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Application of nanomaterials in biosensing for foodborne pathogens detection

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ABSTRACT

Nanotechnology is one of the key fields for electrochemical, optical, and mass sensitive biosensors. The nanomaterials are used as an efficient catalytic tool for electron transfer, immobilization platforms as well as electroactive and optical labels to improve the biosensing performance in terms of high sensitivity, specificity and stability. Nanomaterials or nanoparticles such as carbon nanotubes, metal nanoparticles (Au, Pt, and Pd etc.), nanowires, quantum dots and hybrid nanocomposites are playing a crucial role during the design and development of various biosensing systems for the foodborne pathogen detection. We will discuss throughout this review on the bacterial pathogens associated with foodborne illness such as toxin-producing *Escherichia coli, Staphylococcal enterotoxin B, Botulinum toxins, Salmonella species, Vibrio cholerae, etc.*We will also focus on electrochemical (amperometry, impedance, voltammetry), optical (surface plasmon resonance and fluorescence approach) and mass sensitive (quartz crystal microbalance, microcantilever) biosensing techniques for the detection and characterization of foodborne pathogens. In addition, recent advances in the biosensor development based on various bio-probes such as enzymes, nucleic acids, antibodies, aptamers and phage-display peptides shall be discussed. Herein, next-generation approaches explored for the multiplexed detection of food borne pathogens shall also be enlightened.

Keywords: *Nanomaterials, biosensors, food pathogens,quantum dots, microfluidics.*

INTRODUCTION

Foodborne pathogens are microorganisms (i.e. bacteria, yeasts, molds, fungi and viruses) as well as a number of protozoa, which is responsible for the food spoilage and produce foodborne diseases in human via contaminated food or water (Dwivedi *et al.*, 2011; Invitski *et al.* 1999; Scallan *et al.*, 2011; Velusamy *et al.*, 2010). Because of their ubiquitous presence in nature and rapid growth rate, even under conditions where yeasts and molds cannot grow, bacteria is considered the most important in food spoilage and foodborne diseases. The high-risk pathogens such as *Escherichia coli O157:H7*, *Vibrio cholera, Staphylococcus aureus, Salmonella enteric, Listeria monocytogenes, Bacillus cereus, Clostridium botulinum* (produces paralytic toxin botulin), *Salmonella spp., Yersinia enterocolitica* and *Coxiella burnetii* that causes diseases in humans are mainly transmitted through various contaminated food and water sources. In the recent years, foodborne pathogens, causing diseases have become an important public health problem in global. Raw meat, milk, seeds, fruits, uncooked or canned food, vegetables and water may be the source of bacteria, toxins, which are transmitted during food preparation and enter the food supply chain through cross-contamination by humans. These foods are required to be made free of these bacteria and supply fresh food to humans. Therefore, the safety of food has become a very important concern to human, industry, and their regulatory agencies. The acceptance limit of pathogens depends on its ability to grow in environmental conditions (refrigerated conditions, room temperature or sometimes at high temperatures about 55^0 C). Bacteria important in

food industry have been divided among several groups based on certain characteristics such as thermophilic bacteria, these are capable of growing at 55^0 C (*Bacillus, Clostridium, Streptococcus,*) psychrotropic bacteria, which are capable to grow at the refrigerated temperature (≤ 5^0 C) (*Pseudomonas, Listeria,* etc.). Halotolerant bacteria are capable to survive high salt concentration (≥ 10%), some species from *Bacillus*, *Staphylococcus*, *Vibrio* are included within this group. Aciduric bacteria are able to survive at low pH (≤ 4.0) such as *Lactobacillus, Enterococcus* are included in this group. Most of the bacteria are also considered as biological warfare agents because these pathogens are found to be resistant to environmental conditions and human are susceptible to diseases (Thaselvam *et al*., 2010).

Increasing cases of foodborne diseases among humans are caused by food pathogens. In addition to human health risk and socio-economic aspects due to the microbial contamination which can result in food spoilage and storage of fresh food is another problem in the third-world country. The various agencies/regulatory bodies are monitoring the quality of food in each country. In USA, federal agencies such as the US Department of Agriculture (USDA) and Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) are effectively monitoring the protection of food product, and these systems are acceptable globally. Even though so many regulatory authorities are working with this issue, still various outbreaks of food poisoning and foodborne illness are reported by Center for Disease control USA and every year it is indicating an upward trend (*www.cdc.gov.in*).

In the year 2011, Foodborne Diseases Active Surveillance Network (FoodNet) of US Center for Disease Control and Prevention (CDC) published a report that foodborne diseases caused 48 million people to get sick, 128 000 hospitalizations, and 3000 deaths in America. The main root causes of these deaths were the food pathogens such as *Salmonella, Listeria*, *E. coli* O157 : H7 and *Toxoplasma* (Poltronieri *et al*., 2014). *E. coli*, a Gram-negative bacteria since its discovery have been considered as a non-pathogenic bacterium due to its normal inhabitant in the intestinal tract of humans and warm-blooded animals and birds, but later it was discovered as a causal organism for diarrhea, gastroenteritis, stomach cramps, nausea, and also responsible for several other food and waterborne diseases. This pathogen is transmitted through contaminated food sources like ground beef, uncooked sausages, sprouts, salads, raw milk, vegetables, fruits, contaminated water.

Salmonella, a rod-shaped Gram-negative bacterium is considered a major cause of food and waterborne diseases across the world. The worldwide incidence of foodborne salmonellosis caused by *Salmonella* is very high, and the control measures are not working against *Salmonella*. This is due to the fact that there are more than 2000 serovars of *Salmonella*, potentially capable of causing salmonellosis humans. There are other potential virulent strains like *S. typhi and S. paratyphi* responsible for typhoid and paratyphoid fever in humans. The transmission is caused by fecal-oral route due to consumption of contamination food and water. Some symptoms associated with the infection are diarrhea, fever, chills, vomiting, headache, nausea, prostration and stomach cramps. Food sources for the cause of infection are beef, chicken, shellfish, finfish, turkey, pork, eggs, milk, and products made from them. Another group of bacteria responsible for food poisoning is *Clostridium*. It is a genus of Gram-positive bacteria. It includes *Cl. botulinum* and *Cl. perfringens*. *Cl. botulinum* is responsible for botulism, a form of food poisoning. It produces powerful neurotoxin causing neurological symptoms and gastric symptoms. Based on the type of toxin production *Cl. botulinum* is divided into six types: A, B, C, D, E, and F. A, B, E and F are associated with human foodborne intoxications. Botulism is caused by ingestion of botulin (neurotoxin) contaminated food, which is formed in the food contaminated by the bacteria. Symptoms produced byingestion of contaminated food include dry mouth, nervous system disturbances such as double vision, trouble in swallowing, breathing, speaking followed by nausea, vomiting, constipation and diarrhea. Foods associated with infection are fruits, vegetables, fish, fish eggs, beef stew, chicken, dairy products. Diseases associated with *Cl. perfringens* are mainly

due to type A strain. The enterotoxin produced is responsible for gastroenteritis, its symptoms being associated with the infection are diarrhea, abdominal pain, nausea, vomiting and fever. Food associated with the disease includes vegetables, spices, bean dishes, undercooked meats, meat products, and gravies. *Campylobacter* species are spiral shaped, Gram-negative, microaerophilic bacteria causes human gastroenteritis. *C. jejuni* and *C. coli* are the common causative agents of foodborne illness. Campylobacteriosis is mainly caused by raw milk and improper cooked chicken, poultry or shellfish. The main symptoms associated with this disease are enteric, including abdominal cramps, profuse diarrhea, nausea, vomiting, fever, headache, and chills. Staphylococcal food poisoning caused by toxin of *Staphylococcus aureus*, a Gram-positive bacterium, is considered as one of the most frequently occurring foodborne diseases across the world. *S. aureus* produces seven different enterotoxins: A, B, C1, C2, C3, D and E. The toxin contamination causes gastroenteritis; its symptoms include salivation, nausea, vomiting, abdominal cramps, diarrhea, sweating, chills, headache, and dehydration. Food sources related to thisdisease are pork, baking products, beef, turkey, chicken, eggs, baking products, salads, cream, sauces, and cheeses. Vibrios are gram-negative bacteria; four species under this genus are responsible for foodborne illnesses, *V. cholera* (01 and non-01 serogroups), *V. mimicus, V. parahaemolyticus*, and *V. vulnificus*. Cholera is an acute diarrheal disease caused by *V. cholera* when consumingcontaminated food or water; symptoms include profuse watery diarrhea, vomiting, dehydration, painful muscle cramps, clouded mental status. Food items like oysters, crabs, shrimps, fish, and cooked rice are the carriers of *V. cholera*. Gastroenteritis caused by *V. parahaemolyticus* is responsible for 40-70% of the total bacterial foodborne diseases. The disease symptoms such as nausea, vomiting, abdominal cramps, diarrhea, headache, chills, and fever are observed during infection. This disease is caused by consumption of raw, improperly cooked seafood including fish, oyster, crabs, shrimps and lobsters. *Bacillus cereus*, a Gram-positive, rod shaped important bacterium responsible for gastroenteritis. It produces two types of enterotoxin i.e. emetic and enteric. The symptoms occur due to consumption of contaminated food and include diarrhea, abdominal cramps, nausea, vomiting and fever (in case of emetic form). Consumption of food like vegetables, salads, meats, pudding, casseroles, sauces, soups, rice and starchy food (emetic form) can lead to infection. *Yersinia enterocolitica* is a Gram-negative, short rod shaped bacterium, and is responsiblefor the disease yersiniosis, a zoonotic disease occurring in humans, as well as cattle, pigs, deer, and birds. It is a psychrotroph and can grow at 0˚C. The symptoms occur during infection are severe abdominal pain, diarrhea, nausea, fever and vomiting. The food associated with infection includes raw milk, processed dairy products, insufficiently cooked or raw meat, fresh vegetables, improperly chlorinated water. *Shigella* is another source of diarrhea across the world. It is a Gram-negative, rod-shaped bacterium, causal organism for shigellosis. Its only habitant is in the intestine of humans and some primates. The infection causes abdominal pain, diarrhea mixed with blood, mucus and pus, fever, chills and headache. The infection is transmitted through fecal-oral routes or through fecal-contaminated food and water sources. Most frequently involved food sources are salads (potato, tuna, shrimp, and chicken), shellfish, and dairy products. Hepatitis A, an enteric virus is responsible for foodborne illness. These are difficult to detect and recover from contaminated food. It is a small and non-enveloped, ss-RNA virus packed in a protein shell; normal hosts include humans and vertebrates. The general symptoms of the infection include fever, malaise, nausea, vomiting, abdominal discomfort, and inflammation of liver followed by jaundice. Food source carrying infection includes vegetables, salads shellfish, oysters, clams, mussels, and cockles. Another enteric virus responsible for food-borne illness is Norwalk-like virus/ Norovirus. It is a ss-RNA, non-enveloped virus, causing gastroenteritis, vomiting, diarrhea, nausea, and abdominal pain, and in some cases, loss of taste. Other symptoms include lethargy, headache, coughs, weakness, muscle aches, and low-grade fever may occur. Food vehicles for norovirus are raw fruits and

