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Bioconjugated Nanomaterials for Monitoring Food Contamination

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Abstract

Maintaining food safety and hygiene standards is top priority and challenge for farmers, food industries, governments and food technologists working in the food supply chain. Pesticides, toxins, veterinary drug residues, foodborne pathogens and many other harmful chemicals that may be present in a vast array of food products, due to various stages of their production like packaging and transport, constitute a global health problem that requires powerful and innovative technologies allowing constant and accurate detection of food products from production to consumption. Recent progress in generation of specific synthetic oligonucleotides against food contaminants has provided a new insight into the current sensor technologies, where these functional synthetic oligonucleotides, so-called aptamers, have been successfully combined with nanomaterials for rapid and cost-effective detection of several substances related to the food contamination, such as antibiotics, mycotoxins, heavy metals, carcinogenic dyes, pesticides, pathogens and other plastic products used for food packaging. Unique characteristics of aptamers over antibodies, such

as *in vitro* selection, chemical and thermal stability, small size and ease of labeling have laid the solid foundation for exploring aptamers further in multiplexed food monitoring systems. In this chapter, we reviewed the application of aptamer-conjugated nanomaterials in food safety surveillance as well as the conventional techniques used for food safety monitoring in order to provide a comprehensive and comparative approach.

Keywords: Aptamers, nanomaterials, biosensors, food pathogens, food allergens, mycotoxins

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1 Introduction

Food contaminants, especially pathogens, often go unchecked due to the presence of small quantities initially, and cause diseases in the consumers in later stages due to rapid replication during food transportation or food supply chain. For example, *Staphylococcus aureus* is a foodborne bacterium that causes more than 0.5 million infections in the USA only, and symptoms range from skin burn to pus formation in infected cells (Chang et al. 2013). Pathogens can transmit into the food through contaminated water, nearby soil, and even air. Another problem associated with food safety is the presence of toxins, heavy metals and allergens in food which also cause health problems in consumers. Several food toxins have been identified which are incorporated in foodstuff during growth, storage or processing (Pollet et al. 2011). For example, Selenium enters the food chain when microbes convert selenium from inorganic to organic form (Sors et al. 2005). Excessive uptake of Selenium causes Selenium toxicity or selenosis. This problem has been reported on a large scale in Chinese seleniferous region, and deformity, nail and hair loss, skin lesions, fatigue, garlic-like breath odour, diarrhoea, and irritability are the most common symptoms of this disease.

Incorporation of contaminants from the environment is not the only way through which toxins are added to food. Several food toxins are found in crop plants that are harmful to human health, for example, prussic or hydrocyanic acid is constituted in peach, apple and cherry plant tissues when cyanogenic glycosides are damaged, and come in contact with emulsion enzymes or *beta*-glycosidase. Consumption of food carrying this acid can result in respiratory arrest, trembling, cardiac arrest and muscle non-coordination depending upon its dose (Dolan et al. 2010). The presence of goiter genesis chemical in peaches, canola, cauliflower spinach, sweet potatoes, cassava, peas, and peanuts can cause swelling of throat

or goiter if eaten uncooked. The erucic acid in rape, the most commonly used edible oil crop can cause cholesterol accumulation in muscles leading to a heart problem and lectins in legumes can even cause agglutination of blood cells. Similarly, higher doses of oxalic acid in many leafy vegetables such as celery, broccoli, cabbage, turnip, beans, coffee, beans, peas, tomatoes and potatoes can lead to vomiting, kidney stones, diarrhoea and blood clotting (Dolan et al. 2010).

Food adulterants are an issue that arises due to the professional dishonesty of some traders or farmers who mix inferior quality, some wild-type or closely related species' product with food in order to get more profit. On the other hand, this is a potential threat to food safety as some of the wild relatives of crop plants whose seeds or product is mixed with food containing potential harmful chemicals for human health. For example, in turmeric, moong bean, and chickpea pulse, methanol is added to add a yellow color to these foods, but is carcinogenic and cause stomach disorder. Similarly, malachite green is applied to green peas and green chilies to make their skin glowing that is also carcinogenic (Lakshmi & Pradesh 2012). Adding to the problem is the fact that nobody can identify these toxins, allergens and adulterants with the naked eye, thus, the symptoms appear only after usage of the contaminated food.

From the above discussion, it is evident that food pathogens, allergens, and toxins are a serious threat to human health and cause many disease and complications in consumers if they are part of the food. Therefore, rapid and accurate detection of the food contaminants is a crucial step towards the food safety and public health. Flow cytometry, polymerase chain reaction (PCR) and antibody-based immunoassay platforms (ELISA, LFI, IMS) have

been conventionally used for the detection of food contamination for years (Zhao et al. 2014a).

Recently, aptamers have emerged as potential detection elements which can identify different target molecules ranging from small molecules to whole cells with an affinity and specificity similar to monoclonal antibodies. Aptamers are selected short oligonucleotides or peptide molecules that act as ligands due to their high affinity and specificity towards their targets. Aptamers can be used as probes by labeling, and their low cost of production makes them an excellent choice for sensing applications over the conventional probes, antibodies. A diverse collection of aptamers for hundreds of various targets has been developed from the random pools using either traditional or the modified selection techniques. As the applications vary from medical science to food security, so a broad range of analytical approaches has been developed for the purpose (Song et al. 2012). Aptamers have found applications in food contaminant detection, food packaging and food processing industries, and food monitoring during trading at ports by quarantine departments.

Employment of aptamers in sensor devices is usually achieved through incorporation of nanomaterials, such a gold nanoparticles, quantum dots, carbon nanotubes, graphene, and upconversion nanoparticles into the sensor platform as the signal transducers. Nanotechnology has gained a considerable attention from numerous disciplines owing to the exceptional physical and chemical properties of nanomaterials, such as high thermal conductivity and high surface to volume ratios (Nel et al. 2009; Geim & Novoselov 2007). Coupling of nanomaterials with aptamers has laid the foundation for a new vision in sensing, in which aptamer is used as the sensing reagent or capture molecule while the nanomaterial is used as the signal converter or amplifier. In this chapter, we have reviewed the food

safety monitoring techniques that are mainly based on the aptamers and aptamer-conjugated nanomaterials. The conventional techniques were also mentioned in the chapter in order to provide a comprehensive as well as comparative approach to the topic.

2 Aptamers

Aptamers are short oligonucleotide sequences (ssDNA, RNA or peptide) which can acquire three-dimensional structures in solution to bind with a variety of target molecules like ions, proteins, drugs, cells and even nanoparticles. Aptamers are similar to monoclonal antibodies regarding their binding affinities, but they offer some advantages over antibodies, as listed in **Table 1**, which make them suitable for analytical applications.

