Il Young Jung, Eun Hee Lee, Ah Young Suh, Seung Jin Lee, Hyukjin Lee*
College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Republic of Korea

*Corresponding author; E-mail: hyukjin@ewha.ac.kr

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Il Young Jung, Eun Hee Lee, and Ah Young Suh contributed equally to this work.

Abstract

Oligonucleotide-based biosensors have drawn much attention due to because of their broad applications in in vitro diagnostics and environmental hazard detection. They are particularly of interest to many researchers because of their high specificity as well as excellent sensitivity. Recently, oligonucleotide-based biosensors have been used to achieve not only genetic detection of targets, but are also able to handle the detection of small molecules, peptides, and proteins. This has further broadened the applications of these sensors in the medical and health care industry. In this review, we have highlighted various examples of oligonucleotide-based biosensors for the detection of diseases, drugs, and environmentally hazardous chemicals. Each example is provided with detailed schematics of the detection mechanism in addition to the supporting experimental results. Furthermore, future perspectives and new challenges in oligonucleotide-based biosensors are discussed.
Keywords

Abbreviations

Aβ  Amyloid β

ACNG  Aptamer-coated nanogold

AD  Alzheimer’s disease

ADDL  Amyloid β derived diffusible ligand

AuNP  Gold nanoparticle

Au@PtNP  Nanoparticle consisting of a gold core and a platinum shell

BPA  Bisphenol A

cDNA  Complementary DNA

CEA  Carcinoembryonic antigen

CSF  Cerebrospinal fluid

EDC  Endocrine-disrupting compound

FRET  Fluorescence resonance energy transfer

G4  G-quadruplex
HBV  Hepatitis B virus

HRCA  Hyperbranched rolling circle amplification

HRP  Horseradish peroxidase

LSPR  Localized surface plasmon resonance

MB-AuNPs  Gold nanoparticles with magnetic microbeads inside

mHCR  Multibranched hybridization chain reaction

MWCNT  multiwalled carbon nanotube

NPG  Nanoporous gold

OTA  Ochratoxin A

PCR  Polymerase chain reaction

SELEX  Systemic evolution of ligands by exponential enrichment

ssDNA  Single-stranded DNA

MB-AuNPs  Gold nanoparticles with magnetic microbeads inside

MWCNT  multiwalled carbon nanotube

PDGF  Platelet-derived growth factor

PCR  Polymerase chain reaction
PrP^c  Cellular prion protein

QCM  Quartz crystal microbalance

QD  Quantum dot

EpCAM  epithelial cell adhesion molecule

CTC  circulating tumour cells

mHCR  multibranched hybridization chain reaction

HRP  horseradish peroxidase

CEA  carcinoembryonic antigen

RCA  rolling circle amplification

RT-PCR  Reverse transcription polymerase chain reaction

SELEX  Systemic evolution of ligands by exponential enrichment

SERRS  Surface-enhanced resonance Raman scattering

ssDNA  Single-stranded DNA

HRCA  hyperbranched rolling circle amplification
\( \text{PrP}^c \) - Cellular prion protein

\( \text{Ochratoxin A} \)

\( \text{PrPSc} \) - Scrapie prion protein

\( \text{TAMRA} \) - Tetramethylrhodamine fluorophore

\( \text{THMS} \) - Triple-helix molecular switch

\( \text{cp-DNA} \) - Complementary DNA

\( \text{ADDLs} \) - Amyloid \( \beta \) - derived diffusible ligands

\( \text{CSF} \) - Cerebrospinal fluid

\( \text{MMPs} \) - Magnetic microparticles

\( \text{HBV} \) - Hepatitis B virus

\( \text{NASBA} \) - Nucleic acid sequence-based amplification

\( \text{SDA} \) - Sequence displacement amplification

\( \text{RCA, LAMP} \) - Loop-based isothermal amplification

\( \text{QCM} \) - Quartz crystal microbalance

\( \text{PEC} \) - Photoelectric current

\( \text{AFOB} \) - All-fiber optofluidic biosensing
Introduction

Biosensors are quantitative analytical tools which contain biological materials and physicochemical signal transducers. Biological materials such as DNA/RNA pathogens, proteins, and chemicals can specifically recognize and relate the concentration of the target analyte to a measurable biological response. Signal transducers then convert the response to various reporting signals. If the analyte binds to the receptor, changes in pH, heat, light, mass, and so forth can occur. This change is translated to a reporting signal, and various recognition elements can be used in biosensors such as small chemicals, enzymes, proteins, antibodies, DNA, organelles, microbial cells, plants, and animal tissues.

With increasing demand for biosensors in drug development and medical diagnosis, various biosensors have been developed for particular applications. The value of the global biosensor market was USD 12.46 billion in USD (2013) and is expected to grow by 8.1% per year from 2014 to 2020. Examples of commonly used biosensors in real life...
include the pregnancy test, which detects the human chorionic gonadotropin hCG protein in urine, glucose monitoring devices for diabetes-patients with diabetes, and the tuberculosis (TB)-detection test for identification of the infectious disease in a few minutes. Besides the seabove, applications of biosensors are not limited to the health-care industry and but are also expand-to applicable to food analysis, crime detection, and environmental field monitoring.