vegetables, shellfish, fish, sandwiches, salads, cake icing or salad dressing, and baked products. Table 1 shows the brief description of pathogenic bacteria responsible for food-borne illness.

Table 1. List of pathogens

Microorganism	Basics	Sources	Disease Symptoms	Incubation time
Escherichia coli (E. coli)	A group of bacteria that can produce a variety of deadly toxins.	Meat (undercooked or raw), raw milk, vegetables, fruits, contaminated water.	Severe stomach cramps, bloody diarrhea, and nausea. It can also manifest as non-bloody diarrhea or be symptomless. Must-Know: *E.coli*0157:H7 can cause permanent kidney damage which can lead to death in young children.	May occur from 1 to 10 days after eating contaminated food.
Salmonella	Is a group of non-spore forming, motile bacteria. It is found in both cold-blooded and warm-blooded animals, and in the environment.	Milk, fruits, vegetables, egg, dairy products, raw meat.	Diarrhea, fever, vomiting, headache, nausea, and stomach cramps. Must-Know: Symptoms can be more severe in people in the at-risk groups, such as pregnant women.	12 to 72 hours after eating contaminated food.
Clostridium botulinum	A bacterium that can be found in moist, low-acid food. It produces a toxin that causes botulism, a disease that causes muscle paralysis.	Home-canned and prepared foods, vacuum-packed and tightly wrapped food, meat products, seafood, and herbal cooking oils.	Dry mouth, nervous system disturbances such as double vision, trouble speaking followed by nausea, vomiting, and diarrhea. Botulism can be fatal. It's important to get immediate medical help.	12 to 72 hours after eating contaminated food (in infants 3 to 30 days).
Campylobacter jejuni	It is a thermophilic, obligate microaerophilic bacterium. Its natural inhabitant is intestinal tracts of poultry and warm-blooded domestic animals.	Raw milk, and raw or under-cooked meat, poultry or shellfish.	Fever, headache, and muscle pain followed by diarrhea, abdominal pain and nausea.	2 to 5 days
Staphylococcus aureus	This bacterium is carried on the skin and in the nasal passages of humans. It's transferred to food by a person, as a result of poor hygiene.	Dairy products, salads, milk, (cooked ham, raw meat and poultry) egg.	Nausea, stomach cramps, vomiting, and diarrhea.	Usually rapid - within 1 to 6 hours after eating contaminated food.
Vibrio cholerae	Bacteria that cause cholera, a disease that can cause death if not treated.	Raw and undercooked seafood or other contaminated food.	Diarrhea, vomiting, and leg cramps. Loss of body fluids can lead to dehydration .	6 hours to 5 days after eating contaminated food.
Listeria monocytogenes	It is a motile, facultative anaerobic bacterium, one of the most virulent food pathogen.	Soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables; raw meat and poultry.	Fever, chills, headache, backache, sometimes abdominal pain and diarrhea.	2 days to 3 weeks
Bacillus cereus	It is an endemic, rod shape, and motile bacterium, present in gut.	Meats, milk, vegetables, fish, rice, pasta, and cheese.	Diarrhea, abdominal cramps, nausea, and vomiting.	30 min to 15 h
Clostridium perfringens	It is a rod shaped, anaerobic, spore forming bacterium. It is ever-present in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects, and soil.	Undercooked meats, meat products, and gravies.	Abdominal cramps and diarrhea.	8–22 h
Vibrio parahaemolyticus	It is a curved, rod-shaped, facultative aerobic, non-spore forming bacterium, found in brackish, saltwater.	Raw, improperly cooked, or cooked, recontaminated fish and shellfish, andoysters.	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills.	4 h–4 days
Hepatitis A virus	It is a non-enveloped, ss-RNA virus packed in a protein shell,	Sandwiches, fruits, fruit	Fever, malaise, nausea, anorexia, and abdominal	Unknown

Microorganism	Basics	Sources	Disease Symptoms	Incubation time
	normal host- humans and vertebrates.	juices, milk and milk products, vegetables, salads, shellfish, and iced drinks.	discomfort, followed by jaundice.	
Yersinia enterocolitica	It is rod shaped bacterium, causes zoonotic disease in humans, as well as a wide array of animals such as cattle, deer, pigs, and birds. Its portal of entry is the gastrointestinal tract.	Meat (mostly pork),oysters, fish, and raw milk.	Enterocolitis, diarrhea and/or vomiting;fever and abdominal pain.	1–3 days
Shigella	It is a facultative anaerobic, non-spore-forming, non-motile, rod-shaped bacterium. It is naturally found in humans and apes.	Salads, raw vegetables, dairy products, and poultry.	Abdominal pain, cramps, fever, vomiting, and diarrhea containing blood and mucus.	12–50 h
Pseudomonas aeruginosa	It is an aerobic, bacillus bacterium with unipolar motility. An opportunistic human pathogen It is found in soil, water, skin flora, and most man-made environments throughout the world, can colonized many natural and artificial environments.	Fruits, plants, vegetables, falafel with salads (Lettuces, Tomato, Cucumber).	Pneumonia, septic shock, urinary tract infection, gastrointestinal infect, skin and soft tissue infection.	24-72 h
Aeromans hydrphila	It is a rod-shaped bacterium mainly found in areas with a warm climate. This bacterium can be found in fresh or brackish water. It can survive in aerobic and anaerobic envir onments.	Seafood, meats, dairy, shrimps, shellfish, fish and even certain vegetables such as sprouts.	Fever and chills, abdominal pain, nausea, vomiting, and diarrhea, gastroenteritis, cellulitis	1-2 days
Mycobacterium avium	It is a saprotrophic organisms presen t in soil and water; entry into hosts is usually via the gastrointestinal tract, but also can be via lungs.	Unpasteurized cow milk, meats, water,	Fevers, diarrhea, malabsorption and anorexia, Crohn's disease.	2-3 weeks

The important step towards the foodborne pathogen management is the efficient on time detection and identification of pathogens, during the food production and processing line. Portable, rapid and sensitive methods for real-time microbial detection and source identification would be welcomed by the producers, processors, distributors, regulators, and consumers. Of course, this will help reduce and prevent foodborne diseases; thousands of lives will be protected and will cost $6.5 to $34.9 billion annually. There is a strong need for rapid and sensitive detection methods. With the advent of biotechnology, foodborne pathogen detection has developed reliable methods. Improvements in the area of immunology, molecular biology, sample preparation, automation in technology has the impact on the development of faster and convenient methods ofdetection. The advantage of DNA assays is their high specificity as they detect definite target sequences by hybridizing them to a complementary probe sequence. The polymerase chain reaction (PCR) can be used to enhance the DNA based assays. In PCR, double stranded DNA is denatured into single stranded DNA, and specific primers anneal to these DNA strands with a DNA polymerase. These steps are repeated, resulting in the doubling of the initial number of target sequences with cycles. The amplified products can be analyzed by electrophoresis gel. The various PCR methods are used for the detection of foodborne pathogens (Ikeda *et al.*, 2007; Toze, 1999). PCR has potential advantages over culture methods, because it detects the low number of microbes, viruses, bacteria, etc. The immunological methods have been employed with antigens and antibodies interactions for the detection of cells, toxin, spores, etc. The polyclonal

antibodies have limitations in terms of specificity (Cahill *et al*., 1995; Iqbal *et al*.2000). Monoclonal antibodies are more useful than polyclonal antibodies for specific detection of a pathogen. With the advent in method for production of monoclonal antibodies, immunological detection is more specific and reproducible and many commercial immunological assays are available for the detection of pathogens (Leonard *et al*., 2003).

Biosensors that are analytical devices for microbial pathogens detectionhavebeen used. It consists of biomolecules (enzymes, antibodies, nucleic acids, cells, receptors, etc.) and integrated with physicochemical transducers such as optical, electrochemical, mass sensitive, thermometric, piezoelectric, magnetic (Lazcka *et al*., 2007). Biomolecules exhibit nano-scale sizes which arecomparable to nanomaterials/nanoparticles, quantum dots, nanorods, nanowires. The integration of biomolecules with nanomaterials may give novel hybrid biomaterials that produce unique properties in recognition of the pathogens. Nanomaterials bring novel impacts due to their unique electronic, optical, catalytic properties and they also are biocompatible for immobilization of biomolecules. Due to these properties, it develops sensitive and reproducible assays for the detection of pathogens (Pumera *et al*., 2007, Willner *et al*., 2007).

