

Aptasensors in Health, Environment and Food Safety Monitoring^{*}

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ABSTRACT

Biosensors have been developed using various types of sensing elements like biomacromolecules (viz. enzymes, antibodies, receptors, nucleic acids, etc.) organelles, tissues, intact cells of both microorganisms and higher organisms. A recent trend is the emergence of aptamers as sensing elements that has the potential to replace all the above ligands. This is possible due to the unique features of aptamers (sensitivity, specificity, reusability, stability, non-immunogenicity), which can be easily exploited in biosensor technology. Aptasensors are thus basically biosensors based on aptamers as ligand molecules. Here we review the various applications of aptasensors in health (specifically in diagnostics), food industry and environmental monitoring.

Keywords: Aptamer; Biosensor; Clinical Diagnosis; Food Safety; Environmental Pollution Control

1. Introduction

Aptamers are small oligonucleotides, peptides and peptide nucleic acids that can bind with high affinity and selectivity to diverse targets like small and macromolecules, from organic, inorganic and biological origin [1]. Aptamers can fold into distinct secondary and tertiary structures, bind to their targets with high affinity (dissociation constants on the order of nano- to picomolar) and recognize their targets with a specificity that challenges antibodies and other biological ligands. Nucleic acid aptamers are selected from a random pool by an iterative process called SELEX, who's principle is similar to that of natural selection in evolution proposed by Charles Darwin over 150 years ago. The enriched pool shows high specificity and sensitivity to their target molecules. Due to their versatility aptamers are also called as "magic bullets" and are excellent example of functional biological molecules which are selected in vitro. Since its discovery aptamer based technologies have been drawn immense attention in various research communities [2]. Apart from its high specificity and sensitivity, aptamers offer a wide range of advantages over other existing molecules viz stability, design flexibility and cost-effectiveness. These features favour their use as bio recognition elements in biosensor development. Aptasensors are biosensors where aptamers are used in place of biological

ligands to sense targeted analytes.

Oligonucleotide (both DNA, RNA), peptide and peptide-nucleic acid (PNA) based aptasensors offer high reproducibility against a wide variety of targets (e.g. proteins, peptides, drugs, small molecules, metal ions, and even whole cells) and are quickly emerging as desirable candidates for high throughput analytical methods that use minute amount of analytes (nano-microlitre). Availability of in-depth knowledge of nucleic acid aptamers in terms of their conformational and ligand binding mechanisms has evoked deep interest among researchers for developing aptamer based bioassays as reflected in the exponential increase of published articles related to aptasensors (Figure 1). Here we attempt to review the recent advancements of aptasensors based on different sensing technologies in diverse fields like, diagnostics, food safety, environmental toxicity studies etc (Figure 2).

Aptasensor based analysis is continuously evolving with various detection schemes ranging from label-free methods such as surface plasmon resonance (SPR) [3] and quartz crystal microbalance (QCM) measurements [4] to label dependant methods such as electrochemistry, fluorescence, chemiluminescence, field effect transistors [5] etc. However currently electrochemical and optical aptasensors [6] constitute the two predominant types under development. These can even distinguish between chiral molecules and are able to recognize a distinct epitope of a target molecule as well [7].

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Figure 1. Publication trend in the field of aptasensors (Data obtained from Scopus; Search strings: "Aptasensor").



Figure 2. Applications of aptasensors.

2. Aptasensors in Health Monitoring

Aptamers can be modified and reused for both diagnostic and therapeutic purposes. Aptamers have more potential than antibodies and aptasensors can be used in detection of various biomarkers, in cancer diagnosis and even detection of pathogenic microorganisms.

2.1. Aptasensors for Biomarker Detection

The detection of biomarkers presents in blood, urine and other body fluids help in the early diagnosis of any disease in general. Aptasensors have been widely used for detection of biomarkers, such as thrombin, Immunoglobulin E, C-reactive protein, IFN gamma or platelet-derived growth factor (PDGF) in body fluids.