Aptamers are selected from randomized oligonucleotide libraries by repetitive cycles of binding, partition, recovery, and amplification steps through a well-defined *in vitro* selection method known as Systematic Evolution of Ligands by EXponential enrichment (SELEX). The artificially synthesized random oligonucleotide pool is considered as the starting point for the selection of an aptamer through SELEX, which usually consists of $10^{14\text{-}15}$ unique sequences that have 30-80 nt central random region flanked by primer binding sites for the amplification step. Random oligonucleotide library of complex structures can be synthesized according to combinatorial chemistry principle to perform a variety of functions such as the production of high-affinity aptamers. Unnatural bases can be also incorporated into the random libraries to increase the final diversity and the affinity of the sequences. The affinity of the selected aptamers depends on the method, formation of the three-dimensional structure upon binding to the target molecule, as well as the target molecule itself. Unlike antibody production, aptamer screening process is completed in weeks, and no animal sacrifice is required for the production. SELEX technique has become the basic process for

screening of aptamers directly *in vitro* against the targets including pathogen cells, small biomolecules, and proteins.

[Please insert Table 1 here]

2.1 Aptamer screening

The concept of joining nucleic acids to protein originated from when it was found that some RNAs bound with viral proteins with high affinity and specificity in adenovirus. In adenovirus, virus-associated short RNA-ligand binding led to translational regulation (O'Malley et al. 1986). This *in vivo* discovery was a first step towards its evolution to an *in vitro* selection procedure which was developed in 1990 by two research groups independently. The aptamer screening procedure, SELEX, was described by Tuerk and Gold in 1990, when they were studying the interactions between T4 DNA polymerase and natural ribosome binding site of the mRNA that encodes the enzyme (Tuerk & Gold 1990). In the same year when SELEX was introduced by Tuerk and Gold, Ellington and Szostak independently applied the same strategy to isolate target specific RNA sequences from a random RNA library, and then named them as "aptamers" (Ellington & Szostak 1990).

The basic aim of the selection process is to obtain the tightest binding sequence from the randomized pool through the exponential enrichment which includes repetitive cycles of target binding, partition of the target oligonucleotide complex, elution of the target bound oligonucleotides, amplification of the target specific oligonucleotides, and conditioning for the next SELEX cycle. By these repetitive cycles of selection and amplification, the affinity and specificity of an initial random oligonucleotide pool for the target is enriched, meaning that the total complexity of the randomized pool is reduced to relatively few sequence motifs. RNA aptamer selection process begins after the ssDNA library is converted into a

dsDNA library with the help of sense and antisense primers consisting of T7 promoter at 5′ end. The dsDNA is then *in vitro* transcribed with the assistance of T7 RNA polymerase to form a randomized RNA library to start SELEX process. The selected RNA of each round is reverse transcribed into DNA and amplified for the following round of selection (Thiel et al. 2011). An illustration of the standard SELEX procedure is presented in **Figure 1** (Yüce et al. 2015).

[Please insert Figure 1 here]

Additional steps can be inserted into each round of SELEX procedure to enhance further the stringency of the process. For example, negative-SELEX steps are performed to prevent the enrichment of non-specific oligonucleotides during the selection, in which the pool is incubated with the naïve immobilization surface lacking the target of interest. The non-specific oligonucleotides are those that bind to the immobilization surface (magnetic beads, columns or chips) or blocking agents (BSA or ethanolamine) instead of the specified target. Counter-SELEX, on the other hand, can be applied to remove oligonucleotides that bind with the substrates that are structurally similar to the target of interest (i.e. glycated hemoglobin, non-glycated hemoglobin).

Enrichment of the start pool is monitored over the course of selection to decide where to stop SELEX, which is followed by sequencing of the enriched final pool. Although Sanger sequencing method has been immensely used in SELEX, next generation sequencing (NGS) techniques have recently paved the way for a new era in aptamer sequencing because of the greatness of the received data allowing a thorough evaluation of the final pool so the SELEX process. In the current state, NGS data is processed using the tools such as AliBee (Sarell et al. 2014), Clustal W (Jing & Bowser 2013), Clustal Omega (Wilson et al. 2014), MEME ChIP (Ma et al. 2014), mfold (Sung et al. 2014), RNAfold (Belter et al. 2014) or some

local algorithms (Gupta et al. 2014). Processing of the NGS data from SELEX has been a general problem in the field due to lack of knowledge, expertise, and bio-informatics tools, however, there is a considerable progress being made to develop software, online prediction tools or scripts capable of sorting huge aptamer data sets, including, AptaMotif (Hoinka et al. 2012), AptaCluster (Hoinka et al. 2014) and Aptaligner (Lu et al. 2014).

Sequence analyzing is followed by the chemical synthesis of the selected sequences and the evaluation of the binding kinetics, which is typically identified by surface plasmon resonance, pull-down assays or fluorescent-based techniques. Recently, Microscale thermophoresis (MST) and backscattering interferometry (BSI) methods have been also shown as valuable as the previous techniques to assess the ligand and analyte interactions (Olmsted et al. 2011; Seidel et al. 2014).

Since the inception, SELEX procedure has been substantially modified to increase the final specificity, binding affinity, and to reduce the labor, material consumption as well as the time spent for the process. Within the concept, magnetic bead-based selection, bead or chip-integrated microfluidic selection platforms, NGS-based screening procedures, surface plasmon resonance-based real-time selection, affinity column-based immobilization free selection and many other combined techniques have been introduced in the recent literature (Yüce et al. 2015). Among them, the magnetic bead-based selection has received the greatest attention due to ease of handling, ease of coupling with the target molecules (covalent or non-covalent), and the ability to be employed in automated and microfluidic based-selection platforms. For example, Tok and Fischer (Tok & Fischer 2008) used only single magnetic bead functionalized with *Clostridium botulinum* neurotoxin-related targets Hc-peptide and the Toxoid to be used in SELEX, in which they traced the single bead under

the fluorescence stereomicroscope using fluorescently labelled random pool, and obtained target-specific aptamers with affinities in nanomolar range.

Post-modification of the selected aptamers can be performed to increase their stability, affinity or shelve-live/serum half-life. Also, labeling with classical fluorophore molecules or fluorescent nanoparticles such as quantum dots and upconverting nanoparticles, allow their further utilization in biosensing and imaging applications as fluorescent probes. Because the binding ability of the antibodies compromises after labeling or modification, their application in sensing is limited by their surviving capacity. Aptamers can be also modified with biotin or thiol tags via *in vitro* transcription or PCR on demand. Additionally, aptamers could tolerate the change in thermal conditions, enabling the recycle of the sensing element whereas antibodies go irreversible denaturation and aggregation upon heating that prevent the multiple uses of the biomaterial in sensing applications.

3 Conventional Methods for Food Contamination Detection

The conventional methods used for the identification of food contaminants include antibody-based immunoassays, different types of polymerase chain reactions, and flow cytometry.

3.1 Immunoassay-based methods

The antibody-based contaminant detection is one of the most commonly used methods, and the fundamental principle of this method is the binding of a specific antibody to the target, followed by the antigen-antibody complex detection. Antibodies are produced in a living system in response to the specific pathogen, and they are being produced in large-scale by using different pathogens and toxins in laboratory animals. Antibodies are very specific and can identify a specific pathogen attached to the food in the presence of other

pathogens, but they can only work if the pathogen gives stable expression of the antigen (Wacoo et al. 2014).