In this review, the application of nanomaterials in recent developments of the detection of foodborne pathogens has been reviewed (Table 2). We covered here the optical, electrochemical as transducers and microfluidics system application in pathogen detection. Optical transducers can be described in various classes like absorption, fluorescence, chemiluminescence, reflection, surface plasmon resonance, etc. The main advantage of this technique is the real-time binding affinity reaction. Electrochemical biosensors have an important role in the detection methods, which can be classified in different methods such as amperometry, voltammetry, impedance measurements, and potentiometric method. Microtechnology takes advantage of the unique strategy of miniaturization of the electrochemical system. Microfluidics devices enable sample handling, low volume consumption of sample and reagent, high reproducibility due to automation; reduce the response time on a single integrated system.

Table 2. Nanomaterial based sensor for the detection of food pathogens.

Pathogen	Nanomaterial used	Mode of detection	Detection limit	Ref
E. coli O157:H7	AuNPs	AuNP based Immunoassay ; amperometry	Buffer - 6 CFU/strip , Milk-50 CFU/strip; 10^2-10^7 CFU/mL	Lin *et al*., 2008
		AuNP-aptamer based detection; colorimetric based detection	10^5 CFU/mL	Wu *et al*., 2012
	Peptide Nanotubes	Ab immobilized on PNPs modified SPCE; cyclic voltammetry	-	Cho *et al*., 2008
	Magnetic nanoparticles	Magnetic nanoparticle- Ab conjugate coupled with interdigitated array microelectrode	1.6×10^2–1.6×10^7 beef sample	Cho *et al*., 2008
	PbS	Multiplex nanoparticle-based DNA electrochemical biosensor; square wave voltammetry	1×10-12 mol/L	Fernandes *et al*., 2014
Salmonella	AuNPs	Ab–GNPs conjugated to GCE; electrochemical impedance spectroscopy	1×10^2 CFU/mL; $1 \times 10^2 - 1 \times 10^5$ CFU/mL (pork sample)	Yang *et al*., 2009
	MNPs	Ab-MNPs and Ab-TiNPs; UV-VIS spectroscopy	100 CFU/mL (milk sample)	Joo *et al*., 2012
	CNTs	Ab-CNTs conjugated to GCE; electrochemical impedancespectroscopy	1.6×10^4 CFU/mL	Jain *et al*., 2012
	QDs	Ab-Magnetic beads coupled with Ab-biotin and streptavidin-QDs; fluorescence spectroscopy	10^3 CFU/mL; 10^3-10^7 CFU/mL (chicken carcass water)	Yang *et al*., 2005 Kim *et al*.,, 2013
Salmonella typhi	AuNPs	Ab-Bacteria-Ab(gold conjugated); anodic stripping of Cu	98.9 CFU/mL; $1.3\times 10^2 - 2.6 \times10^3$ CFU/mL	Dungchaia *et al*, 2012
		Thiolated DNA immobilized on AuNPs-SPE; Differential Pulse Voltammetry	1.0×10^{-11} -0.5×10^{-8} M	Das *et al*., 2014
Salmonella	AuNPs	AuNP-aptamer based detection;	10^5 CFU/mL	Wu *et al*., 2012

Pathogen	Nanomaterial used	Mode of detection	Detection limit	Ref
typhimurium		colorimetric based detection		
Listeria monocytogenes	MNPs	Ab-Magnetic nanoparticles; magnetic flux measurement by high-transition temperature SQUID	5.6 × 10^6 cells/20 µL and 230 cells/1 nL	Grossman *et al.*, 2004
	TiO_2 nanowire bundle	Ab functionalized on gold microelectrodes	10^2-10^7 CFU/mL	Wang *et al.*, 2009
	Magnetic nanoparticles	Ab functionalized on magnetic nanoparticle, EIS measurement	10(3) to 10(7) CFU/ml	Kanayeva *et al.*, 2012
Campylobacter jejuni	MNPs	Ab-funtionalized with nanoparticle , EIS measurement	1×10^3-1×10^7 CFU/mL	Huang *et al.*, 2010
Staphylococcus aureus	CdS	Multiplex nanoparticle-based DNA electrochemical biosensor; square wave voltammetry	1×10^{-12} mol/L	Fernandes *et al.*, 2014
	CNT	HRP based fluorescence immunosensor	0.1 ng/mL. 0.1- 100 ng/mL,	Yang *et al.*, 2008
Vibrio cholera	CNTs	mAb-PEDT-MWCNT on GCE and Ab-ganglioside at liposome; voltammetry	10^{-16} g/mL; 10^{-14}-10^{-7} g/mL	Zhao *et al.*, 2004
Clostridium botulinum	Graphene nanosheets, AuNP, AgNP	Sandwich immunoassay based immunosensor format	10 pg/ml - 10 ng/ml	Narayanan *et al.* 2015
Aeromans hydrphila	MWCNT	Gold electrode based thiolated DNA sensor;Carbon paste electrode with MWCNT based DNA sensor	2.0 µg cm^{-3} ; 0.8 µg cm^{-3}	Ligaj *et al.*, 2014
Pseudomonas aeruginosa	Gold Nanorods	Ab-GNRs by carbodiimide chemistry; NIR light-mediated staining of live/dead cells	75% decrease in cell viability	Norman *et al.* 2008
Vibrio parahaemolyticus	GNPs	Agarose-GNPs on SPCE; amperometry	7.4 × 10^4 CFU/mL; 10^5-10^9 CFU/mL	Zhao *et al.*, 2007
Mycobacterium avium	Au NPs	Ab-DSNB-sulfur-AuNPs; SERS-based immunoassay	100 ng/mL in buffer and 200 ng/mL in pasteurized whole milk	Kaittanis *et al.* 2007
	MNPs	Ab-protein G-Magnetic nanoparticles; SQUID	15.5 CFU/mL; 15.5-775 CFU/mL	Yakes *et al.* 2008

BRIEF DISCUSSION ABOUT NANOMATERIALS

The conventional methods commonly used in the detection and identification of pathogens are based on microbiological and biochemical identification. These methods are sensitive, cost effective, can recognize in a qualitative and quantitative way, the type of organism and the number of colonies, but these are not efficient as it is necessary to have a step of enrichment to detect a low number of pathogens also in food and water samples (DeBoer *et al.*, 1999). Conventional methods are based on: i. Culture and colony counting methods (involve counting of bacteria), ii. Immunoassay - based methods (which involve antigen–antibody interactions) and, iii. Polymerase Chain Reaction (PCR) based methods (which involve DNA analysis). Conventional methods for food pathogen detection, although typically sensitive, theyrequire multiple time-consuming steps like extraction, isolation, enrichment, etc., prior to measurement, which leads to longer testing time from few hours to few days. So, there is a need of development of rapid and sensitive detection methods. Many fast detection methods based on biosensors technologies have been developed and studied.

Recently, researchers have focused on the nanotechnologies and/or nanoparticles based methods/biosensors for the ultrasensitive, specific and fast detection of pathogens. In the field of nanotechnology, most of the nanoparticles are used traditionally and fall into the category of colloids like emulsions, micelles, mono and bi-layers. The nanoparticle possesses colloidal stability via Van der Waals forces. Polymers and surfactants adsorbed on the surface of colloidal particles are attributed towards the steric stabilization of nanoparticles. On the other hand, nanoparticles could be further stabilized by

coating them with molecules that can form chemical bonds (Fendler *et al.*, 2001).

Nanomaterials are widely known as emerging tools with specific physical and chemical properties having quantum-size effects and large surface area that provide unique and different properties compared to bulk materials. The exploration of these properties provides the opportunity to enhance the biosensors properties and improve the detection sensibility. Interesting studies have been reported about the high increase in electronic properties when metallic nanostructures are used for electrode modification. Different nanostructured materials are used with specific forms such as 0D – quantum dots, nanoparticles, 1D – nanowires or carbon nanotubes or 2D – metallic platelets, graphene sheet orientation that is observed in their final properties. Nanoparticles are expected to be more biologically active than bulk particles of same chemical composition due to the high surface area of nanoparticles per mass unit. This characteristic of these materials offers several perspectives for food applications. Many novel applications of nanotechnologies/nanoparticles have been reported with the use of nanoparticles as well as the development of nanosensors which are aimed to manage food safety (Lopez *et al.*, 2011; Nasongkla *et al.*, 2006; Valdes *et al.*, 2009; Yih *et al.*, 2006).

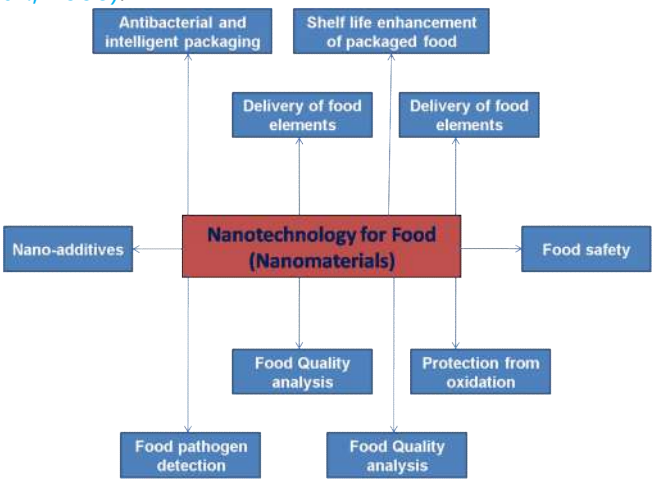

Figure 1. Representation of various uses of nanomaterials in food analysis and pathogen detection

Moreover nanoparticles are extensively used for the sensitive biosensor development- metal nanoparticles, metal oxide nanoparticles, quantum dots etc. Fig 1 represents the various uses of nanotechnology and nanomaterials in food analysis and pathogen detection.