Among various biosensors, oligonucleotide-based biosensors are of much interest to many researchers because since oligonucleotide-based biosensors they can serve the dual role of detection and amplification of target analytes by simple base-pair hybridization and amplification by polymerization. Aptamer is another example of short oligonucleotide for the high affinity binding to the target analytes not by the simple hybridization but more specific to the proteins and chemicals. In contrast to traditional methods such as antibody-based biosensors, oligonucleotide-based biosensors use the base pairings of oligonucleotides. Hybridization between nucleic acids is stable and highly specific. Also oligonucleotide-based biosensors use an aptamer, which is a short oligonucleotide showing strong binding affinity toward target molecules. An Aptamer is useful tool to detect proteins, heavy metals, and other chemicals that do not have a sequence-specific hybridization property. However, an aptamer shows a quite different binding mechanism as compared with the sequence specific hybridization of DNA or RNA. The binding mechanism between aptamers and target molecules is variable according to the systems. The common forces between them are electrostatic and hydrophobic interactions. Many of aptamers selectively bind to the target molecules by their designed structures in addition to their sequences. Therefore, oligonucleotide-based biosensors show good flexibility of oligonucleotide design in upon various applications. Due to Because of their high specificity, stability, and variability for the base-pairing hybridization for detection and diagnosis, oligonucleotide-based biosensors are widely utilized in broad applications of clinical diagnosis and genome mutation detection. However, there are possible drawbacks of oligonucleotide-based biosensors. First, it is necessary to control the temperature during detection. This requires equipment like such as a thermal cycler. Second, it needs a fluorescent dye system is needed, requiring additional steps and equipment to detect, which can detect fluorescence. For detection of target analytes, aptamers should be
synthesized to detect specific target molecules. With systemic evolution of ligands by exponential enrichment (SELEX) technology, aptamers showing high binding affinity toward the target can be isolated. In SELEX, three repeats 3-cycles are constantly repeated: adsorption, recovery, and reamplification [4]. With the unique design of detection systems with various aptamers, the applicability of oligonucleotide-based biosensors has been further broadened. In this review, oligonucleotide-based biosensors are categorized according to their applications—that is, clinical diagnostics (inherited diseases, pathogenic infectious diseases), drug analysis, and environmental monitoring. Among numerous biosensors, we have focused on introducing oligonucleotide-based biosensors with extremely sensitive, rapid detection, and relatively low cost of production.

2. Content

2.1. Oligonucleotide-based biosensors for external diagnostic application

2.1.1. Biosensors for the detection of human diseases

An in vitro diagnostic (IVD) is a tool for detecting disease through analysis of a body fluid such as blood, saliva, sputum, or tissue. In everyday life, in vitro diagnostics IVD have been used for pregnancy tests or glucose-level tests. In addition, in vitro diagnostics IVD can be used for detecting various infectious diseases such as Ebola and Middle East respiratory syndrome MERS and can be a monitoring tool for checking blood cholesterol level. The in vitro diagnostics IVD market is growing by about 8% per year. The U.S. in vitro diagnostics IVD market is largest in the world, occupying accounting for almost 50%. However, peculiarly, the Asian market is growing at a rate that is fastest due to its rapid developing rate [5].

Cancer

Circulating tumour cells (CTCs) have recently gained attention as predictive and prognostic
biomarkers [6]. Highly sensitive detection of circulating tumor cell CTCs in the blood can allow early diagnosis of cancer and cancer metastasis [7-11]. However, it can be a challenging task to detect circulating tumor cell CTCs when their concentration in peripheral blood is very low, thus the development of highly sensitive and specific detection methods is necessary [7-11]. Although some polymerase chain reaction (PCR)-based methods have already been applied to detect cancer cells, they are complicated, costly, and time-consuming and cannot be used to directly detect whole cancer cells because of a cell lysis step [7-10, 12-23].

Xiaolei Zuo et al [24] designed DNA nanostructured biosensors based on multibranched hybridization chain reaction (mHCR) for multivalent capture and detection of cancer cells (Fig. 1a) [24]. In their study, epithelial cell adhesion molecule (EpCAM), which is expressed significantly on the surface of epithelial cancer cells, was used as a diagnostic biomarker for cancers. An initiator partially hybridized to the aptamer of epithelial cell adhesion molecule EpCAM was added to the mixture of two sets of DNA hairpins (H1 and H2). Then, a long chain with multiple biotins and branched arms was generated by mHCR reaction. When cancer cells are present, they conjugate with mHCR products. DNA tetrahedral probes (TSP) immobilized on gold electrode surfaces can be used to capture cancer cells. Multivalent binding of cancer cells to the surface can be realized through multiple branched arms, which enhances the capturing efficiency. In addition, multiple avidin-horseradish peroxidase (HRP) conjugate HRP attached to the biotin labels amplify the electrochemical signal, which increases the detection sensitivity. Electrochemical methods, which use the electrocatalytic properties of HRP for H2O2 reduction, were used for the rapid and sensitive detection of cancer cells captured on the surface of gold electrodes [24-29]. Electrocatalytic current was increased proportionally to the number of cancer cells. The electrochemical current with 1000 cancer cells was 450 nA in the method without amplification (Fig 1b, A), but significantly increased to 8600 nA in the method with mHCR amplification (Fig. 1b, B) [24]. Furthermore, the detection limit of the method with HCR amplification was improved with as few as four cancer cells, whereas the conventional method required 24 or more cancer cells (Fig. 1b, A, B). These results suggest that the detection sensitivity is enhanced by the amplification of multiple HRPs attached to the mHCR products. In addition, almost no differences were observed between
the number of cancer cells captured on the surface of the gold electrodes before and after the washing steps, suggesting there was improved capturing capacity and efficiency due to multivalent binding. Therefore, the synergetic effect of multivalent binding and signal amplification by the mHCR reaction provides highly sensitive and specific detection of cancer cells.