Similar to antigen-antibody interaction, binding properties of aptamers depends on the conformational plasticity and three-dimensional folding of the nucleic acid sequence that results in a 3D-structure "aptatope", the equivalent of an "epitope".

Aptasensor assays can be exploited for detection of biological molecules with various working principles.

The sensitivity and longevity of a aptasensor was significantly increased by altering the size of nucleotide bases and modification of the capping to prevent nuclease activity. Pu *et al.* developed a smart polymeric transducer and aptamer/intercalating dye system that allows the label-free detection of thrombin with high sensitivity and specificity [8]. Further the methodology didn't require any chemical modification of the probes or the analytes. The minimum amount of thrombin that could be measured by the fluorescence intensity changes was estimated to be 0.1 nM. This offers a new strategy to detect a wide spectrum of analytes and would be highly beneficial in future clinical applications.

A method based on combination of *ex situ* polarization modulation FTIR measurements of the RNA monolayer and *in situ* Surface Plasmon Resonance Imaging (SPRI) measurements of DNA hybridization adsorption onto the surface have been developed to detect protein factor IXa .This system can detect single stranded RNA having surface density of 4.0×10^{12} molecules/cm² and a surface ligation efficiency of $85\% \pm 10\%$ [9].

Single-walled carbon nanotubes typically functionalized with gold nanoparticles increases the surface area to capture a large amount of primary aptamers and amplify the detection response. Multi-labeled Platinum-nano particles (PtNPs)-redox probes-reduced glutathione-S nanocomposites display satisfying electrochemical redox activity and high electrocatalytic activity of PtNPs and bienzyme, which exhibit high sensitivity for detection of proteins like platelet derived growth factor (PDGF) and thrombin. The linear range of PDGF was found to be 0.01e35 nM with a detection limit of 8 pM, while the linear range was 0.02 to 45 nM with a detection limit of 11 pM for thrombin are obtained [10]. Numerous aptasensors available for biomarker detection are summarized in **Table 1**.

2.2. Aptasensors in Cancer Diagnosis

Early diagnosis is critical in cancer for prevention, improvement of patient comma missing between survival and disease disease prognosis etc., which in many cases is currently restricted by non availability of sensitive and specific methods of diagnostics.

Detection and identification of cancerous cells rely on the identification of specific markers that appear in case of lymphoma and leukemias. Recognition of cancer markers with specific probes help in identifying the potential risk factor. In blood plasma proteins or free DNA molecules are treated as tumor prognosis markers. A number of recent studies have successfully used aptasensors for detecting tumour markers which are summarized in **Table 2**. Feng *et al.* reported label-free cancer cell detection with an electrochemical sensor based on the first clinical oncology trial II used aptamer AS1411

Biomarkers	Types of aptamer	Detection type	Sensitivity range	References
Thrombin	DNA-Toluidineblue-graphene (Tb-Gra) nanocomposite	Layer by layer technology	0.001 nM to 80 nM	[11]
	DNA labelled with the HCoPt-RGs conjugates	Sandwich type	$1.0 \times 10^{-12} \text{ to } 5.0 \times 10^{-8} \text{ M}/\\ 3.4 \times 10^{-13} \text{ M}$	[12]
	DNA dual labelled with AuNPs and HRP	Sandwich type	0.1 to 60 pM/30 fM	[13]
	DNA SA-ALP and biotinylated labelled	Sandwich type	1×10^{-15} to 1×10^{-8} M/ 0.33 fM	[14]
	DNA labelled with Alexa 532	DNA charge transport	5 pM to 5 nM/1.2 pM	[15]
	DNA	Label free detection	0 to 0.02 $\mu M/0.1~nM$	[8]
IgE	DNA labelled with single PPyNanowirebased microfluidics	One step electrochemical deposition method	0.1 to 100 nM/0.01 nM	[16]
	DNA attached to carboxyl (COOH)-modified nanocrystalline surface	Direct and label-free detection	0.03 to 42.8 μg/mL 0.03 mug/mL	[17]
	DNA labelled with avidin monolayer	Direct detection	2.5 to 200 μg /L 2.5 μg /L	[18]
	DNA streptavidin labelled	ELISA-like array		[19]
Retinol Binding Protein 4 (RBP4)	single stranded DNA	Label free detection	0.2 to 0.5 μg mL - 1/75 nM	[20]
C reactive protein (CRP)	Biotinylated RNA	Direct detection	0.005 ppm	[21]
	DNA	Sandwich type	10 microg/L to 100 mg/L	[22]
N-terminal pro-brain natriuretic peptide	DNA	Sandwich type	0.01 to 500 ng /mL, 0.77 pg/ mL	[23]
IFN gamma	DNA thiolated/MB redox tag	Direct detection	10 nM to 0.06 nM	[24]
VEGF	RNA, DNA, fluorescentlylabeled	optical array, Fluorescencepolarization anisotropy	10 - 100 nM	[25]