Carbon nanotubes (CNTs)

The most common carbon nanomaterials include carbon nanotubes (CNTs), graphene, carbon dots, fullerenes, nanodiamonds and carbon nanofibers. The superior optical and electrical properties of carbon nanomaterials make them good candidates for analytical tasks. According to their intrinsic structures, CNTs can be catalogued into single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) with well-ordered hollow graphitic nanomaterials.

Conceptually, the CNTsare considered as cylindrical structures made of single or multiple sheets of graphene. CNTs are the new form of carbon derivatives and offer unique geometrical, mechanical, electronic and chemical properties. CNT powders have already many commercial applications and are now considered as in their growth phase of their product life cycle (DeVolder*et al.*, 2013, Wang, 2005). Researchers have reported the use of CNTs as components of biosensors and medical devices due to dimensional and chemical compatibility of CNTs with biomolecules, such as DNA and proteins. At the same time, CNTs are also implemented in fluorescent, photoacoustic imaging, and localized heating using near-infrared radiation. CNT-modified electrodes used in biosensor applications have shown high electron-transfer reactions in both small and large biological molecules (Sato *et al.*, 2008). Biofunctionalization of CNTs using covalent linkers or direct immobilization strategies for biomolecules such as antibodies provides additional selectivity of CNT-based biosensors. The three-dimensional cylindrical shape and very large surface area of CNTs allows the substantial amount of biomolecules to be incorporated into the biosensor (Wang *et al.*, 2004).

Graphene

Graphene is another form of carbon material and possesses novel properties. Unlike graphite, graphene is highly conductive (mobility: 200000 $cm^2V^{-1}s^{-1}$), transparent (transmittance: B 97.7%) and has high mechanical strength (Young's modulus: B1.0 TPa) which leads to its utility for

electronic, electrochemical, and optical sensing (Geim, *et al*., 2007; Yin *et al*., 2013). Graphene oxide (GO) is a derivative of graphene which possesses many distinct characteristics that are very different from those of graphene; it contains many oxygen containing groups (–C–O–C–, C–O–H,–COOH, etc.) that act to inhibit electron transfer. However, it is still mechanically strong, flexible, transparent, and biocompatible owing to its hydrophilic nature. In particular, graphene sheets can be obtained using a number of methods including mechanical exfoliation, chemical vapor deposition (CVD) on metal or Si substrates and the chemical or electrochemical reduction of graphene oxide.

Graphene sheets are widely used in the synthesis of nanocomposites and in thefabrication ofvarious microelectrical devices, field-effect transistors, ultrasensitive sensors and electromechanical resonators. Recently, the biological applications of graphene have also started to be taken into consideration (Geim, *et al*., 2007; Liu *et al*., 2010). Recently, it was reported that the properties of graphene based sensors could be exploited by incorporating nanoparticles (e.g. metallic, oxide, and semiconductor nanoparticles) to form graphene–nanoparticle hybrid structures (Bai, *et al*., 2012).

Gold nanoparticles (Au NPs)

Gold nanoparticles (AuNPs) have wide application in electrochemical sensors due to their good biological compatibility, conducting capability, and the high surface-to-volume ratio have become more and more widely used inelectrochemical sensors (Shen *et al*., 2007). The majority of the biosensors based on NPs that have been developed are based on gold NPs, due to their unique optical properties and ease of derivatization with different biomarkers in aqueous solution (Boisselier, *et al*., 2009; Sperling, *et al*., 2008). They have been used in combination with a variety of techniques, such as electrochemical sensing (Sharma *et al*., 2008; Upadhyay *et al*., 2009), DNA/genosensor sensing (Das *et al*., 2014). Biomolecules (Antibodies/antigen/proteins) can be directly immobilized onto AuNPs and help to improve the sensitivity of instruments using the sandwich assay (Sharma *et al*., 2010). AuNPs are typically kept in an aqueous solution, show high biocompatibility and have lower cellular toxicities compared to other nanomaterials used for biomedical applications (Connor *et al*., 2005). In addition to this, AuNPs can be used to modify the electrode surface in biosensors based on the electrochemical principle. AuNPs may be deposited or attached to the electrode transducers, and they provide an efficient and three-dimensional loading-platform with large surface area for immobilizing biomolecules. Furthermore, AuNPs help to improve the electron transfer rate between the biomolecule and the electrode surface. In the recent years, AuNPs based hybrid nanoparticles such as Au-silicon oxide (SiO_2) hybrids, Au-carbon nanosphere hybrids and Au-layered calcium carbonate ($CaCO_3$) hybrids with excellent biocompatibility and stability have been reported (Cui *et al*., 2008; Li *et al*., 2010).

Silver nanoparticles (Ag NPs)

Silver nanoparticles have applications in many fields; some important applications include their use as catalysts, as optical sensors to detect analytes upto zeptomole concentrations, in textile engineering, electronics, optics, and most importantly in the medical field in form of bactericidal and as a therapeutic agent.

Quantum dots (QDs)

Quantum dots are considered as nanocrystalline semiconductors possessing unique properties due to their quantum confinement effects. QDs behave differently from bulk solids due to their confinement effects. If it is in two-dimensional structures it is known as quantum wells, if it is in one dimensional they are called quantum wires, if it is zero dimensional, then they are known as quantum dots. They have very broad continuous absorption spectra from ultraviolet to visible depending upon the particle size. Quantum dots have small sizes between 2-10 nm, unique optical properties, multicolor emission single light source, large brightness excitation coefficient and high photo stability. These QDs nanocrystals exhibit narrower emission peaks, higher emission intensity, and longer shelf life than dye molecules. Fluorescence technique is routinely used to detect or in imaging the cells in a biological research which relies upon the availability of sensitive fluorophore. Quantum dots and nanomaterials are

widely used in biological application such as bioimaging, biodetection and drug delivery in place of fluorophore. To overcome the use oforganic fluorophores inorganic quantum dots are used as fluorophore which specifically binds with biomolecules. The spectroscopic features of QDs make them very easy to use as an alternative to traditional fluorophore in a range of detection for multiplex bioanalysis.

Conjugated polymeric nanoparticles (CNPs)

Nanoparticles based on conjugated polymers are emerging as multifunctional nano-scale materials that possess great potential and offer exciting opportunities the areas of imaging agents, biosensors, photonic and optoelectronic devices. These conjugated polymer nanoparticles (CPNs) possess excellent properties like tuning easily for desired applications through the choice of conjugated polymers and surface modification. The facile synthesis, tunable properties, less toxicity and more biocompatibility compared to the other inorganic nanoparticles, further making them highly attractive in the material choice. There has been reported a sensitive multiplexed method, multicolored FRET (fluorescence resonance energy transfer) for the monitorization of pathogenic bacteria within 30 min with silica NPs (Wang *et al*., 2007).

Magnetic nanoparticles (MNPs)

Another important class of nanoparticles is the nano-scale magnetic materials whichare an important source of labels used in biosensing applications due to their strong magnetic properties, which are not commonly found in biological systems. Due to small size, modulation of the composition and magnetic properties of these materials,they areuse in a variety of instruments and formats for biosensing (Lee *et al*., 2007). New instrumentations using nano-scale magnetic materials are promising for the use of in point-of-care sensors in a variety of applications. Superparamagnetic nanoparticles made of iron oxide and polymeric coatings are clinically approved as contrast agents in magnetic resonance (MR) and used in pre-clinical, targeted molecular imaging applications (Weissleder *et al*., 2008).

In addition to the above discussed nanomaterials, new nanomaterials like antibody-coated microspheres have also been used for the simultaneous detection of Cholera toxin (CT), SEB and other spiked clinical samples by a microflow cytometer (Kim *et al*., 2009). Antibodies conjugated to mesoporous silica-based nanomaterials have been widely explored for the detection of bacterial targets as well as for the enhanced loading of biomolecules (enzymes or other probes) (Eum *et al*., 2014; Syed *et al*., 2014). It should be emphasized that although the many types of nanomaterials are discussed in this review, the use of two or more kinds of nanomaterials in a single assay is becoming more and trendier, due to their complementary properties.