The carcinoembryonic antigen (CEA) is another well-known tumor marker associated with many cancers (e.g., liver, colon, breast, and colorectal cancers) [30-33]. An aptamer with high binding affinity toward CEA was used for biosensing. Recently, a wide variety of biosensors for the detection of CEA have been developed with signal amplification techniques such as polymerase chain reaction (PCR) and rolling circle amplification (RCA) [34]. Although RCA has the advantages of simplicity and efficiency, the sensitivity of RCA-based biosensors is generally lower than that of PCR [35].

Liang and coworkers [34] developed ultrasensitive hyperbranched rolling-circle amplification (HRCA)-based colorimetric biosensors for CEA as a way to overcome this limitation. In the HRCA reaction, specific DNA fragments can be amplified exponentially through replication of circularized probes under isothermal conditions. In the presence of the target protein (CEA), the CEA aptamer binds more specifically to CEA than to complementary DNA (cDNA), forming the CEA-aptamer complex [34]. Therefore, free cDNA is allowed to hybridize with the DNA template (padlock probe), which is subsequently ligated and circularized by Escherichia coli DNA ligase. After formation of circularized padlock probes, multiple single-stranded DNA (ssDNA) strands were produced by HRCA reaction. ssDNA-These strands are consequently adsorbed onto gold nanoparticles (AuNPs), and induce electrostatic repulsion between ssDNA-adsorbed AuNPs. Thus, when sodium citrate salt is added, the salt-induced AuNP aggregation can be prevented, and the solution remains the red color unchanged. However, in the absence of the target protein (CEA), the CEA aptamer hybridizes with the short DNA primer (cDNA). Thus, there is no free cDNA to hybridize with the DNA template (padlock probe), and nothing can trigger the HRCA reaction. As a result, the amount of ss-DNA strands is not enough to prevent salt-induced AuNP aggregation. When salt is added, the AuNPs are caused to aggregate, which leads to the color change of the solution. The absorption intensity ratio $A_{520}/A_{660}$ is
used for evaluation of the difference between the aggregation of AuNPs [36]. In the presence of CEA, the color of the solution is red and the absorption peak is observed around wavelength of 520 nm. In the absence of CEA, however, there is a significant peak shift from 520 nm to 660 nm, accompanied by a chromatic transition from red to blue. In addition, a linear relationship between the ratio ($A_{520}/A_{660}$) and the logarithmic concentration of CEA was observed, which supports the proposed mechanism.

**Neurodegenerative diseases**

Prion proteins cause neurodegenerative diseases that affect humans or animals by disrupting the structure of the brain [39]. The mechanism of Prion propagation is associated with the conversion of normal prion proteins (cellular prion protein, PrP$^c$) into pathogenic isoforms of prion proteins (scrapie-type prion protein, PrP$^{sc}$) [40]. However, scrapie-type prion protein PrP$^{sc}$, the hallmark of prion diseases, can only be observed at the late stage, which makes early diagnosis of the diseases difficult [41].

Over the last few decades, several biosensors have been developed that use the capacity of PrP$^c$ to bind to nucleic acids (i.e., DNA and RNA) [42-46]. Xiao et al. [36] developed an aptamer-based fluorescence biosensor for the detection of PrP$^c$. Fig. 2a explains the strategy for this contribution involved is shown in Fig. 2a. There are three main components of the aptamer structure: (1) the core sequence located in the loop, (2) three guanine bases at the 3' terminal, and (3) tetramethylrhodamine fluorophore (TAMRA) modification at the 5' terminal. In the absence of target PrP$^c$, the guanine bases at the 3' terminal are close enough to TAMRA, and quenched the fluorescence of TAMRA via electron transfer. In the presence of the target PrP$^c$, however, the aptamer specifically interacts with PrP$^c$ and pushes TAMRA away from guanine, recovering the quenched fluorescence.

The quenching efficiency was measured using the fluorescence emission intensity of TAMRA at 580 nm (Fig. 2b) [36]. The quenching efficiency was as high as 76.6 %, indicating that the proposed strategy successfully showed good applicability for the detection of PrP$^c$. Circular dichroism (CD) can be used to estimate the conformational
change of the TAMRA-containing aptamer because the spectrum depends on the conformation. In the absence of complementary-DNA, the TAMRA-containing aptamer shows differences in the circular dichroism CD spectrum with from that of its single-stranded complementary-DNA (cp-DNA) as shown in Fig. 2c [36]. In addition, when the denatured TAMRA-containing aptamer was incubated with various concentrations of PrP\(^c\), the recovered fluorescence intensity exhibited a linear relationship in a range from 1.1 to 44.7g/mL with high correlation (\(R^2 = 0.991\)) and a low detection limit (0.3g/mL).