In an immunoassay, antibodies are usually attached to a solid base, and samples are added to see the interaction, followed by probe-based detection (Song et al. 2012). The major immunochemical assays are described below:

3.1.1 Enzyme-Linked Immunosorbent Assay (ELISA)

Sandwich type ELISA has the highest efficiency as it utilizes two antibodies to detect its target. In ELISA, the primary antibody is attached to ELISA plate, and antigen such as a pathogen or toxin is added and adhered to the antibody while unbound contaminant is removed. Now, a secondary antibody conjugated with the enzyme is added which finds the antigen. In this way, the antigen is sandwiched between two antibodies and the antigen is detected by the change of substrate color in the presence of an enzyme (Zhao et al. 2014a). The most commonly used enzymes in ELISA include beta-galactosidase, alkaline phosphatase and horseradish peroxidase (Yeni et al. 2014). Some foodborne pathogen and toxins that have been identified by ELISA include *Salmonella* spp. (Lee et al. 2015), staphylococcal enterotoxin (Cremonesi et al. 2007), *Escherichia coli* toxins i.e. enterotoxins and botulinum toxins (Aschfalk & Müller 2011), *Vibrio parahaemolyticus* (Kumar et al. 2011), α, β and ε toxin of *Clostridium perfringens* (Aschfalk & Müller 2011), *E. coli* (Carvalho et al. 2014), *Campylobacter* spp and *Listeria monocytogenes* (Gómez-govea et al. 2012). Several food types in which ELISA has provided successful detection include fruits, vegetable, fish, beef, pork and cheese (Law et al. 2014).

3.1.2 Lateral flow immunoassay (LIF)

Although, ELISA has been the most popular immunoassay technique, it requires a skilled workforce and various equipment. Therefore, some simple, cheap, rapid and yet reliable method of contaminant detection is needed. In recent years, LFI-based techniques such as immuno-filtration, immune-chromatography and dipsticks are becoming popular for food pathogen and mycotoxin detection (Wacoo et al. 2014). In LFI, the test sample streams along the immunoassay device comprising of four sections via capillary action. A colored reagent (antibody) loaded on gold particles is mixed with food sample solution containing contaminant while flowing through the tube. Most of LFI assays give visual results in 2-10 minutes which is faster than ELISA (Gómez et al. 2010). Usually, immune-chromatographic strips are developed for quick and visual detection, and various food contaminants have been identified by using these strips such as Avian Influenza Virus (Peng et al. 2008), *Streptococcus suis* serotype 2 (Ju et al. 2010), Porcine circovirus-2 (Jin et al. 2012), *Campylobacter jejuni* (Xu et al. 2013), *E. coli* (Sajid et al. 2015), Salmonella (Fang et al. 2014), *Riemerella anatipestifer* (Hou et al. 2015) and many other food contaminants. Nowadays, several commercial strips are available for rapid detection of food contaminants.

3.1.3 Immunomagnetic separation (IMS)

Immunomagnetic separation method uses immunomagnetic beads (IMBs) for microbe identification. This assay is quite selective in nature that allows the growth of target pathogen while the growth of non-target pathogens is suppressed. In this method, target cells are incubated with IMBs for one hour, separated by a magnetic separator, followed by several cycles of washing to eliminate the non-specific interactions. IMBs do not interact with the sample in assay and conjugation of various range of molecule to the magnetic beads provides an additional advantage (Zhao et al. 2014a). Unlike ELISA, which can take a

number of days for identifying the pathogens, a highly efficient IMBs based fluorescence assay was developed which identified *E. coli* O157: H7 within 8 hours (Decory et al. 2005). A unique way of this approach is coupling immune-affinity with mass spectrometry based on matrix-assisted laser desorption that helped in the detection of staphylococcal enterotoxin B (SEB) (Schlosser et al. 2007). Another unique method is to combine IMS with PCR for the detection of *Alicyclobacillus acidoterrestris* in apple juice. The immune-capture took 60 min with IMPs quantity at 2.5 mg mL^{-1} followed by pathogen detection with IMS-PCR that showed highly specific detection of *A. acidoterrestris* in 3-4 hours only (Wang et al. 2013). However, IMS method does not produce a pure culture of microbes and requires to be coupled with other methods for reliable detection. Another disadvantage of this method is a requirement of large sample even up to 500 times more than the other assays during capture by sample recirculation to reduce detection time and enhance the sensitivity (Fedio et al. 2011).

3.2 PCR-based methods

Standard PCR, multiplex PCR, and quantitative PCR are the most commonly used nucleic acid amplification-based contaminant detection methods in food safety monitoring.

3.2.1 Standard PCR

In PCR, DNA strands are denatured at around 95 °C followed by annealing of specific primers at 55-65 °C, and finally extension step makes several copies of targeted DNA at 72 °C by DNA polymerase enzyme, after several cycles of these three stages. The amplified DNA is separated by gel electrophoresis under voltage difference and action of negative charge; followed by DNA documentation as intensity bands through the gel staining. Reliability of this detection method can be enhanced by sequencing which is quite an expensive method

to date. However, this method is quite specific, sensitive and rapid as compared to many assay based detection methods (Khan et al. 2014).

The PCR-based assay detects the food pathogens by amplification of the genes coding for the toxins that are produced by a particular organism. For instance, *Salmonella* was identified in 150 samples of fish (n=6), chicken (n=80), and beef (n=64) by 12-hour length PCR. A total of 35 samples were positive by PCR against 32 by a culturing method with an evident advantage of less time consumption and along with 100 and 97.5% sensitivity and specificity, respectively. The amplification was done by Salm4 and Salm3 primers that amplified a 389-bp DNA fragment in *invA,* a conserved gene of Salmonella spp (Hegazy AI 2014). In another study, *Salmonella typhimurium* bacteria was identified in wheat, rice, snacks, juices, fish and meal by PCR-based amplification of same *invA* gene (389 bp) by specific primers after 10 hour pre-enrichment (Khan et al. 2014).