Table 1. Example of aptasensors in various biomarker detection.

Table 2. Example of aptasensors used in cancer detection.

Types of Cancer Marker/ Cancer cells	Types of Aptamer	Detection type	Sensitivity Range	References
HeLa cells, K562 cells, MDA-231 cells	DNA	Label free detection	10 - 100 nM	[26]
Ramos cancer cell, CEM cells	DNA	ECL array with a novel cycle-amplifyingtechnique	5 - 50 nM.	[29]
Ramos cancer cell	DNA	Label free detection	100 to 1000 cells $mL^{-1}/$ 58 cells mL^{-1}	[30]
PDGF-BB	DNA labeled with biotin	A sandwich conjugate modified electrode	$1.0 \times 10^{-13} \text{ to } 1.0 \times 10^{-11} \text{ M}/ \\ 2.7 \times 10^{-14} \text{ M}$	[27]
PSA	DNA labeled with FITC	Aptamer blotting assay	40 to 100 nM	[31]
MUC1	DNA labeled with single PPy nanowire-based microfluidic	One step electrochemical deposition method	2.66 nM	[32]
Glutathione	RNA	SPR analysis	1.2 μM	[33]
Leukemia cells	DNA conjugated aptamer molecular beacons	a magnet-quartz crystal microbalance system	1×10^4 to 1.5×10^5 cells mL^{-1} 8 $\times 10^3$ cells mL^{-1}	[34]
VEGF	RNA conjugated CNTs	FET-type biosensor based on CNTs-aptamer	400 fM	[35]
MUC2	DNA labeled with QD	Aptamer-based detection With quantum-dot based fluorescence readout	250 nM	[36]
Multi-marker or Ramos cells, CCRF-CEM cells, Toledo Cells	DNA-conjugated FRET NP	Simultaneous multiplexed analysis	Not specified	[37]

and graphene-modified electrode [26]. An aptamerperylenetetracarboxylic acid (PTCA) nanocomposite was utilized as nanoscale anchorage ligand to effectively capture cells on electrode surface through the specific binding between cell surface nucleolin and the aptamer AS1411. The aptasensor could distinguish cancer cells from normal cells and detect as low as 1000 cells/ml and can be regenerated and reused with ease.

Platelet-derived growth factor B chain (PDGF-BB) is a potential cancer biomarker and is known to be related to tumor growth and transformation. Recently, Chai *et al.* developed a electrochemiluminiscence (ECL) based aptasensor for PDGF-BB by assembling N-(ami- nobu-tyl)-N-ethylisoluminol functionalized gold nanoparticles (ABEI-AuNPs) with aptamers as nanoprobes which showed high sensitivity and specificity [27]. The biotinylated aptamer capture probes were first immobilized on a streptavidin coated gold nanoparticle electrode. The detection limit limit of the sensor was as low as 2.7×10^{-14} M.

In a recent study, Zhao *et al.* reported an electrochemical aptasensor for simultaneous determination of two tumor markers MUC1 and VEGF [28]. Since the cDNA immobilized on an electrode surface can hybridize with both MUC1 aptamer and VEGF165 aptamer to form a long double strand with ferrocene, the probe cannot give electrochemical signal. Nevertheless, the presence of the two markers inhibit the hybridization of cDNA with the aptamers, thus the distance between ferrocene and the electrode is changed, and a "signal-on" electrochemical method to detect two tumor markers is fabricated. It was proven experimentally that electrochemical aptasensors not only detect the two markers but also can discriminate their co-existence.