OPTICAL BASED/FLUORESCENCE/ELECTRO-CHEMILUMENISCENCE DETECTION OF FOODBORNE PATHOGENS

Optical biosensorshavea wide range of applications in the field of analytical techniques, particularly due to their high specification, sensitivity, cost effectiveness, and small size. This property of selectivity of the biological sensing element leads to the great opportunity for development of devices with high specificity for real-time analysis in complex mixtures, eliminating the extensive sample pretreatment steps or large sample volumes requirements. The working principle of optical biosensors is based on the measuring change in amplitude, phase, frequency or polarization of light. Optical biosensors are rapid, highly sensitive, reproducible, and simple-to-operate analytical tools. All these properties of optical biosensors lead them to an exponential growth during the last decade. Among optical biosensors, applications of fiber-optic biosensors, fluorescence detection, electro-chemiluminescence and surface plasmon resonance biosensors are rapidly increasing (Fig 2). Colorimetric assay based on pathogen detection using nanoparticles offers the advantage of observing the signals by the naked eye or by a cheap colorimetric detector

without expensive instruments. Recently, a colorimetric enzyme–nanoparticle conjugate system for the detection of *E. coli* bacteria with the detection sensitivity of 10^2 bacteria mL^{-1} in solution and 10^4 bacteria mL^{-1} in a field-friendly test strip format has been reported (Miranda *et al.*, 2011). Wu *et al.* reported that the AuNPs based aptasensors for the detection of *E.coli* and *Salmonella typhirium* with naked eye wouldtake only 20 minutes (Wu *et al.*, 2012). A multiplex detection platform has been developed to detect three different pathogens using Raman and UV–Visabsorption spectroscopy. Gold (Au), silver (Ag), and Ag–Au core–shell nanoparticles were used to functionalize the anti-*Salmonella typhimurium* aptamers, anti-*Staphylococcus aureus* and anti-*Escherichia coli* O157:H7 antibodies respectively and labeled with unique Raman reporter molecules. The developed method used total detection time under 45 min for *E. coli* O157:H7 vs. *S. typhimurium* and strain level for *E. coli* O157:H7 vs. *E. coli* K12 obtained a limit of a detection ranging between 10^2 and 10^3 CFU/ml (Ravindranath *et al.*, 2011). A fiber-optic portable biosensor using fluorescence resonance energy transfer (FRET) was developed for *Salmonella typhimurium* (*S. typhimurium*) detection in ground pork samples. Labeled antibody–protein G complexes were formed by the incubation of anti-*Salmonella* antibodies labeled with FRET donor fluorophores (Alexa Fluor 546) and protein G labeled with FRET acceptor fluorophores (Alexa Fluor 594). The biosensors were tested in two different solutions: PBS doped with *S. typhimurium* and homogenized pork sample with *S. typhimurium*. The lowest detection limit was 10^3 cells/ml in PBS, while in homogenized pork samples limit of detection of 10^5 CFU/g was found (Ko *et al.*, 2006). A rapid and sensitive *Listeria monocytogenes* detection technique has been developed. This method utilizes hyperbranching rolling circle amplification (HRCA) combined with gold nanoparticle (AuNP) based colorimetric method to offer an isothermal, specific, and sensitive assay for the detection of *L. monocytogenes*. The detection limit obtained was 100 aM for synthetic hly gene targets and about 75 copies of *L. monocytogenes*were detected (Fu *et al.*, 2013).

Optical biosensors are the most popular category of biosensors for the detection purposes due to their exceptional selectivity and sensitivity. Fibre-optic is the first commercially available technology among all optical biosensors. The fundamental mechanism behind fibre-optic biosensors is the use of fluorescently labelled bacterial/viral pathogens or toxins that get an excitation by the laser wave of approximately 630 nm and finally fluorescent signal is generated and detected by the fluorescent detector. Most routinely used fluorescent marker is fluorescein isothiocyanate (FITC) (Li *et al.*, 2004), but lanthanides are also reported for this purpose (Selvin, 2002). Another category of optical biosensors is the Surface plasmon resonance (SPR) sensor, which works on the phenomenon of optical illumination of metal surface that is one of the target sources for food pathogen detection. Binding of food pathogen to metal surfaces leads to a change in resonance to longer wavelengths and magnitude of the shifted wave length which is directly related to the concentration of the targeted pathogen. SPR biosensors are capable of detecting molecules even in femtomolar range (Bhunia *et al.*, 2008; Rasooly *et al.*, 2006). The network approach uses antibodiesconjugated with AuNPs and peroxidase and has demonstrated extremely high sensitive detection of 3 CFU/mL for *E. coli* O157:H7 and 15 CFU/mL for *S. typhimurium* in liquid food samples (Cho *et al.*, 2013). A remarkable sensitive gold nanoparticle-based lateral flow immunoassay for the detection of *Escherichia coli* O157:H7 was reported with the detection sensitivity of 10^2 colony forming units per mL by taking advantage of the optimized AuNPs and the separate incubation of the AuNPs/antibody/E. coli O157:H7 complex (Cui *et al.*, 2015). Surface plasmon resonance (SPR) biosensor based on the spectroscopy of grating-coupled long-range surface plasmons (LRSPs) combined with magnetic nanoparticle (MNP) assay is reported for the rapid and highly sensitive detection of bacterial pathogen *Escherichia coli* O157:H7 which could be detected at concentrations as low as 50 cfu mL^{-1},thatis 4 orders of magnitude better than the detection limit achieved by regular grating-coupled SPR with direct detection format (Wang *et al.*, 2012). The

simultaneous and quantitative detection of four species of bacteria, *Escherichia coli* O157:H7, *Salmonella choleraesuis* serotype typhimurium, *Listeria monocytogenes*, and *Campylobacter jejuni*, using an eight-channel surface plasmon resonance (SPR) sensor based on wavelength division multiplexing has been reported. Detection curves obtained shows SPR response versus analyte concentration for each species of bacteria in the buffer at pH 7.4, apple juice at native pH 3.7, and apple juice at an adjusted pH of 7.4, and mixture containing all four species of bacteria in the buffer. The limit of detection in the tested matrices for each of the four species of bacteria ranges from 3.4×10^3 to 1.2×10^5 cfu/ml (Taylor *et al.*, 2006).

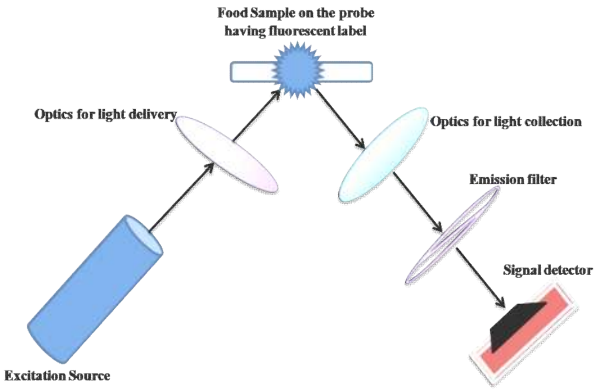

Figure 2. Typical Fluorescence measurement set-up

Multifunctional nanoparticles i.e. fluorescent magnetic nanoparticles with a core–shell structures are synthesized and bioconjugated with gentamycin, which can detect diluted *E. coli* cells at a concentration as low as 1 x 10^3 CFU mL^{-1} (Chen *et al.*, 2013). A novel detection protocol for three foodborne bacterial pathogens (*S. typhimurium*, *E. coli* O157:H7, and *S. flexneri*) with biolabeling of corresponding antibodies by fluorescent CdTe quantum dots with different emission wavelengths was reported. Their result showed that the detection limit was found to be 10^3 CFU mL^{-1} (Zhao *et al*, 2009). Quantum dots as fluorescent labels used for the detection of *Salmonella* in chicken carcass wash water (Yang *et al.*, 2005). Yang *et al.*, developed an immunosensor using CNTs for the detection of SEB by conjugating anti-SEB primary antibody immobilized on CNTs, incubating it with the HRP-labeled secondary antibody and detecting the fluorescence produced by HRP (Yang *et al.*, 2008). Phage display based 12-mer peptide that has the affinity to bind towards staphylococcal enterotoxin B (SEB), which causes food poisoning was reported. These peptides could detect 1.4 ng of SEB per sample whilein a fluorescence-based immunoassay using fluorescently labeled SEB-binding phages (Goldman *et al.*, 2000). Based on a similar principle, array biosensors were also developed for simultaneous detection of *Bacillus globigii*, MS2 phage and SEB (Rowe *et al.*, 1999). Ferracci *et al.*, achieved label-free, real-time detection of botulinum neurotoxin B in food and human serum. In the developed method, VAMP2 was immobilized onto a plasmon resonance chips surface and cleavage of this protein by botulinum neurotoxin B and was monitored. This sensor provided detection limit of 2 pM of the toxin within 10 minutes (Ferracci *et al.*, 2011). An fiber-optic biosensor capable of detecting *B. anthracis* spores was developed and spiked in common powders at concentrations of 3.2 x 10^5 spores per mg or higher within an hour (Tims and Lim, 2004). Multi-well plates containing Ped-2E9 cells were encapsulated in a collagen matrix and the amount of alkaline phosphatase released from infected cells was measured colorimetrically; the statuses of live or dead cells were confirmed using laser cytometry. This device can measure *B. cereus* having the concentration of 10^2 –10^4 CFU per g in meat and rice samples P (Banerjee and Bhunia, 2010).

In electro-chemiluminescent (ECL) detection a reporter molecule like ruthenium (II) trisbipyridal or a fluorescent nanoparticle such as AuNPs is tagged with biomolecules (e.g., antibodies, DNA). After electrochemical stimulation it undergoes electron transfer reactions, which results in the emission of light allowing the detection of very low concentration of analytes (Drummond *et al.*, 2003).

Yang *et al.*, (2009) demonstrated an immunoassay based on gold nanoparticles, gold nanoparticle-based enhanced chemiluminescence (ECL) detecting the increased levels of SEB in food samples using a CCD detector and conventional plate reader. The immunosensor assay has a ten-fold lower detection limit (0.01 ng SEB per mL) compared to traditional ELISA (Yang *et al.*, 2009).

Highly sensitive registration of magnetic nanoparticles by their nonlinear magnetization method is used in a sandwich-type immunosensor

for the determination of staphylococcal toxins in complex media. The signal is studied from the entire volume of a nontransparent 3D fiber structure employed as a solid phase. The method shows a near-linear dose–response curve in a wide range of ~3 decades, and it can detect staphylococcal enterotoxin A (SEA) and toxic shock syndrome toxin (TSST) without any sample preparation in milk (Orlov *et al*., 2013). Diana Pauly *et al*., developed a multiplexed fluorescent magnetic suspension assay for the simultaneous detection of five bacterial and plant toxins such as abrin, ricin, staphylococcal enterotoxin B (SEB) and botulinum neurotoxins type A and B (BoNT/A, BoNT/B) in complex matrices (Pauly *et al*., 2009).