3.2.2 Multiplex PCR

Multiplex PCR is a particular kind of reaction which can amplify more than one locus linked to multiple microbes, hence, helps in simultaneous detection of various microorganisms by a single PCR reaction. This method is just similar to the previous method except that several specific primers are combined in a single PCR assay. The problems associated with multiplex PCR include interaction between different primers so primer designing and concentrations should be kept in mind while designing the primers in addition to a same annealing temperature (Zhou et al. 2013). Several multiplex PCR assays have been reported in which foodborne pathogens have been detected, for instance, a rapid multiplex PCR simultaneously detected five important pathogens including *Listeria monocytogenes*, *Shigella Flexner*, *S. Enteritidis*, *E. coli* O157: H7 and *Staphylococcus aureus* in artificially

contaminated pork. This highly specific, efficient and sensitive method also found that 80% meat samples were positive for these pathogens by using five primer sets specific to these microbes (Chen et al. 2012). A novel GeXP analyzer-based multiplex PCR was developed for detection of six pathogens including *Listeria monocytogenes*, *S. entries*, *E. coli* O157: H7, *Campylobacter jejuni*, *Staphylococcus aureus* and *Shigella spp*. simultaneously. They fused chimeric primers unique to both pMD19 plasmid and microbes, and PCR products were separated by capillary electrophoresis. DNA bands were visualized with fluorescence spectrophotometry while pMD19-T plasmid was used as a control in each reaction. This method did not need any enrichment and was specific, sensitive, rapid and high throughput (Zhou et al. 2013). Recently, a triplex multiplex PCR identified three major raw poultry pathogens i.e. *Shigella, Listeria monocytogenes,* and *Salmonella in* 5.2%, 6.2%, and 26.9% of the samples studied respectively (Zhao et al. 2014b).

3.2.3 Quantitative PCR

Quantitative PCR (qPCR) or real-time PCR is an advanced reaction that can amplify and quantify or detect the target DNA simultaneously, thus, providing an advantage of observing the effect of environmental conditions on the microbe. As no post-amplification treatments are required, it reduces the effort and time needed for food contamination detection (Law et al. 2014). The newly developed qPCR identified Shiga toxin producing E-coli (STEC) in dairy products and minced beef with equal accuracy level to the methods mentioned above (Derzelle et al. 2011). A highly reliable and fast “most probable number (MPN) qPCR” was developed which quantified *E-coli* O157: H7 and *Listeria monocytogenes* in fresh-cut vegetables. The method was validated by comparing the results with simple MPN method followed by biochemical assay and phenotyping. MPN-qPCR identified as low as 1 CFU g^{-1} of

both pathogens within 48 hours of artificial inoculation of minimally processed vegetables. Whereas, qPCR alone detected 101 CFU g^{-1} of both pathogens after two hour enrichment as vegetables are perishable commodity, so such method requiring short time can help to monitor their food safety (Russo et al. 2014).

3.2.4 Flow cytometry method

In contrast to most of the immunoassays in which samples are fixed to the slide, flow cytometry is based on passing the samples through a liquid dye or sensing medium making it just like an automated fluorescence microscopy. In Flow Cytometry Method (FCM), the sample is carried by laminar flow through a light whose wavelength is matched to the absorption spectrum of the stained dye in the sample cells. The fluorescence emitted by pathogen appears in the form of scattered light; that is collected by detectors and converted to an electric signal. Analysis of scattered light from pathogen cells provides the insight about size, structure and shape of microorganisms. FCM can analyze the thousands of cells simultaneously at a single cell level, showing its effectiveness (Mandal et al. 2011). However, real-time pathogen detection by this method has several limiting factors including the limited performance of instrument for particle size resolution, the complexity of sample matrix and ruggedness of the system (Buzatu et al. 2014). Moreover, FCM studies have been much limited to bacteria till date (Ambriz-aviña et al. 2014). A unique and improved FCM protocol eliminated the background noise and improved signal strength. This provided high sensitivity for detecting *E. coli* O157: H7 and other bacteria, and it required few minutes after enrichment (Buzatu et al. 2014).

4 Monitoring Food Contamination through Bioconjugated Nanomaterials

Conventional methods for detection of contamination were based on various media, cultures, assays and instruments which gave results in qualitative and quantitative forms. However, these techniques are comprised of series of steps to be followed to get the final results. In this way, these methods required several days to confirm the presence of a particular contaminant because the results were based on the pathogen ability to multiply and form detectable colonies (Biswas et al. 2008). Preparation of culture medium, inoculation, and counting of grown colonies to interpret the presence of a particular contaminant in a food sample makes the conventional techniques time consuming and labor intensive. Another complication related to the conventional methods is the separation of target pathogen or other contaminant from the food as food is a complex entity consisting of different macromolecules ranging from carbohydrates to fats (Doyle & Erickson 2006). Even after the separation process, the contaminant/pathogen concentration may be too low for efficient and accurate detection that may lead to a wrong conclusion about the type of contaminant present in food (Bhunia 2008).

The above-mentioned limitations of the conventional contamination monitoring methods have resulted in the development of highly efficient and specific aptamer based biosensor platforms. These systems have several advantages over conventional ones; such as aptamers are cheap, stable and can be easily modified. Other benefits include portability, low toxicity, purity, reproducibility, multi-pathogen or multiplexed detection, high level of specificity and efficacy, and real-time or fast detection (Song et al. 2012). In recent years, several nanoparticles have been conjugated with aptamers for more accurate, efficient and real-time detection of food contaminants. The nano-materials which have been conjugated with aptamers for the purpose include gold nanoparticles, carbon nanotubes, quantum dots, graphene, and upconversion nanoparticles. The unique properties of these

particles such as high stability, physical and geometric properties and larger surface area make them excellent material for improving the interaction of aptamers with biological molecules e.g. food contaminants. Further, nanomaterials are easy to be conjugated with aptamers and do not require any other reagent for signal transduction. Conjugation of NPs with nucleotides further prevents the nanoparticle aggregation due to electrostatic repulsion. A major advantage of this conjugation approach is that it prevents any direct modification of the aptamer sequence involved in the binding process. Some of the nanomaterials such as quantum dots, graphene oxide and upconversion nanoparticles, in addition to above advantages, helps in accurate and rapid detection of food contaminants by enhancing or quenching the signal from contaminants (Javier et al. 2008). The fast detection of contaminants in foodstuff can help to screen large trade consignments for a number of targets simultaneously so that much needed early delivery of food to the consumer is achieved. Below we have described nanoparticle-conjugated aptamers for detection of food contaminants.

4.1 Detection of foodborne pathogens

Keeping in view the advantages provided by aptamers conjugated with nanoparticles, several detection systems have been reported. For example, a non-PCR-based aptamer-conjugated gold nano-particles (GNPs) system with low cost and ultra-sensitivity for *Staphylococcus aureus* bacterium identification has been developed. The pathogen signals were measured by utilizing the resonance light scattering, and the single cell detection was achieved within 1.5 hours only. Two aptamers SA17 and SA61 were conjugated with GNPs showed higher binding ability and specificity with *S. aureus*. The GNP-based conjugation provided greater surface area for interaction with the sample including pathogen. These

results were further confirmed by scanning electron microscopy (SEM), and fluorescence microscopy and the quantification was done through immunofluorescence assays (IFAs). Both GNPs conjugated-aptamers were able to identify six different strains of *S. aureus* (ATCC: 12600, 25923, 6538DR, 6538P, 6538, 29213), but did not show any cross react with 13 other bacteria studied, showing highly specific nature of the selected aptamers. However, SA61 showed a weaker interaction with *P. aeruginosa* and *S. epidermis* than *S. aureus* strains. It was also found that immobilization of both aptamers on GNPs significantly altered their functional structure. This advantageous change was documented in terms of K_d (binding constant) values as they changed from 129 and 35 nM in SA61 and SA17 to 9.9 and 3.03 nM for SA61-GNPs and SA17-GNPs respectively. This showed a visible enhancement in pathogen signal due to conjugation of GNPs and increased affinity in comparison to free aptamers. The whole process is illustrated in **Figure 2** (Chang et al. 2013).

