensors

CNT can be incorporated with conductometric and impedimetric biosensors. This enables the monitoring and measurement of both electron transfer rate and also charge transfer resistance results from a bio-recognition event.

Dong et al. proposed a new electroanalytical method based on poly(amidoamine)-MWCNT-chitosan label-free electrochemical impedance immunosensor for the detection of *S.* Typhimurium.[191] The anti-*Salmonella* antibody was immobilized on the electrode using gold nanoparticles. The electron transfer resistance resulted from the binding event between *Salmonella* cells and antibodies were measured. This innovative combination of nanomaterials increases the stability, shelf-life and overall sensitivity of the sensor with a detection limit of 5×10^2 cfu mL^{-1}. This immonosensor also showed high specificity when tested for interference effect with other bacteria such as *E. coli* and *S. aureus*. When the particular sensor was adapted for food analysis, it gave a satisfactory result with recovery rate ranging from

94.5% to 106.6% within 1 hour assay time in fat-free milk samples. Besides that, detection of whole bacterial cells was made possible with the reduced graphene oxide (rGO) and carboxy-modified MWCNTs nanocomposite electrode as reported by Jia et al.[160] They developed CNT based impedimetric aptasensor for the detection of *Salmonella* bacteria. This rGO-MWCNT nanocomposite was covalently immobilized with amino-modified aptamer specific for *Salmonella* via amide bonds. In the presence of the bacterial cells, the anti-*Salmonella* aptamer hybridizes with its target and causes blockage of electron transfer and results in a large increase in impedance. *Salmonella* cells can be quantified by this aptasensor with detection ranges from 7.5×10^1 to 7.5×10^5 cfu mL^{-1} and detection limit of 2.5×10^1 cfu mL^{-1}.

A conductometric disposable electrochemical immunosensor was also developed for a specific, reproducible, stable and sensitive simultaneous detection of *E. coli* O157:H7 and *E. sakazakii*.[205] MWCNT/sodium alginate (SA)/carboxymethyl chitosan (CMC) composite films were coated on screen-printed carbon electrodes to enhance the sensitization of the electrode. This sensor that capable to perform simultaneous detection of different type of pathogens are efficient to quantitatively detect a panel of biomarkers in a single run. The antibodies of respective pathogens were labeled with Horseradish peroxidases (HRP) and immobilized on the screen-printed electrode. MWCNT and HRP enhance the CV response and under optimized conditions, this immunosensor showed a linear range of detection from 10^4 to 10^{10} cfu mL^{-1} with a low detection limit of 4.6×10^3 cfu mL^{-1} and 3.3×10^3 cfu mL^{-1} for *E. sakazakii* and *E. coli* O157:H7, respectively. Besides immunosensor, the DNA-based conductometric biosensor was also studied by some researchers. Fernandes et al.,[206] developed another novel conductometric genosensor based on MWCNT–chitosan–bismuth and lead sulfide nanoparticles for the detection of pathogenic *Aeromonas*. Two different types of DNA probe namely, a signalizing DNA probe and thiol-modified oligonucleotide fixing DNA probe were immobilized on the surface of the electrode. These probes hybridize target *Aeromonas* DNA and the hybridization signal was measured using DPV. The detection limit of this genosensor was 1.0×10^{-14} M. This method was able to detect *Aeromonas* cells up to 10^2 cfu mL^{-1} in naturally contaminated water samples. The result obtained was also validated using PCR and this genosensor was reported to be more sensitive for the detection of this targeted gene.