An electro-chemiluminesence assay has been reported for the detection of biothreat agents in selected food samples and screening of *Clostridium botulinum* outbreak strains associated with type A botulism. The detection achieved by this ECL assay is 40 pg mL^{-1} for BoNT/A complex, 10 pg mL^{-1} for SEB and 40,000 CFU mL^{-1} for *Bacillus anthracis* spores in milk products (Sachdeva *et al*., 2014). A fluorescence based biosensor for the femtogram-level detection of *Clostridium botulinum* neurotoxin type A (BoNT/A) in food and water matrices was reported based on sandwich immunoassay using nanoporous substrate and fluorescent supra-nanoparticles. The detection limit obtained in the buffer was 21.3 fg mL^{-1} in orange juice 145.8 fg mL^{-1} and in tap water 164.2 fg mL^{-1} (Bok *et al*., 2013). ECL based detection system is commercially available on 96-well assay plates (MesoScale Dignostics, Gaithersburg, MD, USA).

ELECTROCHEMICAL BIOSENSOR

An electrochemical biosensor measures current or potential changes due to interactions that occur on the sensor electrode surface (Fig 3). The electrochemical method of detection facilitates the miniaturization of the system as well as it can be used in complex and turbid media of the food samples. They are generally classified according to the parameter observed, such as current, in case of amperometry, potential in potentiometric methods, impedance in impedimetric studies and conductance in conductometric methods (Bard & Faulkner, 2000; Lazcka *et al*, 2007). The working principle of an amperometric sensor is based on electrode reactions, which involve a three electrode system - a reference electrode, a working electrode, and an auxiliary electrode. The reference electrode is non-polarizable, Ag/AgCl electrode or calomel electrode are frequently used as reference electrodes; the working electrode is a polarizable electrode made of a noble metal or carbon and the auxiliary electrodes used must be larger than the working electrode in order to obtain a rate limiting reaction on the surface of the working electrode. Amperometric biosensors work on a constant application of apotential and the current obtained by the reduction or oxidation of an electroactive specie and is recorded during the detection process. The current generated is linearly proportional to the analyte concentration. In amperometric immunosensors, the advantage is the selectivity of antigen-antibodies reactions, the amplification feature by using enzyme as a label and the ease with which small amount of enzyme generated product can be detected amperometrically. By using different immunoassay approaches, the concentration of the analyte bound to the antibody, on an electrode surface, can be determined by monitoring the labeled enzyme catalyzing the reaction with its substrate and mediator. In the DNA based biosensors, a single-stranded DNA probe is immobilized on the electrode surface, which on hybridization to a specific complementary region of the target DNA produces the electric signal. The hybridization can thus be detected via the change in the current signal generated by the electroactive indicator that preferentially binds to the DNA duplex.

Potentiometric sensors are consisted of an ion-selective membrane, an internal reference electrode and a bioactive material, e.g. an enzyme. The ion selective membrane shows the selectivity of the electrode and it is also known as working electrode or ion-selective electrode. The potential difference across the ion-selective electrode is measured using an external reference electrode under zero current condition and is linearly

dependent on the logarithm of the activity or concentration of the analyte.

In voltammetric techniques, a potential (E) is applied to an electrode, and the resulting current (i) present in the electrochemical cell is monitored. It works on three electrodes: system reference electrode, working electrode and auxiliary electrode. The current is passed through the working and auxiliary electrode and is measured in between the working and reference electrode.

The differential pulse voltammetry (DPV) is another technique of voltammetry, in which the potential is applied in form of a pulse. In this technique, a series of potential pulses of increasing amplitude are used, and the current is measured near the end of each pulse; this allows time for the charging current to decay. It is performed in an unstirred solution. In DPV, the potential is scanned in a series of pulses, each pulse is fixed at small amplitude (10 to 100 mV) and is further superimposed on a slowly changing base potential. The current is measured twice; is the first measurement takes place just before the application of the pulse and the second at the end of the pulse. The difference obtained during current measurements from these points for each pulse is calculated and plotted against the base potential.

In square wave voltammetry (SWV) the excitation signal consists of a symmetrical square-wave pulse of amplitude, E_{sw}, viewed as superimposed on a staircase waveform of step height ΔE, where the forward pulse of the square wave coincides with the staircase step. The current is measured two times: the first measurement is made at the end of the forward potential pulse (i_{for}), while the second measurement is recorded at the end of the reverse potential pulse (i_{rev}). Its difference ($i_{for} - i_{rev}$) is used to obtain the net current, i_{net}. The current difference between these two points is then plotted against the applied potential during square wave voltammogram. The peak height obtained is directly proportional to the concentration of the redox active species and measures the detection limits as low as possible. Square-wave voltammetry has several advantages, including high sensitivity and suppressed background currents.

Electrochemical impedance spectroscopy (EIS) deals with the study of adsorption processes, mechanism of electrochemical reactions, and the dielectric and transport properties of materials used for the creation of sensors/biosensors. EIS provides the opportunity to study the process of immobilization of biocomponents and to characterize electric features of biocomponent/ electrode interface. In this technique, a low-voltage sinusoidal AC signal (typically 2-10 mV) is applied, and the current response is determined, which is used to calculate the impedance at each of the probed frequencies. The current amplitude, potential signals and the resulting phase difference between voltage and current, which depends on the system being studied, dictates the system impedance. The impedance spectrum has an imaginary component and a real component due to which its mathematical treatment has become quite difficult and cumbersome. Impedance spectra are often fitted to equivalent circuits of capacitors and resistors, such as the Randles circuit, which is commonly used to interpret simple electrochemical systems. EIS method has been employed for the detection of DNA hybridization; antibody-antigen binding can be directly detected allowing the development of immunosensor.

Piezoelectric quartz crystal (PQC) based biosensor are mass sensitive detectors; its principle is based on an oscillating crystal which resonates at a fundamental frequency. In these types of sensors, the analyte detection is based on the recognition of adsorbate where selective binding results in a mass change identified by a corresponding change in the acoustic parameters of the PQC. The crystal is coated with biomolecules (such as an antibody, DNA, enzyme) and exposed to its respective analyte, which brings a quantifiable difference in the resonant frequency of the crystal, which is correlated to mass changes at the crystal surface. Majority of acoustic wave biosensors utilize piezoelectric materials as signal transducers. These piezoelectric materials have great applicability in biosensors as they can generate and transmit acoustic waves in a frequency-dependent manner (Griffiths *et al.*, 1993). The optimal resonant frequency for transmission of the acoustic wave is influenced by

the physical characteristics of the piezoelectric material. The most often used piezoelectric materials are lithium niobate ($LiTaO_3$) and quartz (SiO_2)(http://www.sensorsmag.com/articles/1000/68/main.html). To achieve an active surface, the surface must be chemically stable in order to be usedin a piezoelectric biosensor. It helps with theloading capacity of biomolecules (Babacan *et al.*, 2000). Acoustic wave biosensors are label-free, provide analysis for antigen–antibody interactions, DNA interaction, and also offers the option of several immunoassay, DNA assay formats, which increases detection sensitivity and specificity. Chen *et al* has developed a circulating-flow piezoelectric biosensor. It is based on Au nanoparticle amplification and verification,it is used for real-time detection of a foodborne pathogen, *Escherichia coli* O157:H7. They have immobilized thiolated probe specific towards *E. coli* O157:H7eaeA gene onto the piezoelectric biosensor surface. The piezoelectric biosensor was able to detect 10^2 to 10^6 CFU/ml (Chen *et al.*, 2008). A quartz crystal microbalance (QCM) DNA sensor was developed based on the nanoparticle amplification method, for detection of *Escherichia coli* O157:H7. A thiol – ss-DNA probe specific to *E. coli* O157:H7 eaeA gene was immobilized using self-assembly process on the QCM sensor. For the hybridization assay ss-DNA probe was exposed with the complementary target DNA and mass change and therefore, frequency change of the QCM was observed. The detection of bacterial cell concentration for *E. coli* O157:H7 was found from 2.67×10^2 to 2.67×10^6 CFU/ml (Mao *et al.*, 2006). Ryu *et al.,* developed a piezoelectric crystal biosensor used for detection of *L. monocytogenes*. *L. monocytogenes*-specific DNA was amplified using the polymerase chain. A SH modified DNA probe was used for coupling to the gold electrode surface, and the hybridization was monitored in the quartz crystal microbalance (QCM) system by adding the complementary ss-DNA of 161 nucleotides obtained via the single-strand PCR. 5 µg of the complementary ss-DNA was detected using the developed sensor (Ryu *et al.*, 2001). A piezoelectric biosensor based immunoassay for *Escherichia coli* O157:H7 detection has been reported. It utilized affinity-purified antibodies immobilization on the monolayer of 16-mercaptohexadecanoic acid (MHDA) on a quartz crystal Au electrode surface. The immobilized antibodies bind to the target bacteria and decrease the sensors resonant frequency; this shift in frequency was correlated with the bacterial concentration. The detection range of the immunosensor is 10^3–10^8 CFU/ml within 30–50 min (Su *et al.*, 2004).