[Please insert Figure 2 here]

Another GNPs-conjugated aptamer assay for rapid, simple, and cost-effective detection of *S. typhimurium* has been reported. SELEX identified 96 candidate sequences for pathogens which were classified into 6 families (S1-S6) on the basis of high sequence homology (75-85%). One aptamer per family was selected, and its binding affinity to *S. typhimurium* was studied by fluorescence spectroscopy. S2, S3, and S6 aptamers showed high binding affinity to the pathogen and S6 aptamer with the highest value. Aptamer S6 was conjugated with GNPs in colloidal gold solution, and the aptamer was denatured/renatured for the target specific 3D structure. Then aptamer-conjugated GNPs solution was treated with high concentration of salt (1.0 M) in the presence or absence of the target pathogen. GNPs helped in maintaining the balance between van der Waals attraction and electrostatic repulsion in solution. Thus, it provided stability to the detection system. Furthermore, high

mobility of GNPs into a porous membrane, and less aggregation due to their binding with an aptamer, the aptamer-GNPs complex remained stable when *S. typhimurium* was added. When S6 aptamer was used in the GNP-based colorimetric procedure, even 106 CFU mL^{-1} of the pathogen was successfully detected; which was more efficient than the aptamer alone (Moon et al. 2014).

Carbon nanotubes (CNTs) especially single-walled Carbon nanotubes (SWCNTs) have been conjugated with aptamers for simple detection of food pathogens. SWCNTs have exceptional thermal, electrical, physical and mechanical properties. The unique properties of SWCNTs include outstanding mechanical properties, even better than commercially available carbon, steel or aramid fibers; a low defect density or sound structural integrity; exceptional electrical conductivity of metallic SWCNTs i.e. 1000 times better than most commonly used copper. An SWCNT-conjugated aptamer system involving ssDNA based Apt22 aptamer has been developed which showed high specificity and binding affinity for *Salmonella paratyphi* with the K_d value of 47 ± 3 nM. For the conjugation, SWCNTs were combined with aptamer detection probe P0, which was labeled with DNAzyme. In the first step, SWCNTs-COOH complex was sonicated for 30 min in DMF until a homogeneous black solution was obtained; and diluted into working solution to get SWCNTs suspensions. In the second step, SWCNTs suspension was hatched with P0 (500 nM) for 4 hours at 37 °C. Then, centrifugation of the solution was done at 22,000 × *g* for 15 min to obtain P0/SWNTs containing supernatant. Finally, the target pathogen and hemin were incubated with the supernatant for 15 h at 37 °C to react. For detection, H_2O_2 and luminol were quickly mixed with the final mixture and fluorescence spectrophotometer was used to measure pathogen signal. This novel aptamer-based system conjugated with DNAzyme and SWCNTs by non-covalent self-assembly showed high specificity to the pathogen with 103 CFU mL^{-1}

detection limit. A further advantage of this system is that it does not need the probe designing, synthesis and gene extraction (Yang et al. 2013). Aptamer-SWCNTs complex has also been used for detection of one of the most common foodborne pathogen *E. coli* (So et al. 2008).

4.2 Detection of food toxins

Aptamers and aptamers conjugated with nanoparticles have also been used to detect toxins in food. Danofloxacin is a synthetic fluoroquinolone which is used in poultry and other animal husbandry systems as an antibacterial drug. It remains as a residue in the animal body and is transferred to humans as toxin via food. This can affect the nervous system, liver, muscles, and skin. SELEX was used to develop highly affinitive and specific RNA aptamers for Danofloxacin having $2^{/}$ -fluoro-$2^{/}$-deoxyribonucleotide modified pyrimidine. The truncated aptamer (minimum sequence of the aptamer that is required to bind any other molecule), dano-I showed binding ability with danofloxacin, but, did not show any binding affinity with tetracycline, thus, helped in discriminating danofloxacin from the rest fluoroquinolones. Dano-I (36 and 24 mer) aptamers showed affinity to danofloxacin, but a 21 mer truncated Dano-I, and dano-II aptamer did not show any binding (Han et al. 2014).

Ochratoxin A (OTA) is nephrotoxic while the International Agency for Research on Cancer has declared it carcinogenic to humans. More than 50% of humans are exposed to OTA by eating cereals and cereal products. SELEX procedure was used to screen aptamers, called A08 and B08 that had high binding affinity against OTA mycotoxin rather than the other toxins. The K_d values for both selected parameters were 290 ± 150 and 110 ± 50 nM, respectively. These aptamer sequences and the truncated versions were modified with SYBR® Green I fluorescence schemes for OTA detection. This fluorescent-based platform

was highly selective, sensitive and rapid which was capable of identifying 9 nM toxin (McKeague et al. 2014).

A chromatographic strip assay for rapid identification of OTA by conjugating aptamers with GNPs has been developed. GNPs were prepared, and thiolated aptamers were coated on GNP surface by a well-known self-assembly method. Two DNA probes were fixed on streptavidin by utilizing the 5' biotin followed by the assembly of the strip. A plastic plate was used as a base on which nitrocellulose and glass fiber membranes; sample and absorbent pads were placed as layers in said sequence. Now, both DNA probe conjugated with streptavidin were transferred to the strip and GNP-aptamer probe was attached to the glass fiber membrane as presented in **Figure 3**. Working principle of the strip was based on the competitive reaction between the target OTA and test line or DNA probe 1. When OTA toxin was present in the sample to be tested, the aptamer-GNP conjugate was unable to hybridize with DNA probe 1; and thus the red color of the strip became weaker. But aptamer-GNP bound to DNA probe 2 even at lower OTA concentrations in the control line which validated the results. This strip had a limit of detection (LOD) value of 1 ng mL^{-1} in qualitative tests, but it also showed promise in semi-quantitative analysis.

[Please insert Figure 3 here]

A quantitative calibration curve was constructed by using a scanning reader with an LOD of 0.18 ng mL^{-1}. This detection limit was better than ELISA, antibody-based strip and aptamer without GNP conjugation; thus showing the greater efficacy of nanoparticle conjugates as compared to other detection systems. This strip did not show any binding to other food toxins including fumonisin B1, mycotoxins deoxynivalenol, microcystin-LR and zearalenone (ZEN) showing specificity of the strip for OTA. In addition to higher efficiency and specificity of this nanoparticle-based detection system; other advantages of this strip were its stability

after 30 days, 96%-110% recoveries, fastness (less than 10 minutes for detection), reliability and cost effectiveness (Wang et al. 2011).