Many researchers also reported the use of both conductometric and impedimetric measurement simultaneously for the detection of foodborne bacteria. For instance, Abdalhai et al.[207] designed a genomic DNA MWCNT-chitosan-bismuth functionalized electrochemical biosensor system for the detection of *S. aureus* DNA extracted from pure cultures and fresh beef samples. The fixing and capture DNA probes were modified by thiol and amino groups, respectively. The fixing probe was complementary with *S. aureus* target sequence, whereas the capture probe was bound to the lead sulfide nanoparticles and target sequence to build a sandwich structure. Upon the hybridization of target gene, the sandwich structure dissociates and releases lead sulfide nanoparticles ions which can be quantified using CV and EIS. As the concentration of the complementary target DNA increases, the concentration of dissolved Pb^{2+} also increases. This genosensor exhibited high sensitivity and rapid response with a detection limit of 3.17×10^{-14} M. When this sensor was tested with artificially spiked beef homogenate, a detection limit of 1.23 ng/mL was recorded. A similar approach was adapted for the detection of *Salmonella* spp. using only carboxylated SWCNTs (SWCNT-COOH) modified glassy carbon electrode.[193] *Salmonella* monoclonal antibodies were covalently attached to CNTs by using diimide activated imidation coupling chemistry. In the presence of *Salmonella* cells, changes in charge transfer resistance and impedance were observed after the formation of the antigen–antibody complex. In the study, CNTs behave as molecular wires allowing electrical communication between the underlying electrode and the conjugated antigen–antibody complex which gives a measurable signal. This electrochemical impedance immunosensor showed a sensitive detection with a low detection limit of 1.6×10^4 cfu mL^{-1}.

Potentiometric CNT-based biosensors

Potentiometric biosensor measures the potential change in solution resulting from biorecognition event using an ion-selective electrode. However, the applications of potentiometric biosensors in food analysis is not popular, although some innovative designs of these biosensors are capable of giving reasonable sensitivity and specificity.[88] For instance, Zelada et al.[196,208,209] have extensively researched the adaptation of CNT-based biosensors for bacterial detection. They reported that CNT is capable to act as an ion-to-electron transducer due to its remarkable double layer capacitance that aids the charge transfer between CNT/solution interface and ions surrounding the target analyte.[210] Moreover, the high surface-to-volume ratio of the CNT enables the binding of aptamers which provides a selective detection and enhances the electrochemical properties of CNT by switching the surface charge on the SWCNT layer upon target-recognition event.[122,210,211]

Zelada et al.[209] have also successfully reported the usage of a CNT-based potentiometric biosensor for the detection of *Salmonella enterica* serovar Typhi cells using amino modified aptamer immobilized of carboxylated SWCNT. The interaction between amine spacer and carboxylic group aids the formation of amide bond through carbodiimide-mediated chemistry. This SWCNT not only acts as a sensing layer, but it is also capable to transduce the signals results from the binding of target analytes. In the presence of bacteria, aptamer binds to their cell walls and induces a conformational change leading to a charge transfer between H^+ ions that surround the cell wall and CNTs. The potential increases with increasing concentration of bacterial cells and reached a plateau at a bacterial concentration of 10^6 cfu mL^{-1}. This aptasensor was highly sensitive to detect even a single bacterial colony (1 cfu) present in 5 mL of PBS

solution within 60 s of assay time. When tested with other bacteria such as *E. coli* and *Lactobacillus casei*, this aptasensor also exhibited a high degree of selectivity and proves a facile and sensitive potentiometric analysis for the bacterial detection. Similarly, *S. aureus* has been detected using label-free potentiometric detection using Electromotive Force (EMF) measured in a SWCNT-aptamer biosensor system.[208] The real-time EMF bacterial binding generated a linear signal with increasing concentration of bacteria upon the binding to the aptamer covalently bound to the nanotubes with a detection limit of 8.0×10^2 cfu mL^{-1}.

Design and fabrication of carbon nanomaterials-based electrode for electrochemical biosensors

A variety of designs have been proposed to fabricate carbon nanomaterial-based electrodes for electrochemical biosensors, with the aim of enhancing the biosensor's functionality at an affordable cost. The major design factors that need to be considered include the dimensions and types of the electrode, which will then determine the methods available to integrate the carbon nanomaterial with the electrode.