Alzheimer’s disease (AD) is a degenerative disorder of the brain that accounts for the majority of the cases of dementia characterized by progressive cognitive impairment among people over older than the age of 65 years [47]. The neuropathological process of AD is associated with the formation of amyloid-\(\beta\) (A\(\beta\)) peptide, also known as senile plaque, and neurofibrillary tangles of hyperphosphorylated tau (\(\tau\)) protein have been used as biomarkers for early diagnosis [48]. There are two main strategies for the detection and quantification of these soluble AD biomarkers. The first strategy is the measurement of the total concentration of A\(\beta\) or tau \(\tau\)-protein in cerebrospinal fluid (CSF) or plasma [49,50]. However, this strategy showed unreliable results in terms of no significant differences in the levels of such markers between healthy individuals and AD patients/subjects [51]. The other strategy provided a more feasible solution, because since it targets established pathogenic biomarkers such as cleaved \(\tau\)-protein, hyperphosphorylated \(\tau\)-protein, and A\(\beta\)-derived diffusible ligands (ADDLs) [52-54]. However, in the early stage of the disease, the concentration of such markers in the cerebrospinal fluid (CSF) is extremely low, which makes it very hard to identify them accurately with conventional methods such as ELISA or blotting assays.

To solve this problem, Georganopoulou et al. [52] developed a new technique for highly sensitive detection of soluble AD biomarkers in CSF [53-54]. In the method described in their work, ADDLs were recognized by magnetic microparticles (MMPs) with monoclonal anti-ADDL antibody attached Ab, and then sandwiched with AuNPs functionalized with DNA barcode and polyclonal anti-ADDL antibody Ab. The sandwich complexes were purified by magnetic separation (Fig. 3a). After repeated washing and dehybridization steps, a large number of barcode-DNA strands were released,
followed by the isolation and quantification through a scanometric method. (Fig. 3a, b). By use of the silver amplification technique, ADDLs of sub-femtomolar concentration were identified, and the detection sensitivity of the system was significantly enhanced by six orders of magnitude over the conventional ELISA method.

A calibration curve was obtained before analyzing the ADDL concentrations were analyzed in the subject samples (Fig. 3c) [52-54]. The assay exhibited a broad analytical concentration range from the order of $10^{-1}$ to $10^2$ fM, with a detection limit as low as 100 aM. In addition, the signal plateau was achieved at higher ADDL concentrations (greater than $>500$ fM). As a result, the bio-barcode assay can provide analytical detection of ADDLs from the lower attomolar to the upper femtomolar range. CSF samples obtained from AD patients and healthy individuals were used as a positive and a negative control, respectively (Fig. 3d). The ADDL concentration in the CSF was estimated by comparison of the grayscale intensity in the scanometric assay with the calibration curve. The median ADDL concentrations for the two groups were 200 aM and 1.7 fM, respectively ($P < 0.0001$, unpaired $t$ test), which suggested that ADDL levels were significantly lower in the control group when compared with the AD group.

**Infectious diseases**

Hepatitis B is a devastating infectious disease caused by the hepatitis B virus (HBV). Especially in Asian countries, a large number of patients have been infected with HBV and the numbers are growing rapidly. Exposure to HBV can cause both acute and chronic inflammation of the liver, and consequently leads to cirrhosis and liver cancer [55, 56]. Therefore, it is necessary to develop a method for early HBV detection to possibly prevent disease progression. Recently, there have been many developments of methods for the detection of HBV, including chemiluminescence [57], spot hybridization [58], nested-PCR [59, 60], reverse transcription PCR (RT-PCR) [61], and oligonucleotide-based biosensors [62, 63]. Although several PCR-based techniques have been developed, these methods have several limitations due to the lack of amplification specificity and the requirement for highly sophisticated equipment [64]. Thus, there is a growing need for the
development of a new isothermal detection method with improved sensitivity and specificity, such as NASBA (Nucleic Acid Sequence Based Amplification), SDA (Sequence Displacement Amplification), RCA (Rolling Circle Amplification), or LAMP (Loop-based Isothermal Amplification). Furthermore, there has been increasing interest in the quartz crystal microbalance (QCM) analytical method for biosensing, because it can provide a rapid response, high sensitivity, and high stability associated with the operating frequency of quartz crystals.

For example, Yao and coworkers [65] have designed an RCA-based QCM biosensor that can be used to directly detect HBV genomic DNA [65]. After the RCA reaction, amplified RCA products can hybridize with the captured probes immobilized on the gold electrode surface by covalent bonding (Fig. 4a, b) [65]. In the presence of the target sequence, they hybridize with the circular probe, which is subsequently ligated by E. coli DNA ligase (Fig. 4a) [65]. The circular probe is constructed with 5' and 3' terminal sequences complementary to the target DNA sequences. Thus, the ends of the circular probe can be joined together only when the circular probe is hybridized with a perfectly matched target sequence. With a single-base mismatch in the target sequence, the RCA reaction cannot be initiated and the QCM frequency shift signal cannot be observed. After formation of the circular probe, the primer sequence is isothermally extended to generate a long single-stranded–DNA sequence with use of the Phi29 DNA polymerase by the rolling-circle amplification RCA. Even a single-base mismatch strand can be differentiated from the target strand with the two definite advantages of high amplification efficiency of Phi29 DNA polymerase and the remarkable precision of E. coli DNA ligase. The relationship between the QCM frequency shift and the concentrations of HBV strands was investigated in the optimized condition (Fig. 4c) [65]. A positive linear relationship was observed between the two variables in the concentration range from $10^3$ to $10^8$ copies per milliliter, with a high correlation coefficient of 0.995. In addition, the frequency shift significantly increased along with the HBV target concentration over the range from $10^4$ to $10^8$ copies per milliliter. In contrast, there was an extremely low frequency shift in the control group, and virtually no increase in the frequency shift was observed over the same range.