In addition to above strip based system, GNP-conjugated aptamers have also been reported for rapid, highly sensitive, specific and reliable OTA detection by utilizing fluorescence resonance energy transfer (FRET) aptasensor. This detection system was based on hybridization of a dye-tagged ssDNA aptamer conjugated with GNPs. The hybridization of the aptamer-conjugated GNPs with the dye-labeled ssDNA aptamer provided the advantage of fluorescence quenching of FAM. When OTA is present in a food sample, it gives fluorescence as a consequence of hybridization, and a quadruplex-OTA complex is formed; this complex now detaches from GNPs. The fluorescence intensity is directly proportional to the OTA concentration in food sample which ranged from 5×10^{-12} to 5×10^{-9} g mL^{-1}, with an LOD value of 2×10^{-12} g mL^{-1}. The above system successfully measured OTA concentrations in naturally contaminated maize, and it was validated by using a commercially available ELISA method (Duan, Wu, Ma, et al. 2012).

Staphylococcal enterotoxins are secreted in food by *S. aureus*, a food-borne bacterium. Staphylococcal food poisoning occurred when SEs contaminated food is eaten, and it leads to severe abdominal pain, diarrhea, and emesis. A novel aptamer APTSEB1 showed high binding affinity and specificity against *staphylococcal enterotoxin* B (SEB) which isolated SEB from a complicated mixture of enterotoxins (DeGrasse 2012). A GNP-based enhanced chemiluminescence (ECL) biosensor has been developed for rapid and reliable detection of SEB in food. Use of GNPs gives rise to a stronger signal as compared to an alone antibody or aptamer. This enhanced ECL provides the additional benefit to detect minor quantities of SEB in samples; thus helps screening the foodstuff for this toxin with increased sensitivity. According to this system, the anti-SEB primary antibody was conjugated with GNPs through

physical adsorption and this complex was fixed on a polycarbonate surface (Minghui Yanga, Yordan Kostova, Hugh A. Bruckb 2009). The above system has also been replicated by GNPs conjugated with ssDNA aptamer for SEB detection, with low cost and in less time. Several aptamer sequences linked to SEB were got by SELEX and five of them selected for further analysis. Now, aptamers were conjugated with GNPs and a colorimetric procedure was followed to detect SEB. The main structure of the aptamers was found to be in stem-loop and hairpin form. One of the GNP-conjugated aptamer, i.e. aptamer No. 15-1, detected the even lower concentration of SEB like 10 ng mL^{-1}. This aptamer also showed higher selectivity for both SEC1 and SEB toxin as compared to the non-conjugated aptamer (Liu et al. 2013).

In another study, aptamers selected through SELEX procedure were functionalized with graphene oxide (GO) nanoparticles. This GO-conjugated ssDNA aptamer system detected T-2 toxin in food with high binding affinity and sensitivity. The high binding affinity of the aptamer Seq.16 to the T-2 toxin was recorded by circular dichroism spectroscopy and dissociation constant for the interaction was 20.8 ± 3.1 nM. This aptamer was used to develop an aptamer-based fluorescent method for detection of T-2 toxin in beer with detection range varying between 0.5 to 37.5 μM (Chen et al. 2014). T-2 is a trichothecene mycotoxin that cause diarrhea, hemorrhaging, and feed refusal in livestock when ingested through contaminated grains like cereal crops (McCormick et al. 2015).

4.3 Detection of food allergens

Allergy caused by peanuts is a notorious and life threatening food contamination as it can lead to death by causing a lifelong disorder. *Arachis hypogaea* 1 (Ara h 1), a 63,000 Da molecule, is an important peanut allergen, and it is found as a homotrimeric complex in

food. The particular 3D structure of this protein makes it stable, viable and thus allergic even if it goes under heat and enzyme treatment. This protein has protease-resistant fragments which make its denaturation and protease digestion difficult. Therefore, rapid and efficient detection system is required to isolate the infected food from healthier one and to ensure food safety or consumer protection.

Although several immunoassays are available for Ara h 1 detection such as ELISA, dipstick assay, lateral flow assay and immuno-sensors, all these methods are based on antibodies whose generation is difficult and these assays are time-consuming as well (Pollet et al. 2011). Recently, a GNP-based capillary electrophoresis-based-SELEX method was used to develop Ara h1 specific ssDNA aptamers that additionally showed no affinity for Ara h 2 and other similar proteins. The dissociation constant of the aptamer having the highest binding affinity and specificity, i.e. A1 aptamer, was 450±60 nM, 419±63 nM and 353±82 nM as measured by capillary electrophoresis, fluorescence anisotropy and surface plasmon resonance, respectively. This selected aptamer was utilized to develop another biosensor called as FO-SPR or fiber optic-based surface plasmon resonance for detection of the same allergen in different food materials. FO-SPR device's robotic arm was used for binding in three steps as it was programmed for the purpose. Optimization was done with tris(hydroxyamino)methane-glycine-Potassium (TGK) buffer and a baseline measurement was made, followed by Ara h 1 molecules' capture by the aptamer in 100 µL of food sample for 10 min. In the 2nd step, FO-probe was dipped in TGK buffer having a rabbit polyclonal antibody (10 µg mL^{-1}) which had binding ability for Ara h 1. In the final step, the fiber was immersed in GNP solution for 10 min. Before the third step, GNPs were functionalized by adsorption of protein A on their surface. This was done by mixing 5 ml GNPs (1 OD, pH of 7.0) with 100 µM protein A in 100 µL of 0.2 M Na_2CO_3 buffer and the whole process is

shown in **Figure 4**. Protein A helped in the recognition of the antibody's Fc portion and along with GNPs amplified the signal by the FO-SPR sensor if the antibody was present. The FO-SPR assay proved to be a valuable procedure as it took less time and had larger linear dynamic range though the most signal amplification was got in SPR by the addition of GNPs. Gradual augmentation of the signal with increasing Ara h 1 concentration verified the multistep process. The dose response and signal amplification was also observed in the candy bar matrix; when GNPs were added. SPR was used to study the detection signal at each step, and when only antibody was used, the signal was unable to discriminate the Ara h 1 protein below the threshold level of 423 nM. But, GNP conjugation helped in the detection of Ara h 1 below this level and even in food sample matrix. The Lower sensitivity of the aptamer was a limitation, but this assay provided a strong advantage of the complete elimination of any manual handling (Tran et al. 2013).