In electrochemical sensing, the dimensions of the electrodes are an important aspect of the manufacture of field-portable biosensing instruments. Electrodes with smaller physical dimensions offer higher detection sensitivity due to the formation of controlled-diffusion profile near the electrode surfaces.[11,212] Hence, micro-sized electrodes (microelectrodes) offer practical benefits such as decreased ohmic potential drop, fast establishment of steady state signal and a current signal increase due to enhanced mass transport at the electrode boundary. Moreover, device miniaturization also reduces the device cost and the sample volume required for analysis (microliter range or less).[69] For electrochemical sensors, an alternative way to control mass transport is using rotating disk electrodes. However, the application of disk electrodes, that is, hydrodynamic electrodes to control the rate of mass transport were less described in literatures. This is due to the integration of microfluidic systems into the biosensor design which limits the practicality of rotating disk electrodes.

The two main types of electrodes used in commercial biosensors are screen-printed electrodes (SPE) microelectrodes and interdigitated array (IDA) electrodes[17,213] (Figure 6). SPE carbon nanomaterial-based electrodes consist of working, reference and auxiliary electrodes patterned on the microelectrode system. The working electrode usually uses conductive carbon paste and this electrode can be modified with biomolecules or nanostructured materials using drop-casting and electrodeposition techniques.[216] Alternatively, interdigitated array (IDA) electrodes consists of electrode (metal fingers) pairs in parallel which is separated and interdigitated with insulators.[217] The sensitivity of IDAs can be amplified by modulating the digit width/gap ratio and height of the IDA digit,[218] whereby favorable changes in sensitivity are caused by an increase in the surface area available for redox species to interact with electrodes. Moreover, the oxidation current of redox molecules usually gives an amplified signal which increases the signal-to-noise ratio and lowers the detection limit.[219] Signal enhancement up to 1000x has been observed in IDAs, and is dependent on the dimension of the electrode (digit width/gap ratio) to promote the diffusion of redox species.[17]

SPEs and IDAs are prepared using two types of fabrication process which are thick-film and thin-film fabrication processes, respectively.[220] The thick film technology is widely used for the fabrication of low cost and mass production of disposable SPE electrode which involves patterned pressing of the carbon paste at desired dimensions.[213] IDAs can be fabricated using the thin film technique which increases the sensitivity of sensors, such as photolithography method that involves carbonization of light-sensitive polymeric coatings (photoresist) on a silicon wafer surface.[216] When the photoresist coating is exposed to UV light through a mask with the desired electrode pattern, functional patterns are created on the surface of the polymeric substrate.

The carbon nanomaterials may then be integrated onto either the surface or the bulk of the electrode. The most common form of integrating the carbon nanomaterials onto the surface of the electrodes are by simple drop casting or spin coating. Using this technique, the layer of carbon

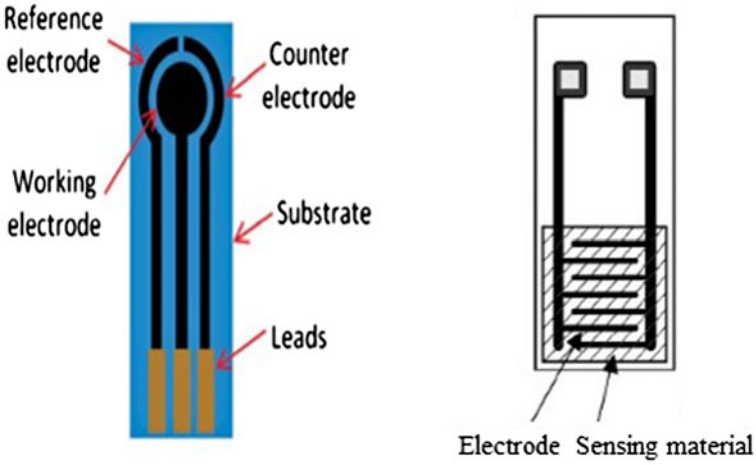

Figure 6. The examples of commercially available (A) SPE[214] and (B) IDA[215] electrodes.

nanomaterials is physisorbed on to the electrode surface. The electrode stability and shelf life are the limiting factors in sensor electrodes prepared using drop casted or spin-coated carbon nanomaterials, as the physisorbed carbon nanomaterials may dissociate easily. Alternatively, to integrate the carbon nanomaterials throughout the electrode (bulk integration), the carbon nanomaterial must be uniformly dispersed through the electrode precursor material, before carrying out the thin film fabrication process.