The recent outbreaks of Ebola and Middle East respiratory syndrome (MERS) were
worldwide issues. Infectious diseases such as Ebola, Middle East respiratory syndrome MERS, and severe acute respiratory syndrome SARS spread very quickly and easily. When outbreaks become pandemic, societies and their economies fall into chaos. It is necessary to detect these diseases early to prevent them from reaching epidemic status. RT-PCR is the most widely used method to diagnose infectious diseases. However, this method has multiple drawbacks. It requires a reverse transcription (RT) step for cDNA synthesis. Furthermore, a thermo-cycler is required for the annealing and amplification of target genes [66]. However, padlock probe recognition shown in this system has various advantages. First, it has single-base specificity, meaning that it can distinguish a single base difference. Second, the amount of RCA product is directly proportional to recognition of the target pathogen. Because, circularized molecular padlock probes MPPs (cMPPs) formed only through recognition of the pathogen strand make the RCA product [67]. Furthermore, these direct proportional relationship between the RCA product and target recognition makes it possible to develop a biosensor showing with high sensitivity without an additional purification step [68]. The assay which uses the RCA reaction induced by the target pathogen can be implemented by simple modification of the device [69].

Here we show describe an oligonucleotide-based biosensor for detection of infectious pathogens. The name of this biosensor is called DhITACT, which stands for “DNA Hydrogel Formation by Isothermal Amplification of Complementary Target in Fluidic Channels” [70]. This system utilizes uses a dumbbell-shaped padlock probes that can selectively hybridize with single-strand DNA/single-stranded RNA targets. After binding with pathogen strands, they form a closed circular structure by ligation and rolling-circle amplification RCA can take place occur to generate long tandem repeats of DNA products. Once sufficient amplified DNA strands have been produced, they form self-entangled DNA hydrogels in microfluidic channels (DhITACT) with high viscosity. This results in selective blockage of matching channels with the target pathogen template (Fig. 5a). This system has good sensitivity relative to conventional RT-PCR. Complete blockage of flow can be visualized with the naked eye at 10 pM (6 × 10⁹ copies per milliliter mL⁻¹) and fluorescence detection allows for results in a sensitivity of 0.1 pM (6 × 10⁷ copies per milliliter mL⁻¹) (Fig. 5b). All these processes happen at room temperature and require less than 3 hours of operation. This assay provides is a novel method which detects viral or
bacterial DNA/RNA within a few hours by the naked eye.

2.1.2. Biosensors for the detection of drugs in the body

A drug test is an analytical method for detecting illegal drugs or their metabolites in a human sample, such as blood, sputum, or urine sample. Drugs testing can be used in various situations. For example, it is used to detect prohibited drugs such as cocaine and steroids from— in sportsmen and sportswomen during competitions and outside competitions games. Also, in everyday life, it measures the alcohol level in blood or breath air to for catching drunken drivers. Among various drugs, cocaine addiction is a serious worldwide problem and cocaine is the second most used illegal substance in both Europe and the USA [71]. Multiple assays have been developed for the detection of cocaine, but most of these methods are time-consuming and require expensive laboratory equipment [72]. Therefore, the development of simple, sensitive, and rapid detection methods is necessary. For example, aptamers have been used as a new biosensor platform for the detection of cocaine. Aptamers are single-stranded ssDNA or single-stranded RNA oligonucleotides that bind to specific molecular targets with high affinity.

Here we introduce examples of DNA-based biosensors for detecting cocaine. The first example utilizes quantum dots (QDs) immobilized with cocaine aptamer. The aptamer changes its shape from linear to hairpin when it binds to cocaine. That change makes causes Cy5 to be detached from QD Quantum-dots, resulting in the inducing signal turning off. Therefore, the presence of cocaine can be determined by observing the decrease of the Cy5 fluorescence signal [73]. Quantum-dots {QDs,} which are semiconductor nanocrystals, have various advantages. For example, QDs have stable photoluminescence with a broad absorption range while having a sharp emission range. In addition, they have adjustable photoluminescence due to a similar reason [74]. QDs have been used as fluorescence resonance energy transfer (FRET) donors in many detection systems [38]. QDs can be used helping a FRET detection system provide as novel and sensitive biosensors for cocaine. As shown in Figure 6, if there is no cocaine, the
Cy5 fluorescence signal can be detected due to FRET between 605QD and Cy5. Since QDs serve as FRET donors to provide energy to neighboring Cy5 molecules, strong Cy5 fluorescence signal can be obtained. As Fig. 6a shows, the presence of cocaine leads to the formation of a cocaine–aptamer complex and the associated conformation change results in the subsequent reduction of FRET between 605QD and Cy5. Time-correlated photon counting graphs showing fluorescence bursts depending on the existence of cocaine are presented in Fig. 6b. Figure 6b, graph -A shows Cy5 and 605QD bursts from the 605QD–aptamer–Cy5 complex in the presence of cocaine. In contrast, there is no Cy5 signal in Fig. 6b, graph -B because of the absence of Cy5 linked to the aptamer. While Figure 6b, graph -C shows the linear correlation of the Cy5 signal depending on the ratio of Cy5 and 605QD, Cy5 fluorescence signal reduction is observed depending on the concentration of cocaine (Fig. 6b, graph -D). Greater reduction of the fluorescence signal is achieved with more cocaine.