[Please insert Figure 4 here]

Two DNA Aptamers, named as Aptamer 2 and 40, which showed high affinity and selectivity to flour allergen Lup an1 have also been developed by SELEX. The binding of the selected aptamers was monitored by ELISA and enzyme-linked oligonucleotide assay (ALISA). The selected aptamers were highly specific as they did not have any affinity for other conglutin proteins of lupin and other flour ingredients (Nadal et al. 2012). Lysozyme, also called as Gal d4, is an egg-white allergen that can cause allergy even in trace amounts. Capillary Electrophoresis-based SELEX (CE-SELEX) method has been used to generate ssDNA aptamers which showed high specificity and binding to lysozyme allergen. Aptamers, Apta1, Apta3, and Apta8, showed higher binding towards the lysozyme. The final aptamer showed K_d values of 52.9 ± 9.1 nM, 6.1 ± 0.5 nM and 2.8 ± 0.3 nM as measured by capillary electrophoresis, SPR and fluorescence anisotropy respectively (Tran et al. 2010). But, no

nanoparticle-conjugated aptamer based detection system has been reported for both of above-mentioned food allergens till date. However, GNP-conjugated aptamer for rapid and accurate detection of another life-threatening peanut allergen i.e. *Arachis hypogaea* 2 (Ara h2) has been developed (Liu et al. 2010). This sensor comprised of an important IgE-binding epitope having binding ability to Ara h2 which was attached to a GNPs film on a pyrolytic graphite electrode. The LOD for the sensor was 5 pg mL^{-1} that was 60 and 100 times lower than HRP-Ab2 based amperometric and label-free impedimetric immunosensors, respectively.

4.4 Detection of Other Food Safety Hazards

Some genotoxic or carcinogenic dyes such as Sulforhodamine B and Melamine are suspected to be included in the processed foodstuff in order to expand the apparent protein content. An aptamer targeting Sulforhodamine B was develop in 1998 by Wilson and Szostak research group (Wilson & Szostak 1998) that showed 660±60 nM affinity towards the dye in solution while the affinity was even lower around 190±20 nM for immobilized Sulforhodamine B. Later in the same year, the same research group published an RNA aptamer for Sulforhodamine B target, but this time with a greater binding ability in solution around 310 nM (Holeman et al. 1998). Although the aptamers were selected for general applications in biotechnology, such as DNA staining, these aptamers might also be useful for food safety monitoring purposes. On the other hand, Malachite Green (MG) is used in aquaculture to prevent antifungal diseases in fish. MG and its metabolite named as Leucomalachite green (LG) has been reported to induce toxicity extending to gene mutations in soil fungi and bacteria as well as in fish (Gopinathan et al. 2015). The first MG aptamer, called Clone MG-4, developed by Grate and Wilson in 1999 (Grate & Wilson 1999)

is recently characterised by Stead et al (Stead et al. 2010), which showed significant affinity towards the MG rather than its metabolite LG. Recently, an electro-chemiluminescence sensor platform based on the abovementioned aptamers were designed using QDs and the luminol GNPs as the signal transducers, where the detection limit was found as low as 0.03 nM. Besides, the procedure was successfully applied to the real fish samples, indicating a high potential for the food analysis (Feng et al. 2015).

Human exposure to heavy metals, which is a grave threat to both environment and living organisms, could be through direct ingestion, such as direct water intake from the heavy metal contaminated soil or consumption of the crop products that are grown in heavy metal accumulated soil. A high-affinity DNA aptamer , named as Ars-3, was selected by Kim et al (Kim et al. 2009) for the detection and elimination of arsenic from Vietnamese groundwater. Ars-3 aptamer assays was successfully applied to the field samples where the arsenic concentrations between 28.1 to 739.2 μg L^{-1} were eliminated after 5 min of exposure.

Bisphenol A (BPA) is a potential hazard to foetuses, infants, and young children which has been used in common polymer plastic products since 1930s, as a monomer. An aptamer for BPA developed by Lee and his colleagues detected BPA at very low concentrations (Lee et al. 2011). Another BPA targeting DNA aptamer, named as called #3, R11, was also developed within the same year (Jo et al. 2011) that does not recognize the Bisphenol B, showing only one methyl group difference with BPA)

5 Multiplexed biosensors

The simultaneous detection of several foodborne contaminants can save time and effort; also ensures fast and efficient monitoring of the food materials. A DNA-based electrochemical microarray platform identified four foodborne microbes (*E. coli* O157: H7, *S.*

aureus, *Salmonella spp*. and *L. monocytogenes*) simultaneously (Farabullini et al. 2007). But the dependence of such detection systems on unstable enzymes has shifted the attention towards nanoparticle-conjugated aptamers for simultaneous detection of various food contaminants. Lab-on-a-chip and Lateral flow devices are being used by aptamer-based food analysis procedures. A procedure which combines both lab-on-a-chip and multiplexed detection has been utilized by designing a Polydimethylsiloxane or PDMS/paper/glass chip that detected and *S. aureus* and *Salmonella enteric* simultaneously. Aptamers were labeled with fluorescent material and were attached to graphene oxide (GO) nanoparticles that quenched its fluorescence. When pathogen cells were present, aptamers separated from the GO surface, and the pathogen was detected by increased fluorescence. This method provides one-step and 'turn on' method for detection of several pathogens together, and this microfluidic device takes around 10 min with an LOD of 11.0 CFU mL^{-1} for completion on biochip (Zuo et al. 2013).

Recently, a multiplexed aptamer system has been reported for simultaneous detection of *S. typhimurium* and *V. parahaemolyticus.* This detection system used unique amorphous carbon nanoparticles (CNPs) as acceptor and red and green light emitting quantum dots (rQDs and gQDs) as donors; and QDs provide dual fluorescence resonance energy transfer, as illustrated in **Figure 5**. Aptamer Apt1 conjugated with gQDs was specific for *V. parahaemolyticus* and Apt2-conjugated with rQDs recognized *S. typhimurium*. Use of CNPs showed strong quenching effect on the fluorescence of both QDs. However, when a triplex complex is formed between aptamer, QDs and target, a decrease in quenching by CNPs was observed. The QDs-based fluorescence was directly proportional to the pathogens' concentration between 50 to 106 CFU·mL^{-1}. This assay detected 35 and 25 CFU mL^{-1} of *S.*

typhimurium and *V. parahaemolyticus* respectively in only 2 h 20 min in chicken and shrimp food samples (Duan et al. 2015).

[Please insert Figure 5 here]

Multicolor upconversion (or upconverting) nanoparticles (UCNPs) have also been conjugated with aptamers for simultaneous detection of food contaminants as they add more sensitivity to the luminescence process. An extremely sensitive aptamer conjugated magnetic nanoparticles (MNPs) based system for simultaneous detection of *S. aureus* and *S. typhimurium* has been reported. This system utilized UCNPs as dual color fluorescence which added a higher level of the sensitivity to the detection process. Aptamer 1 and 2 specific for *S. typhimurium* and *S. aureus,* respectively, were conjugated to the surface of MNPs. In the next step, UCNPs modified with aptamer 1 and 2 were attached to the captured bacteria to form a sandwich complex. The correlation between the signal and pathogen concentration was 0.9964 and 0.9936; and LOD was 5 and 8 CFU mL^{-1} for *S. Typhimurium* and *S. aureus,* respectively. This system also detected the pathogens in real water samples mainly due to the improved sample concentration through magnetic separation and the sensitivity by UCNPs (Duan, Wu, Zhu, et al. 2012).