3. Aptasensors for Food Safety and Environmental Pollution Control

Food maybe contaminated by chemical compounds, toxin or pathogen leading to various food borne ailments. According to World Health Organization (WHO) food borne and waterborne diarrhoeal diseases kill about 2.2 million people annually out of which 1.9 million are children [38]. This growing public health problem demands the development of highly sensitive technologies for rapid detection of food contaminants. In this context, biosensors have a potential to emerge as a major tool in maintaining food safety. Traditional techniques are largely based on antibody-conjugated biosensors. However, many food contaminants are not conducive for antibody generation. Also antibodies suffer from batch to batch variation and short shelf lives necessitating the development of a simpler, consistent and cost effective approach for food safety testing.

Aptamers can address many of the challenges of a traditional biosensor without compromising on specificity and affinity. Based on the type of target, aptamers used in food safety testing and environmental pollution control can be broadly classified under two groups-aptamers against small molecule contaminants and those against pathogens.

3.1. Aptasensors against Small Molecules

Small molecule contaminants include antibiotics, toxins, pesticides and heavy metals that may be present in a variety of food products and environmental samples. For example, theophylline is a commonly used bronchodilator for asthma patients. However, its overdose leads to severe toxicity like, seizure, nausea etc. RNA based electrochemical aptasensors have been developed to monitor its level in serum [39]. Also aptasensors for detection of drugs like cocaine has been developed based on surface enhanced raman scattering spectroscopy (SERS) [40].

3.1.1. Aptasensors against Antibiotics

Antibiotics are often administered to farm animals along with their feed for prophylactic and therapeutic purposes. However, a large percentage of these antibiotics remain unmetabolized and accumulate in the tissues or are excreted out in the environment. The occurrence of antibiotics in the environment might lead to antibiotic resistance which may be transmitted to humans via the food chain [41].

3.1.1.1. Chloramphenicol (Cam)

Chloramphenicol is a popular bacteriostatic antimicrobial drug effective against a wide variety of gram positive and gram negative bacteria. It inhibits peptide bond formation by binding to peptidyl transferase loop of 23S ribosomal RNA. However it has lost its favour due to resistance and serious side effects like aplastic anemia. Burke et al. initially reported the use of RNA aptamers against chloramphenicol. These aptamers screened in vitro through SELEX resembled the Cam binding site in 23SrRNA. Various studies indicated that the aptamers structurally contained two symmetrically arranged A-rich bulges [42]. However, RNA aptamers are susceptible to nuclease attack and requires transcription and reverse transcription making it difficult to screen via SELEX. Recently Mehta et al., developed DNA aptamers to detect Cam having higher selectivity and affinity ($K_d \sim 1 \ \mu M$). Interestingly the two best aptamers screened by them had G-rich nucleotide regions distinguishing them from the rest [41]. These screened aptamers have now been used to develop label free electrochemical biosensors for detection of chloramphenicol. The aptamers were immobilized onto a gold electrode by self assembly approach. The developed

electrochemical aptasensor was highly sensitive (detection limit of 1 nM) and specific to chloramphenicol over its other derivatives [43].