Miniaturized SPE electrodes is an excellent transducer for portable microfluidic systems, but the reproducibility of the electrodes varies from batch to batch.[220] There are many reports on the application of the carbon nanomaterials-based SPE sensors for the detection of foodborne bacteria. Viswanathan et al.[194] successfully fabricated a disposable MWCNT-polyallylamine modified SPE electrochemical immunosensor for simultaneous detection of *E. coli* O157:H7, *Campylobacter* and *Salmonella* foodborne bacteria within 4 hours of assay time. In the assay, quantum dots conjugated antibodies specific to respective pathogens were immobilized on the surface of modified SPE electrodes. Upon the specific antigen–antibody interaction, the quantum dots were released after the dissolution step and gives three well-distinguished current peaks which were quantified using Square Wave Anodic Stripping Voltammetry. This sensor exhibited low detection limit of 10^2 cfu mL^{-1} and showed reproducible detection when exposed to milk samples. Moreover, another novel SWCNT-modified SPE aptasensor was developed for detection of *E. coli* O157:H7 by Housaindokht et al.[221] The aptamer immobilized on the SPE captured the *E. coli* cells and with the help of methylene blue as a redox probe and the decrease in DPV was recorded after the bio-recognition event. The detection limit of the aptasensor was 1.7×10^1 cfu mL^{-1}. Besides that, a GO based SPE electrode for the sensitive detection of *S.* Typhimurium using bacteriophage as a bioreceptor immobilized using EDC-NHS cross-linkage of the SPE electrode was reported recently.[222] This GO-based SPE sensor used impedimetric technique with a detection limit of 1.2×10^1 cfu mL^{-1} within 40 minutes of assay time. The above studies showed the flexibility of the SPE electrodes for immobilization and surface modification with different types of bioreceptors and carbon nanomaterials, respectively.

Carbon microelectromechanical systems (CMEMS) and carbon nanomechanical systems (CNEMS) devices are examples of IDA that are commonly used for foodborne bacterial detection. Although, the IDA electrode has high reproducibility, however the cost is the limiting factor for the purpose of commercialization.[220] For foodborne bacterial detection, carbon nanomaterials-based IDA was popularly used. For instance, a label-free impedimetric graphene-interfaced interdigitated microelectrodes were developed for a sensitive detection of *E. coli* O157:H7.[153] The graphene was spin-coated and covalently attached on the silicon substrate followed by patterning of the substrates through photolithography. The specific antibodies were immobilized on the substrate by drop-casting and the capturing of complementary cells will produce a capacitance response due to the changes in charge carriers hole density in graphene. This sensor exhibited a detection limit of 10^1 cfu mL^{-1} without using any chemical mediators.

Although the usage microelectrodes are promising in the field of electrochemical sensing, the issues of sensitivity, selectivity and interference with food sample matrices still exist as barriers. The non-selective binding causes clogging problem and reduces shelf-life and reliability of the sensor.[223]

Current status and prospects of carbon-based electrochemical biosensors

A summary of the available electrochemical techniques and the application of carbon nanomaterials for the detection of pathogenic bacteria have been introduced. Moreover, a brief overview of the limitations of the different current detection methods and the various factors leading to the selection of electrochemical detection as a superior detection method with carbon nanomaterials as the material of choice in biosensor devices have also been presented. Electrochemical detection is a promising alternative technique compared to conventional foodborne bacterial detection techniques due to its rapid analytical time and accuracy. The advancements in nanoscale fabrication technology also allow for increasingly compact devices which will increase usability by reducing the amount of sample and analytical reagents required for the analysis.