In another study, three foodborne bacteria were detected simultaneously by Aptamers-UCNPs conjugates. UNCPs played the role of luminescence labels were developed by doping procedure using many rare earth ions. The aptamers were screened by SELEX targeting *S. typhimurium*, *V. parahemolyticus,* and *S. aureus* bacteria. Aptamers-UCNPs complex was used for the capture and quantification of above three target bacteria simultaneously and successfully on the basis of independent peaks for each bacterium. The quantity of bacterial detection ranged between 50−106 CFU mL^{-1} but, it was later improved to 15, 10 and 25 CFU

mL^{-1} for *S. typhimurium*, *V. parahemolyticus,* and *S. aureus,* respectively by concentration effect of magnetic separation (Wu et al. 2014).

Pathogens are not the only food contaminants which have been detected by aptamer-conjugated nanomaterials, aptamer conjugated UCNPs and GO nanoparticles have been developed which simultaneously detected two notorious food mycotoxins. This system was based on dual FRET between GO nanoparticles as acceptor and the multicolor UCNPs as donors. BaY0.78F5:Yb0.7, Tm0.02 and BaY0.78F5:Yb0.2, Er0.02 UCNPs were functionalized with aptamers specific to Fumonisin B1 (FB1) and OTA toxins. The strong upconversion fluorescence of both UCNPs was completely quenched by the GO, due to a nice overlap between the absorption spectrum of GO and fluorescence emission of UCNPs. On the other hand, when both toxins were present, the aptamer bound to their related toxin leading to changes in aptamer formation that kept aptamer bound UCNPs away from the GO. Fluorescence intensity of both UCNPs was found to be directly proportional to FB1 and OTA concentrations in maize samples which helped in direct detection and quantification of both toxins. The detection range was found to be between 0.1-500 ng mL^{-1} for FB1, and 0.05 to 100 ng·mL^{-1} for with LOD of 0.1 and 0.02 ng·mL^{-1} for FB1 and OTA, respectively (Wu et al. 2012).

6 Summary

Food-borne pathogens cause severe infections across the world; while food toxins and allergens also cause health problems in humans. The resultant health problems range from minor health issues to diseases which could lead to fatalities. Therefore, efficient and reliable food monitoring system is required for food safety and public health. Conventionally, Flow cytometry, PCR and particularly immunoassay-based detection of food

contaminants have been carried out. But, time consumption, difficult handling, multiple steps, labor need, and non-stability of proteins used in these systems have limited the scope of the food safety monitoring. In recent years, aptamers have emerged as reliable and efficient probes for environmental food monitoring as they are inexpensive, stable, easily modifiable, portable, less toxic, pure, reproducible, and can allow real-time and multiplexed detection. Successful conjugations of aptamers with various nanoparticles have combined the additional advantages of high stability, physical and geometric properties, larger surface area for them. This also results in prevention of aptamer modification and provides enhanced detection signals which make them excellent biosensors. A list of aptamers that are selected for food safety monitoring purposes is presented in Table 2, as a comprehensive summary of the literature reviewed.

[Please insert Table 2 here]

In this book chapter, we have reviewed the latest literature on aptamer-conjugated nanoparticles for rapid and accurate detection of major food pathogens (*S. aureus, P. aeruginosa, S. epidermis, S.typhimurium, S. paratyphi, E. coli*), toxins (Danofloxacin, Ochratoxin A, Fumonisin B1, staphylococcal enterotoxin B, T-2 toxin) and allergens (Arachis hypogaea 1 or Ara h 1, Ara h2, flour allergen Lup an1, and Gal d4). The nanoparticles conjugated with aptamers included gold nanoparticles, carbon nanotubes, quantum dots, graphene oxide, and UCNPs. Among these, GNP-based assays proved to be most frequent one due to its easily binding with aptamers through thiol interaction, color transform upon binding with the target as well as the signal enhancement ability. Multiplexed or simultaneous detection of the contaminants have been mostly achieved by aptamers that are conjugated with graphene oxide, quantum dots and UCNPs, which has added more power to biosensor platforms as it reduces the time and another resource requirement. In

future, these multiplexed systems based on fluorescent nanoparticles like UCNPs or QDs could lead the field as they offer the fastest detection platforms with minimal sample consumption and labor.

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Captions to Figures

Figure 1. Illustration of a standard SELEX round. A) Incubation of the target molecule with the random oligonucleotide library, B) Elution of the target bound oligonucleotides and elimination of the non-specific binders, C) Amplification of the target bound oligonucleotides in which the tightly binding oligonucleotides dominate the pool, known as enrichment, D) Following X-cycles of the SELEX (A, B, C), the enriched pool is sequenced and analyzed for the consensus sequences whose binding abilities are tested through the well-known analytic methods like Surface Plasmon Resonance (Yüce et al. 2015) Copyright© 2015, Royal Society of Chemistry

Figure 2. Flowchart of S. aureus detection using aptamer-conjugated GNPs. (a), Aptamers were conjugated onto 60-nm GNPs with thiol-DNA adaptors. (b), Aptamer-GNPs in the direct detection of S. aureus. 109 aptamer-GNPs were incubated with S. aureus cells. After removal of unbound aptamer-GNPs, bound aptamer-GNPs were eluted, and their light-scattering signals were analyzed. (c), Bead-based amplification for the detection of S. aureus. SA61-aptamers (biotin-aptamer 1) were conjugated onto 60-nm GNPs, and SA17-aptamers (aptamer 2) were conjugated to magnetic beads. Aptamer 1-GNPs and aptamer 2-magnetic beads interacted with S. aureus and the resulting complexes were isolated with a magnet. Bound biotin aptamer 1 was eluted by heating and further incubated with an excess of reporter-GNPs (conjugated with DNA adapter) and streptavidin (SA)-coated magnetic beads. The reporter-GNPs were then captured with SA-magnetic beads in the presence of biotin-aptamer 1. The bound reporter-GNPs were eluted with NaOH and their light-scattering signals were analyzed (Chang et al. 2013). Copyright© 2013, Rights Managed by Nature Publishing Group

Figure 3. Working principle of the aptamer-based strip (Wang et al. 2011). Copyright© 2010, Elsevier B.V.

Figure 4. Schematic representation of multi-step protocol for Arah1 protein detection in food matrix samples. Allergen from the food matrix is captured by aptamer on FO-SPR sensor tip followed by sandwich assay formation with polyclonal secondary antibodies which last lyare used for further enhancement of the signal (Tran et al. 2013). Copyright© 2012, Elsevier B.V

Figure 5. Schematic illustration of the dual fluorescence resonance energy transfer from QDs-apts to CNPs for the simultaneous detection of pathogenic bacteria (Duan et al. 2015). Copyright© 2014, Springer-Verlag Wien

Captions to Tables

Table 1. General differences between aptamers and antibodies

Table 2. List of aptamers selected for food safety monitoring purposes