Another cocaine detection system employs chemiluminescence-based cocaine aptasensors. Chemiluminescence is emission of light by a chemical reaction [75]. One example is emission of light by the reaction between luminol and hydrogen peroxide. Chemiluminescence (CL) analysis has various advantages, such as high sensitivity, cost-effectiveness, and simple but rapid detection. These merits make chemiluminescence CL analysis more attractive as a detection tool [76]. Figure 7a shows outlines of this platform. In this system, cocaine aptamers (S1) are attached on the gold nanoparticle (AuNPs) which has magnetic microbeads (MB-AuNPs) inside [77]. Gold nanoparticles (AuNPs) provide nucleic acid strands in biosensing systems, making it complementary assays to recognition of nucleic acid probes [78]. After immobilization of the cocaine aptamer on MB-AuNPs, the aptamer is hybridized with the signal DNA (S2) and horseradish peroxidase (HRP). This hybridization generates double-functional gold nanoprobes (DF-AuNPs) labeled with horseradish peroxidase (HRP). In the presence of cocaine, cocaine competes with signal DNA (S2) that is already bound to the cocaine aptamer. Since the binding between cocaine and cocaine aptamer is stronger than that between of cocaine and signal DNA (S2), signal DNA (S2) is dissociated from the MB-AuNPs complex, which has cocaine aptamer attached. Free signal DNA (S2) conjugated with having horseradish
peroxidase (HRP) can then react with \( \text{Luminol} \) and \( \text{H}_2\text{O}_2 \), generating a chemiluminescence (CL)-signal. However, in the absence of cocaine, HRP cannot react with \( \text{Luminol} \) and \( \text{H}_2\text{O}_2 \) because signal DNA (S2) conjugated with having HRP is linked to magnetic microbeads (MB-AuNPs), meaning that it is not free and is in an unreactive state. The chemiluminescence CL signal of \( \text{Luminol} - \text{H}_2\text{O}_2 - \text{HRP} \) \( \text{p-iodophenol} \) \( \text{PIP} \) system has a linear relationship with the amount of cocaine (Fig. 7b, graph -A). All these results are compared with those obtained without the use of AuNPs. The sensitivity of the aptasensor with AuNPs is much higher than that without use of AuNPs. Figure 7b, graph -A outlines the relationship between the concentration of cocaine and the chemiluminescence CL signal with AuNPs, whereas Fig. 7b, graph -B shows the relationship without AuNPs. The sensitivity of the biosensor with AuNPs is about ten times higher than that of assays without AuNPs.

Sportsmen and sportswomen should take a doping test during competitions such as the Olympic Games. Such a test confirms the existence of prohibited substances in athletes’ blood or urine. A doping list consists of banned drugs and stimulants, and hormones which are substances that enhancing athletic performance artificially. Therefore, there is a need for rapid and accurate detection of various drugs on the doping list. Peptide hormones, growth factors, related substances, and mimetics are included in the major doping lists. For example, platelet-derived growth factor (PDGF) is a prohibited drug for athletes.

Andrew D. Yang Ellington et al. [81] developed a novel detection system for PDGF. This system employs rolling-circle amplification (RCA). A real-time amplification assay is suitable for detection and determination of PDGF. Currently, real-time (RT)-PCR is a representative analytical method for many diagnostic applications [79]. However, there is limitation in adjusting a RT-PCR system to an assay for proteins such as PDGF. Since it requires an additional reverse transcription step, many RT-PCR methods are time-consuming. Instead, RCA can be used for the detection of target proteins [80]. Figure 8a shows the schematic plan of PDGF detection by using RCA. To apply real-time RCA for PDGF...
detection, a conformation change of the aptamer is necessary for it to form a circularized template upon interaction with its protein target (PDGF) \[81\]. Later, the circularized PDGF aptamer is recognized by the T4 DNA ligase, generating a closed circular loop for the RCA. \(\Phi\)29 DNA polymerase recognizes the 3’ end of the primer, and continuously forms long oligonucleotide products. The RCA product can be detected by its binding with fluorescent nanoprobes \[81\]. The RCA product is massive, making possible its detection as discrete single molecules \[82\]. Similar to As in the conventional method, the real-time RCA product can be detected by use of molecular beacons, cleavable probes, or SYBR Green \[83\]. With real-time RCA, it is possible to quantitate the amount of PDGF with a linear correlation. Figure 8b shows the quantitative relationship between fluorescence and two variables: reaction time and the concentration of PDGF in the sample. Both variables exhibit a linear relation with fluorescence intensity. This means that a longer reaction time and higher concentration of PDGF will result in a stronger fluorescence signal.