A lot of effort has been focused on finding successful combinations of biorecognition element and transducer platform for sensitive and specific signal generation. Contributions to the field of biosensors may take on the form of discovering new biorecognition elements, modifying the chemical structure of biorecognition elements with well-known biochemical interactions, discovering new transducer platform materials, improving the stability of immobilized biorecognition elements and understanding the electron transfer process at the electrolyte-biorecognition element or biorecognition element-transducer interfaces. Ultimately, these efforts should translate into enhanced reliability, specificity and detection limit of biosensor devices. In the quest for discovering new transducer platform materials, carbon nanomaterials particularly CNTs and graphene have been explored as a promising sensing platform material due to its exceptional electrical properties and ability to be functionalized.

However, some issues pertaining to device fabrication involving carbon nanomaterials such as the stability of the immobilized biorecognition element on carbon nanomaterial surfaces and the limited understanding of the sensing mechanism in electrochemical devices are caveats for further research opportunities to improve their commercial application. Specific tuning of carbon nanomaterials which involves surface functionalization of the carbon material with polymers, inorganic compound, metal and metal oxide have been reported to increase the electronic properties of carbon, as well as enhance the immobilization of biorecognition element on the carbon nanomaterial sensor electrode surface (by facilitating covalent bonding). A deeper insight into the sensing mechanism is advantageous for optimizing the

device and enhancing the device's performance. Hence, future research is required to further elucidate the sensing mechanism for various analyte-biorecognition element pairs.

The development of carbon nanomaterials for biosensing applications remains an exciting area of research with many opportunities for innovation. Areas of research that would benefit toward the application of CNTs and graphene in biosensor devices include the specific control of functional group types on the surface of carbon nanomaterials, elucidating the mechanism of electron transfer and improving the stability of carbon nanomaterials on the device substrate.

The driving force for commercialization of electrochemical biosensors is the market size, simplicity of the instrument and cost (including maintenance cost). The fabrication of electrochemical-based handheld devices can be complex due to the difficulty in ensuring the reproducibility and stability of the sensor. The immobilization of the biorecognition element on the surface of the biosensing device without losing its function can be challenging. The multiplex detection of various types of foodborne bacterial cells at a time also adds to the complexity of the device. Moreover, the properties of carbon nanostructures also may affect the performance and reliability of the sensing device. Sophisticated sensing device fabrication also may incur more cost which affects its commercialization.

Conclusion

Though electrochemical biosensors have created a breakthrough in foodborne bacterial detection, the culture-dependent and established molecular detection methods remain as the standard analytical methods to assess the safety of food supply. However, the overall assay and detection time is an important factor to be rectified. Therefore, in the past two decades, researchers have taken into consideration these factors to develop fast, direct and reliable detection methods. Electrochemical detection techniques are adapted to ensure food safety as it provides fast, sensitive and reliable results. There are two important factors that need to be considered in implementing electrochemical biosensor for bacterial detection. First, the low concentration (less than 10^2 cfu mL^{-1}) of bacterial contaminants in food products, hence any analytical method should be sensitive enough to detect the low level of bacterial loads and second, it must provide real-time monitoring to avoid delay in detection.

Recent advances in the application of carbon nanomaterials for electrochemical biosensing pave ways for a reliable, rapid, accurate, simple, sensitive, selective and cost-effective detection of foodborne bacterial pathogens. Carbon nanomaterials is a superior platform for the detection of bacterial pathogens due to some attractive physicochemical properties. These include large surface area that enables surface functionalization and immobilization of biological molecules, high electron mobility and excellent conductivity. These superior electronic properties of carbon nanomaterials make them extremely sensitive to small changes in charges resulting from the binding of bacteria to the transducers and causes a noticeable read-out in electronic-sensors. Despite all the advantages, the commercialization of carbon-based electrochemical biosensors remains a challenge due to its high cost, stability issues and complex instrumentation design.

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