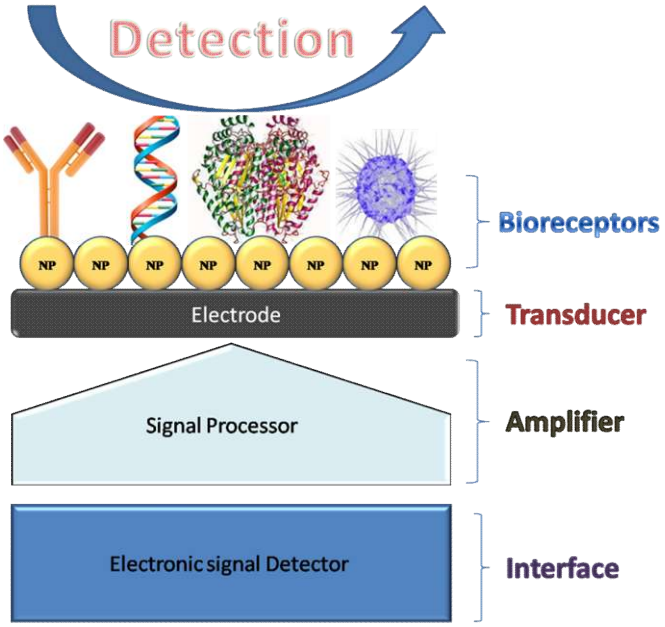

Figure 3. Schematic presentation of electrochemical biosensor

Foodborne pathogens detection by electrochemical methods

E. coli and *Salmonella* are among the most studied species using nanoparticles modified antibodies, DNA or aptasensors for detection and quantification of bacteria in food. *E. coli* can be transmitted to humans by consuming raw or improperly cooked food products such as meat products, yogurt, etc. It can also be cross-contaminated through feces in water, fruits, and vegetables. Electrochemical enzyme linked immunosensing strip has been developed for the detection of *E coli*O157:H7 (Lin *et al.*, 2008). The screen-printed electrode (SPE) was modified with AuNPs and ferrocenedicarboxylic acid and with *E. coli* specific antibody. The detection method is based on horseradish peroxidase (HRP) activity coupled with AuNPs and ferrocenedicarboxylic acid to amplify the amperometric signal. The

detection of *E coli*O157:H7 in the milk sample is detected upto 50 CFU/mL. The peptide nanotubes on SPE have been used for the detection of *E coli*O157:H7 from samples through antigen-antibody interaction (Fig 4) and the current is measured by cyclic voltammetry (Cho *et al*., 2008). Multiplex nanoparticles based DNA electrochemical detection for *E coli*O157:H7 and *S. aureus* has been reported (Fernandes *et al*., 2014). They used lead sulfite (PbS) and cadmium sulfite (CdS) which act as signal reporter and amplifier, and DNA hybridization with target DNA is monitored by square wave voltammetry. The other strains of *E. coli* were also detected by using nanomaterials such as magnetic nanoparticles, carbon nanotubes, AuNPs (El-Boubbou *et al*., 2007; Maurer *et al*., 2012; Zhang *et al*., 2009). Zhang *et al*. reported *E. coli* by anodic stripping voltammetry usingcore-shell Cu@AuNPs as anti-*E coli* antibody labels.

After binding the pathogenic bacteria on nanoparticles, which changes the properties of nanoparticles, it could be detected by EIS methods. The detection of *E coli*O157:H7 is done by immobilizing the anti-*E. coli* on gold nanowires using the *E.coli*O157:H7 count,which was determined by EIS methods (Basu *et al*.2004).

93.8 million cases of gastroenteritis and 155,000 cases of deaths each year in both developing and developed countries are reported and have as cause *Salmonella* species (Macjowicz *et al*., 2010). According to Center for Disease Control and Prevention (CDC) *Salmonella* has been listed as a category B bioterrorism agent. An important member of this genus is *Salmonella typhi*, which is the causal organism for enteric fever or typhoid. For the detection of *Salmonella sp*. in food samples, nanoparticles modified with antibodies or DNA are used. *Salmonella sp*. antibodies tagged with AuNPs were used for the detection (Yang *et al*., 2009). Magnetic nanoparticles are utilized for the detection of *Salmonella* in milk. The antibody conjugated MNPs captured the bacteria in milk and separated the bacteria probe by externally applying a magnetic field. A detection limit of 100 CFU/mL is obtained in milk (Joo *et al*., 2012). Jain *et al*. reported an electrochemical impedance spectroscopy method based on an electrochemical biosensor using CNTs functionalized antibodies onto a glassy carbon electrode (Jain *et al*., 2012). An electrochemical immunosensor for the detection of *Salmonella typhirium* is developed by Dungchai. They have immobilized antibodies on polystyrene for bacteria capturing, followed by adding polyclonal antibody-AuNPs conjugate, which binds the bacteria in the presence of copper enhancer solution and ascorbic acid. The release of copper is monitored with anodic stripping voltammetry (Dungchai *et al*., 2008). The DNA sensor (Fig 5) has been developed for the *Salmonella typhirium vi* gene. Here the thiolated DNA probe is immobilized on AuNPs modified SPE and target DNA hybridization is monitored by differential pulse voltammetry (Das *et al*., 2014). An ultrasensitive electrochemical immunosensor sandwich for the detection of *Salmonella* has been reported using high density gold nanoparticles (GNPs) dispersed in chitosan hydrogel and used to modify glassy carbon electrode. A wide linear range of detection was obtained from 10 to 10^5 CFU/mL with a low detection limit of 5 CFU/mL (Xiang *et al*., 2015).

Listeria monocytogenes is responsible for listeriosis, an infectious disease. For the detection of *Listeria,* a method is reported, which is based on the binding rate between antibody-linked magnetic nanoparticles and bacteria by using high transition temperature superconducting quantum interference device (SQUID). A detection limit is achieved by this method is 5.6 x 10^6 cells/mL and 230 cells/1nL (Grossman *et al*., 2004). Wang *et al*. reported a TiO_2 nanowire bundle electrode for the detection of *Listeria monocytogenes* by using the impedance method (Wang *et al*., 2009). Kanayeva *et al*. have also reported detection by using magnetic nanoparticles and separation of bacteria using amicrofluidic chip (Kanayeva *et al*., 2012). An impedance biosensor using immunomagnetic separation and urease catalysis for sensitive detection of *Listeria monocytogenes* has been developed using an immobilization-free microelectrode as the detector. The detection limit of the developed biosensor for *L. monocytogenes* was 300 CFU/mL in both pure sample and spiked samples (Chen *et al*., 2015). Huang *et al* developed

an electrochemical impedimetric immunosensor for the less detection label of *Campylobacter jejuni* in stool using magnetic nanoparticles (Huang *et al.*, 2010).

Staphylococcal enterotoxin B (SEB) is a water-soluble toxin produced by *Staphylococcus aureus*. The food poisoning due to SEB causes nausea, vomiting, anorexia, and diarrhea even when present at low levels (20-100 ng/person). Sensitive detection of SEB has been developed by using mice anti-SEB monoclonal antibodies tagged with PbS QDs on SPE by square wave voltammetry method. Detection limit is achieved up to 0.01 ng/mL (Sharma *et al.*, 2014). A dual-aptamer-based sandwich immunosensor for the detection of *S. aureus* has been developed using biotin labeled primary anti-*S.aureus* aptamer immobilized on streptavidin coated magnetic beads, which act as a capture probe. A secondary anti-*S. aureus* aptamer conjugated to silver nanoparticles sensitively detects the target. The electrochemical immunosensor detects in range from 10 to 1 × 10^6 cfu/mL with a detection limit of 1.0 cfu/mL (Abbaspour *et al.*, 2015).

Cholera toxin is produced by *Vibrio cholera* and is responsible for watery diarrhea during cholera infection. Electrochemical immunosensor is developed with liposomic magnification by linking an anti–cholera toxinB monoclonal antibody with nafion supported CNT on the glassy carbon electrode (Viswanathan *et al.*, 2006, Zhao *et al.*, 2007). The detection method is based on a sandwich type immunoassay. Development of an electrochemical genosensor using lyophilized gold nanoparticles/latex microsphere reporter label for *V. cholerae* has been reported. The detection range was 1 aM to 1 fM for linear target DNA, detection limit of 1 fM for PCR products, and for LAMP products 50 ng µl $^{-1}$ (Sheng *et al.*, 2015).

The *Clostridium botulinum* produces seven neurotoxins serotypes (A-G). The toxicity caused by botulinum neurotoxins can cause life-threatening illness within a few hours to several days of ingestion. An electrochemical immunosensor is developed for the botulinum neurotoxin E, based on graphene nanosheets aryldizonium modified electrode and enzyme induced silver nanoparticles deposited on gold nanoparticles as a signal amplifier. The detection limit is achieved up to 5 pg/mL (Narayanan *et al.*, 2015).

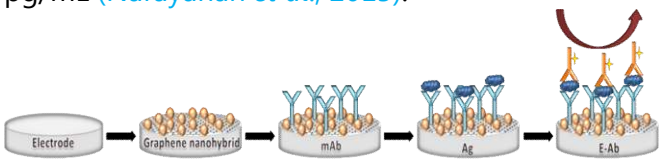

Figure 4.Representation of electrochemical immunosensor.

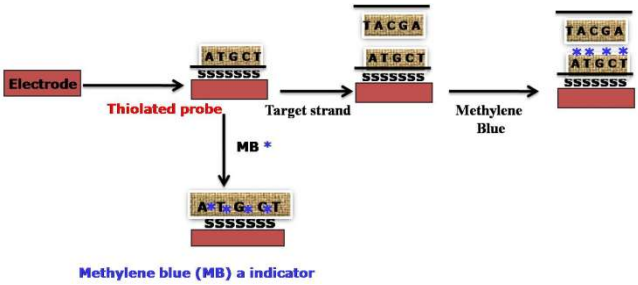

Figure 5. Schematic representation of an electrochemical DNA biosensor.