### 3. Oligonucleotide-based biosensors for detection of environmentally hazardous materials

People are receiving more exposure to hazardous materials, leading to excessive immune responses, cancer development, imbalance of hormones, and abnormal early sexual development. To this end, early detection of environmentally hazardous materials is of interest to many health care professionals and to the public. There are many hazardous materials around us in everyday life. For example, heavy metals, endocrine-disrupting hormones, antibiotics, and toxins are frequently found in standard products and in the environment. Among the heavy metals, mercury, lead, and cadmium are common. They induce toxicity through accumulation in the body. Mercury is toxic to the kidneys and induces anemia by hemolysis. Lead is toxic, especially to the nervous system. Heavy metals usually take a long time to cause health issues, but short-term acute exposure is a serious concern in developing countries. Endocrine-disrupting hormones-compounds (EDCs) include bisphenol A and estradiol and bisphenol A (BPA); which act as a hormone-mimicking substance, which cause an imbalance in the endocrine system, cancer development, and abnormal immune response in addition to reducing the reproductive capability of the individual. Tetracycline is a good example of a commonly used antibiotic. People treat animals or fishes with antibodies to make them more resistant to diseases.
however, antibiotics can accumulate in the human body over time with consumption of meat and fish products. Finally, aflatoxin B₁ and ochratoxin A (OTA) are toxins that cause food poisoning. It is easy to get food poisoning especially in warm and humid conditions, and it is important to detect toxins that can cause food poisoning. Therefore, the detection of environmentally hazardous materials is very important for our protection.

3.1. Heavy metals: mercury, lead

Highly sensitive electrochemical sensors for lead (II) detection have been developed using with use of multiwalled carbon nanotube (MWCNT)s/AuNPs-modified electrodes. Because of the Pb²⁺-induced guanine (G)-rich DNA conformation, the DNA aptamers can bind to Pb²⁺ ions with high sensitivity and selectivity. There are three types of DNA aptamers that can be used for the detection of Pb²⁺ ions: RNA-cleaving 8-17 DNAzyme [84], GR-5 DNAzyme [85], and Pb²⁺-dependent G-quadruplex (G4) oligonucleotide [86]. 8-17 DNAzyme and GR-5 DNAzyme show cleavage activity by catalyzing RNA transesterification when lead ions are present. The Pb²⁺-dependent allosteric G4 aptamer contains guanine (G)-rich nucleic acid sequences, which allow G4 to form stacked arrays of G-quartets with a unique higher-order structure. Since the diameter of Pb²⁺ is smaller than that of many other ions, G4 offer an advantage due to its selectivity [87]. Conventionally used methods for detection of Pb²⁺ ions, such as atomic emission spectrometry and mass spectrometry, have some problems such as multiple sample preparation steps and limited sensitivity. To overcome these barriers, fluorescent [75, 88, 89], electrochemical, photoelectrochemical, and optofluidic sensing strategies have been studied.

Recently, Zhu Y. and coworkers et al. [90] developed an electrochemical sensor for the detection of Pb²⁺-dependent G4. Additionally, AuNP gold nanoparticles and multiwalled carbon nanotubes (MWCNTs) were introduced to improve the conductivity of biosensors. AuNPs and MWCNTs exhibit good electrical conductivity and large surface areas that are appropriate for electrochemical sensors. To construct MWCNTs/AuNPs-modified electrodes, MWCNTs were assembled on the surface of glassy carbon electrodes and AuNPs were coated. The DNA capture probe 5′-CACCCACCCAC-SH-3′ was immobilized on the surface of the AuNPs via Au–SH interaction. When there are no Pb²⁺ ions in the sample, the DNA aptamer probe 5′-(GGGT)₄-3′ can hybridize with the capture probe. In the
presence of Pb\(^{2+}\) ions, the DNA aptamer probe reacts with lead ions, forming a highly stable G4 structure. As a result, methylene blue is detached from G4, resulting in reduced conductivity of the electrochemical sensor\cite{90}. The advantage of this platform compared with other electrochemical sensors is its improved sensitivity. Due to the highly conductive MWCNTs and AuNPs, it can detect very low concentrations of lead ions: the limit of detection is 4.3 \(\times 10^{-15}\) M. Furthermore, it shows linearity with a range of 5.0 \(\times 10^{-11}\) to 1.0 \(\times 10^{-14}\) M.

Photoelectrochemistry is another good strategy for the detection of lead ions, owing to its low background signal and high sensitivity \cite{91-93}. In the photoelectrochemistry sensing system, a-light excites some particles and induces a change in the photocurrent signal. Commonly, QD quantum dots are used for bioanalysis due to their resistance to photo-bleaching as well as their broad absorption spectrum and sharp emission spectrum \cite{74}. As shown in Fig. 9a, ascorbic acid donates electrons to QDs and the generated photoelectric current (PEC) generated can be measured in the presence or absence of lead ions. In the absence of lead ions, DNA aptamer can hybridize with the AuNP-labeled DNA strands and AuNPs can induce the quenching of PEC through an energy transfer between QDs and AuNPs. In contrast, when a sample containing Pb\(^{2+}\) ions is applied, Pb\(^{2+}\) interacts with the aptamer, forming the stable G4 structure \cite{94}. Formation of the G4 structure inhibits the sequence-specific binding of the aptamer with the AuNP-labeled DNA strands, thereby maintaining a high photoelectric current PEC signal. Figure 9b shows the linear relationship between the photocurrent and the concentrations of lead ions. With increasing concentrations of lead ions, the photocurrent level goes up increases. The photoelectrochemical sensor has a strong advantage, because it can handle the water sample directly from the environment without pre-treatment. Compared with other detection methods, it shows has high selectivity for lead ions even with other ions in the sample such as potassium, nickel, cobalt, and sodium ions. Second, the sensor can obtain a linear range from 1.0 \(\times 10^{-10}\) M to 5.0 \(\times 10^{-8}\) M and the limit of detection is 5 \(\times 10^{-11}\) M, which is lower than that of other photoelectrochemistry-based sensors(Fig. 9c).