3.1.1.2. Tetracycline

Tetracyclines are a group of broad spectrum antibiotics which inhibits prokaryotic translation by interfering with the binding of amino-acyl tRNA to the ribosomal A-site [44]. Tetracyclines are often used as a veterinary drug to promote growth in animals. Thus residues of the antibiotic has been detected in meat [45], milk [46], honey [47], eggs [48] etc. On the other hand, tetracyclins, it has also been reported to be hepatotoxic to pregnant women [49]. Traditional detection methods for tetracycline like HPLC and capillary electrophoresis lack specificity and are often time consuming and expensive. Recently, aptamer based systems have been developed for detection of tetracyclines. Berens et al. first screened a RNA aptamer (cb28) against tetracycline which resembled its interaction with the small ribosomal subunit [50]. Later Xiao et al., improved upon the structure of the developed aptamer to obtain a very high affinity ($K_d \sim 0.8$ nM) [51]. A ssDNA electrochemical aptasensor was recently developed by Kim et al having high specifity for tetracycline over its other derivatives [52]. Zhang et al. reported another rapid electrochemical aptasensor for tetracycline using aptamers immobilized over glassy carbon electrodes. The aptasensor was able to rapidly detect tetracycline in milk with very high sensitivity (~1 ng/ml) [53]. A competitive enzyme linked aptamers assay (ELIAA) based aptasensor for tetracycline was developed by Jheong et al. using both DNA and RNA aptamers [54].

3.1.1.3. Aminoglycoside

Aminoglycosides are a class of antibiotics that bind with prokaryotic ribosomes leading to frameshift mutations which produce nonsense peptides eventually causing cell death [55]. Its clinical use has been restricted due to toxic side effects to the kidney and ear [56]. Aminoglycosides include antibiotics like tobramycin, kanamycin, neomycin etc, all having a common streptamine ring. Initially RNA aptamers were developed against tobramycin by Wang and Rando [57]. They later went on to develop RNA aptamers against all aminoglycosides based on the consensus sequence. [58]. RNA aptamers have often been reported to be unstable due to their vulnerability to endonucleases. In the quest to find the right balance between affinity and stability, Rowe et al., screened RNA aptamers to detect aminoglycoside antibiotics in human serum. Serum was separated from nucleases by ultrafiltration through a 3000 Da cut off spin column, so that the aptamers remain stable. This enabled them to develop a reagentless RNA based electrochemical aptasensor which could detect low levels of aminoglycoside antibiotics (2 - 6μ M) in human blood samples [59]. Recently, a ssDNA aptamer against kanamycin has also been screened by Song *et al.* The aptamers conjugated with gold nanoparticles showed dissociation constants in the nanomolar range and were able to detect kanamycin by colorimetric method [60]. Derbyshire *et al.*, has been successful in screening RNA aptamers against the entire class of aminoglycosides. The aptamers were then coated with gold nanoparticle for a rapid and sensitive colorimetric assay based detection of antibiotics [61].

3.1.2. Aptasensors against Toxins

Mycotoxins are the major toxins that may be present in our food. They are a group of naturally occurring chemicals produced by moulds growing on a variety of different crops. Mycotoxins can cause adverse effects on humans from liver cancer, gastrointestinal diseases to kidney damage and immune suppression. A DNA aptamer was developed to detect ochratoxin A-a mycotoxin in food with high specificity [62]. This aptamer has been integrated into several biosensor detection systems including electrochemical, electrochemiluminescent, colorimetric and fluorescent platforms. Bacterial endotoxins (Lipopolysaccharides) are also one of the major contaminants present in commercially available proteins and pharmaceutical products. These can cause severe septic shock in humans and animals [63]. An electrochemical gold nanoparticle based aptasensor for detection of endotoxins from crude biological liquor was developed by Kim et al. [64]. The aptasensor showed excellent sensitivity and selectivity with a detection range of 0.01 to 1 ng/mL.

Seeds of the leguminous herb lupin have been widely used as a low cost protein source. However, there have been an increasing number of cases reporting severe allergic reactions to Lupin seeds. To meet this challenge and detect Lupin allergen levels in food a DNA aptamer based colorimetric sensing system was developed by Nadal et al. [65]. Many toxins are excreted by humans and animals which ultimately end up in water effluents. Endocrine disrupting compounds (EDC) form a major class of pollutants which causes severe health hazard by disrupting normal endocrine functions among human and aquatic organisms. 17β -estradiol is one such compound which has deleterious effects on the male reproductive system [66]. Recently, Yildrim et al. reported a highly selective and rapid fluorescence based DNA aptasensor for detection of low levels of 17β -estradiol in environmental water samples [67].