Aeromonas hydrophila, is associated with human diseases and foodborne infections. This bacterium produces several extracellular toxins such as aerolysin, cytolytic enterotoxin responsible for pathogenesis. The electrochemical DNA sensor for rapid and reliable detection of pathogenic strains of *A. hydrophila* was developed using biosensing device (biosensor I), a gold electrode modified with a mixed self-assembled monolayer of ss-DNA probes and mercaptohexanol. The second biosensor (II) uses multi-walled carbon nanotubes modified carbon paste electrode. The detection of pathogenic strains of *A. hydrophila* in food samples and the optimal DNA concentration in analyzed samples was 2.0µg cm^{-3} (biosensor I) and 0.8µg cm^{-3} (biosensor II) (Ligaj *et al.* 2014). An electrochemical DNA based on a multi-walled carbon nanotubes/chitosan/bismuth complex and lead sulfide nanoparticles for the detection of pathogenic *Aeromonas* has been reported. Lead sulfide (PbS) nanoparticles modified with 5′-(NH_2) oligonucleotides form amide bonds and wereused as signalizing DNA probe (ss-DNA) and thiolated oligonucleotides sequence was used as fixing DNA probe. The detection limit for this biosensor was 1.0 × 10^{-14} M. Detection of *Aeromonas* in spiked tap water was obtained at 10^2 CFU mL^{-1} (Fernandes *et al.*, 2015).

Pseudomonas aeruginosa is a Gram-negative bacterium that usually causes sepsis and

inflammation, urinary tract, colonizes in the lungs and kidney, which can be lethal. It is also responsible for cross infections from hospital and clinical equipment such as catheters. Norman *et al*., synthesized amine terminated gold nanorods labeled with anti-*P. aeruginosa* antibodies to selectively destroy *P. aeruginosa*. They achieved 75% decrease in cell viability with gold nanorods when exposed to near-infrared radiation (Norman *et al*., 2008). *V. parahaemolyticus* is commonly observed among people consuming raw and undercooked shellfish, oysters. A disposable enzyme immunosensor was developed based on a screen-printed electrode coated with agarose-doped AuNPs for the detection of *V. parahaemolyticus*. The obtained detection range was 10^5-10^9 CFU/mL, detection limit 7.4×10^4 CFU/mL (Zhao *et al*., 2007). *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a causative agent of Johne's disease occurring in cattle and Crohn's disease in humans. A one-step nanoparticle-mediated bacterial detection methodwasdeveloped for milk and blood using the magnetic relaxation characterstic of superparamagnetic iron oxide nanoparticles. The detection range attained was 15.5-775 CFUs (Kaittanis *et al*., 2007). Another sandwich immunoassay was developed for fast, low-level detection of MAP, based on surface-enhanced Raman scattering (SERS) with two key components immobilizing monoclonal antibody 13E1 for the detection of a surface protein, MAP2121c, on microorganism and preparing extrinsic Raman labels with 60-nm AuNPs for determining selective binding of captured proteins to generate large SERS signals. The developed sensor can detect 100 ng/mL in PBS and 200 ng/mL in pasteurized whole milk (Yakes *et al*., 2008).

MICROFLUIDICS SYSTEMS FOR FOOD PATHOGEN DETECTION

The various detection systems for food pathogens have been developed based on different approaches but still require a minimized and point-of-care (POC) system which rapidly detects on-site and helps with the preventionof food poisoning. The area of microfluidics analysis systems also called micro total analysis system (μ TAS) or lab-on-a-chip (LOC) is a rapidly developing in the field of miniaturization of the system. Microfluidics is the technology involved with science leading to the development of a system that processes or manipulates the small amount of fluids using microchannels with a dimensional range of tens to hundreds micrometers (Whitesides, 2006). An integrated microfluidic device incorporates the necessary components involved, and the functionality of a typical room-sized laboratory onto a small chip. The miniaturized versions of bioassays has many advantages, such as small requirements for solvents, reagents, and cells (valuable samples and for high-throughput screening), high resolution, short reaction times and sensitivity, portability, low cost, low power consumption, versatile design, and can have parallel operations and can be also used for the integration with other miniaturized devices. The shrinking of a system to the micrometre scale leads to an increased surface area with respect to volume, often by several orders of magnitude (Yoon *et al*., 2012). For a fluid, these effects allow more efficient transfer of mass and heat in microsystems relatively, which leads to better availability of the interface so that transfer can occur. So, both the creation and the homogenization of solute or temperature gradients occur faster as the size of the system is reduced. Fluid behavior in reduced dimensions will also be more influenced by viscosity rather than inertia. Microfluidic systems with simple geometries result in laminar flow. Such behavior occurs if the Reynolds number *Re*, gives the ratio of viscous to inertial forces. When the viscous forces dominate the fluid flow, the flow is said to be laminar. At Re above ~2000 the flow assumes turbulent behavior wherein convective mass transport takes place in all directions. In laminar flow, there would be effective diffusion for moving and mixing solutes on micrometer length scales.

Nanomaterials have unique properties when used with microfluidics technology sensitive devices in bioanalytical or biosensing systems for pathogen detection can be developed. The optical

and electrocatalytic properties of nanomaterials are used in microfluidics devices in order to improve the performances of bioanalytical sensing systems. Nanoparticles are also used as biomarkers for the purpose of detection in microfluidic devices. In SPR immunosensor, after miniaturizing the flow channels into a microchannel, the detection of *E.coli* O157:H7 on SPR lab-on-a-chip obtained up to 10^2-10^3 CFU/mL in milk, juice or beef (Waswa *et al.* 2007). *Salmonella typhimurium* detected in SPR LOC up to 10^4 CFU/mL in apple juice (Taylor *et al.*, 2006).

A microfluidic flow cell embedded with gold interdigitated array of microelectrodes integrated with magnetic nanoparticles-antibody conjugates has been developed for *E.coli* O157:H7 detection in beef samples in just 35 min. The detection limit 1.6 × 10^2 and 1.2× $10^3$of cells present pure culture and beef samples respectively (Varshney *et al.*, 2007). A microfluidic platform has been developed for aqueous environment detection of Botulinum toxin A (BoNT typeA). The system employs a toxin-specific bead substrate conjugate to screen BoNT/A enzyme activity. When the toxin samples are introduced into the channel, it catalyzes the cleavage reaction of fluorescent peptide substrate, which is immobilized on silica beads surface releasing the fluorescently-labeled fragments into bulk solution. The detection limit is achieved upto a very low level, 10pg/mL in buffer solution in 3.5 h (Frisk *et al.* 2008). Lab-on-a chip is developed for CNT based immunoassay detection for SEB. Here they have used four detection elements for immunological assay for SEB in LOC: (i) CNTs used for primary antibody immobilization, (ii) electro-chemiluminiscence for the generation of signal light, (iii) an actively cooled charged coupled device (CCD) detector and (iv) a LOC designed using polymer lamination technology. The advantage of using LOC is that an assay can be performed without laboratory, with a less amount of reagents and minimal exposure of toxins to the users. The detection limit is 0.1 ng/mL of SEB. Here, only 10 µl of sample is required for detection compared to the 50-100 µl for ELISA (Yang *et al.*, 2010). Microfluidic immunoassay platform has been developed for the detection of *E.coli* in different matrices, such as blood, milk and spinach samples. The limits of detection achieved for buffer, blood, milk and spinach samples were 50, 50, 50 and 500 CFUs/mL respectively. This technology can use for on-site real time food quality monitoring (Wang *et al.*, 2012). Polydimethylsiloxane (PDMS) /paper hybrid ,microfluidic system used for integration of aptamer functionalized graphene oxide (GO) nanobiosensor has been developed for multiplexed pathogen detection. For *S.aureus* and *Salmonella enteric,* the detection limit is 800 CFU/mL and 61.0 CFU/mL respectively (Li *et al.*,2013).

CONCLUSIONS AND FUTURE TRENDS

In this review, we have summarized the important role of different nanomaterials and their potential applications in the field of food pathogen detection by different transduction methods, specially optical and electrochemical as well as the integration with microfluidic platform. Although, the conventional methods are available for pathogen detection, an early-warning system for timely recognition is required to prevent the epidemics. Here, nanotechnology will give sensitivity to the application field of devices for the detection of food pathogens. Nanomaterials used in sensors provide a new and powerful model in terms of novel and improved functionality that encompasses a wide variety of applications in diagnostics and biological research. Nanomaterials incorporated into sensors increase the sensitivity, selectivity, and stability of the devices. It also enables the portability of the sensor for on field application. Another application of nanomaterials, other than amplified sensitivity in these devices, is the high loading capacity of the biological components, (*i.e.*, enzymes or antibodies) leading towards a better detection of analytes. All these properties and functionalities will further undergo improvements and refinements in the future due to advancements in nanomaterials synthesis, fictionalization, integration and testing techniques.

Nanomaterial based biosensors can also simultaneously detect multiple targets, as

nanomaterials can amplify biomolecular recognition events due to their large surface-volume ratio, enhancing the biomolecule loading capacity amount and in turn, maximizes the number of binding events. This results in signal enhancement, coupled to the high sensitivity of optical or electrochemical transducers.

The integration of nanomaterials with microfluidic devices brings additional advantages in terms of sensitivity, portability for field condition. This technology offers novel approaches to solve the crucial problems related to field portable systems and reduce the whole process of detection from the sampling process to the end. It will enable us to monitor early spread of food pathogens in samples and water before the outbreak. It needs to design better microfluidic devices which are more user-friendly. This is a challenging area for the integration of nanoparticles and microfluidic devices in one platform. However, there are some issues related to nanotoxicity that remain to be solved. More emphasize must be made on the novel nanomaterials and binding with biomolecules for the development of more sensitive and reliable devices for the food pathogen detection. Future research in this area is very helpful, developing more sensitive and fast biosensors for detection and prevention of epidemics is at a glance.

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Conflicts of Interest

The authors declare no conflict of interest.