3.1.3. Aptasensors against Food Packaging Contaminants

Bisphenol A is used as a monomer compound in plastic polycarbonate products. However, it is potentially dangerous to humans and animals as it disrupts endocrine function by blocking binding of estrogen with its receptor. Using high affinity ssDNA aptamers Jo *et al.* developed a sol-gel biochip assay to detect Bisphenol A and measure its level in water samples [68].

3.1.4. Aptasensors against Heavy Metals

Meat, milk, egg, fish and other foodstuffs are often found contaminated by heavy metals like Mercury (Hg²⁺), Arsenic (As) etc. Hg²⁺ causes severe toxicity to the nervous and endocrine system whereas Arsenic (As) leads to serious ailments like heart problems, skin lesions and cancer. High arsenic contamination levels were reported in Mekong river delta in Vietnam. According to government sources the residual arsenic levels in water had exceeded the allowable limit by 10 µg/L leading to severe arsenic toxicity among the inhabitants. To address this crisis, Kim *et al.* screened DNA based aptamers showing highest affinity for Arsenate (V) and Arsenite (III) [69] which may be used to develop Arsenic based aptasensors in future.

Mercury (Hg^{2+}) ions were successfully detected in water using gold nanoparticles based colorimetric aptasensors. The aptamer was rich in thymine regions which formed dimers in presence of Hg^{2+} ions. The gold nanoparticles were able to differentiate between dimers and short single stranded DNA and functioned as a colorimetric probe. The system had high sensitivity of around 1 nM [70]. Recently, Helwa *et al.*, in their study, immobilized DNA aptamers on polyacrylamide gel. The setup provided rapid visual detection of Hg^{2+} and high sensitivity of around 10 nM [71].

Till date, a number of aptamer based sensors have been developed to detect very low levels of heavy metals in our environment. But not much research has been done on how to reduce the toxicity caused by these heavy metals. In an interesting work reported by Hu *et al.*, aptamer-nanoparticle conjugate was used to reduce Hg^{2+} toxicity. The authors used selenomethionine (SeMet), having a higher binding affinity to mercury, as an antidote and conjugated it with PLGA naoparticle-aptamer complex. Rats fed with mercury contaminated food were treated with the conjugate and mercury accumulation was found to decrease appreciably in the brain and kidney [72].

3.1.5. Aptasensors against Pesticides

Atrazine is one of the most widely used pesticides to inhibit the growth of weeds. However, it can lead to severe reproductive damage in humans. Sinha *et al.* developed a novel strategy to engineer bacterial cells for detection and removal of atrazine from environmental samples. They first screened a series of aptamers against atrazine and then cloned them into *E. coli* cells. This construct then

functioned as atrazine dependent riboswitches. [73].

Insecticides are also often applied to crops to protect them from insect attack. One such example is Acetamiprid, which is a neonicotinoid insecticide. However when leached into the environment it can cause toxicity in humans and animals. He *et al.* screened a series of aptamers via SELEX which bound to acetamiprid with a dissociation constant (K_d) of 4.98 μ M [74]. Recently, Wang *et al.*, successfully developed DNA aptamers which were able to detect upto four highly poisonous organo-phosphorous pesticides including phorate, profenofos, isocarbophos and omethoateas [75]. Although aptamers have been screened against various types of pesticides, its potential as a ligand molecule in a biosensor is largely unexplored.

3.2. Aptasensors against Pathogens

Biosensors capable of rapidly detecting pathogens with high sensitivity and specificity are essential to meet the ever increasing serious clinical and therapeutic challenges. Most importantly, aptasensors are able to target and specifically differentiate pathogens without prior information of their membrane molecules and structural genes. Various attempts to confront this challenge with aptasensors against pathogenic virus and bacteria are discussed herein.

3.2.1. Virus

The development of aptasensors against virus is in its infancy. Currently promising leads are available regarding the screening of aptamers against specific viruses which has the potential to be integrated with suitable biosensor platforms. Tang et al. reported the development of DNA aptamers against a specific protein of vaccinia virus. They infected a mammalian cell line (A549) with the virus and used the aptamers as probes for detection of the viral protein [76]. Those aptamers can be developed in aptasensors by modifying for immobilization purposes and labelled with reporter molecules, fluorophores without hampering their target specificity. Several aptasensors have been developed to detect viral proteins. Minunni et al. fabricated an aptasensor to detect HIV-1 Tat protein by immobilizing an RNA aptamer on a piezoelectric quartz crystal. Sensitivity, specificity, and reproducibility parameters were quantified. The aptasensor was also compared with the available immunosensor with immobilized anti-Tat antibodyies. Both the optimized aptasensor and the immunosensor showed a detection limit of 0.25 ppm [77]. The quartz crystal microbalance (QCM)-based aptasensor has also been compared with the corresponding surface plasmon resonance (SPR)-based aptasensor. The two aptasensors were developed by biotin-avidin linking onto the gold surface of the transducers (quartz crystals

or chips) for the immobilization. Both platforms showed similar reproducibility, sensitivity and specificity. The linear range of SPR (1 - 2.5 ppm) was higher that of QCM (0 - 1.25 ppm) [78]. Another viral aptasensor developed against the heptatitis C virus (HCV) core antigen detector. Lee et al. experimented with the binding affinity of several aptamer sequences against the target candidate. After selection, the specific aptamer was immobilized in a 96-well plate, using the sol-gel-based immobilization method. Then, the immobilized aptamers on the chip were incubated with recombinant core antigens. The aptamer-core complexes were incubated with Cy3-labeled secondary antibodies. The platform was able to detect core-specific interaction with the aptamers, using pure recombinant protein as well as human sera matrixes [79].

3.2.2. Bacteria

Detection of bacteria using aptasensors is a relatively new area. Two different strategy for whole-cell detection, using quantum dots (ODs) and carbon nanotubes (CNTs). have been proposed recently. Aptamer-functionalized ODs have been exploited to detect Bacillus thuringiensis spores [80]. In the study, zinc sulfide-capped cadmium selenide QDs were functionalized with a specific aptamer selected to detect the pathogen. After OD-aptamer incubation with the target, the spores were washed and collected for fluorescence measurement. Several controls with non-functionalized ODs and without spores were tested to measure the non-specific attachment of the ODs to the spores and the fluorescence background noise. The reported sensitivity was 10³ CFU/ml. For specificity purposes, spores from B. globigii (B. subtilisvar. niger) were also tested. The system could differentiate B. thuringiensis from B. globigiiat concentrations above 10^5 CFU/ml. In another study, using aptamer-functionalized singlewalled carbon nanotube field-effect transistor (SWNT-FET) arrays aptasensors was developed to detect E. coli DH5 α [81]. The binding between *E. coli* cells and the aptamer-functionalized FET resulted a difference in conductance (>50%) in culture samples with concentrations between 10^5 and 10^7 CFU/ml. Specificity assays were conducted with Salmonella typhimurium.

Recently, bioterrorism has become a major threat to national security. One of the leading examples is Anthrax caused by the spores of gram positive bacteria *Bacillus anthracis*. Cella *et al.* has developed an aptasensor for detection of protective antigen (PA) of anthrax. The aptasensor consisted of single stranded DNA aptamer functionalized to single walled carbon nanotubes having sensitivity in the nanomolar range (~ 1 nM) [82].

4. Conclusion

Aptamers can easily be integrated to the many existing

biosensor schemes. The plethora of transduction platforms for affinity sensing can readily be adapted to the use of aptameric bio components. Additionally, catalytic aptamers (aptazymes) have also been reported, to offer modes of specific binding event detection. Moreover, it can be anticipated that the use of immobilisable molecular beacons can be exploited in the biosensor field (till date a handful of publications exist); a mode of detection highly suited to aptasensors facilitating reagentless, cost effective one step analysis. Overall, the potential of aptasensors is immense and this exciting and challenging area is on the brink of exponential growth. The ability to develop affinity based detection systems based on tailor made characteristics, like size, toxicity and matrix effects, offers the field of biosensing the opportunity to explore hitherto unexplored horizons in sensor development.

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