The third example of a Pb\(^{2+}\) biosensor uses an all-fiber optofluidic biosensing (AFOB) platform. A biosensor equipped with an optofluidic system can be very useful with real
samples, since a very small volume of sample is required for detection of Pb\(^{2+}\) ions [95]. The all-fiber optofluidic biosensing AFoB system is comprised of four parts: a micro-fluidic system, an all-optical fiber system, a signal processing system, and a signal control system. When a pulsed laser beam (635 nm, 5 mW) is generated, it passes through the silica optical fiber coupler and reaches the tapered optical fiber sensor. An evanescent wave is produced at the surface of the optical fiber sensor and excites the immobilized fluorescently-labeled G4 aptamer. Due to the narrow range of the evanescent wave, it is possible to discriminate bound aptamers from unbound aptamers without an additional washing step.

In this platform, the G4 aptamer is labeled with fluorescent Cy5.5 material and is used as a recognition probe. Another DNA probe with a sequence complementary to the G4 aptamer is bound to the surface of the optical fiber sensor by a covalent bond. When there is a high concentration of Pb\(^{2+}\) ions in the optofluidic system, the fluorescence signal decreases because of competition for the immobilized cDNA probe between the G4 aptamer and the Pb\(^{2+}\) ions (Fig. 10a). Figure 10b shows the fluorescence signal during detection of Pb\(^{2+}\) using the described optofluidic biosensor. The fluorescence signal curve decreases with increasing concentrations of Pb\(^{2+}\), from 0 nM to 1 M. Figure 10c shows the relationship between the fluorescence signal and the calibration plot for the detection of Pb\(^{2+}\). The optofluidic biosensor can achieve a linear range from 1.0 to 300.0 nM with the following equation: 

\[
I' = 0.305 \times \log[\text{Pb}^{2+}] + 0.0705.
\]

It is worth noting that the optofluidic-based aptasensor can offer simple and fast detection in actual water samples. As mentioned before, another beneficial point is the very small volume of sample needed for the analysis. In this platform, a 3.0-µL sample volume is enough for the detection process. Furthermore, reusability of the sensor probe is possible, allowing for cost-effective measurement [96].

Mercury ions are very notorious environmentally toxic pollutants and are a global issue, since they cause severe and irreversible effects in human [97,98]. To detect small amounts of mercury with high sensitivity, ssDNA aptamers with thymine residues can be used. When thymine-T-containing ssDNA encounter mercury ions, the mercury ions bind tightly between the two thymine residues of the DNA due to their high electron density [99]. As a result, a T–Hg\(^{2+}\)–T complex is formed.
In addition to the $T\rightarrow\text{Hg}^{2+}\rightarrow T$ complex formation, fluorescence polarization (FP) can be employed as an appropriate detection tool. The basic principle of fluorescence polarization is the excitation of fluorescent elements using polarized radiation. After excitation of the fluorescent molecules, the emission returns to a fixed plane [100].

Fluorescence polarization (FP) changes depending on the molecule’s rotational relaxation time, increasing with longer rotational relaxation time. Tongfei Shen et al. [A7] designed a biosensor involving an adapted fluorescence polarization (FP) system for the analysis of mercury ions. To enhance the fluorescence polarization (FP) signal, iron oxide nanoparticles were selected as the magnetic core material. Iron oxide nanoparticles are commonly used as magnetic resonance imaging contrast agents [101]. As shown in Fig. 11a, a thymine-rich ssDNA probe is connected to superparamagnetic iron oxide nanoparticles ( MNPs ) through streptavidin–biotin linkage. Additional thymine-containing ssDNA is labeled with fluorescent dye for fluorescence polarization (FP) measurement. Based on the basis of the specific linkage between thymine and Hg$^{2+}$, thymine$T$-rich ssDNA on superparamagnetic iron oxide nanoparticle ( MNPs ) and free fluorescently labeled thymine$T$-rich ssDNA are bridged together through Hg$^{2+}$. The formation of a $T\rightarrow\text{Hg}^{2+}\rightarrow T$ complex allows the fluorescently labeled ssDNA to bind to superparamagnetic iron oxide nanoparticle ( MNPs ), thus increasing their molecular weight. In response to the increased molecular weight, the fluorescence polarization (FP) signal is also increased in the sample. When different concentrations of the Hg$^{2+}$ sample we are tested, the fluorescence-polarization (FP) method-based biosensor based on the fluorescence polarization method showed a linear relationship over the range from 2.0 nM to 1.0 mM (Fig. 11b). The limit of detection was estimated to be 0.49 nM. Compared with previous studies, this study revealed a low limit of detection ( LOD ) and a wide range of linearity are exhibited. Furthermore, it is possible to reuse the magnetic particles with similar reproducibility for six cycles [102].

Ling Zhang et al. [110] have proposed another heavy metal detection system for mercury ions. In their detection system, a novel surface-enhanced resonance Raman scattering (SERRS) method was applied. Biosensors with optical detection techniques such as colorimetric analysis [103], fluorescence detection [104-106], plasmon resonance spectroscopy [107], and surface enhanced Raman scattering [108] have been widely studied. However, the sensitivity of these measurement platforms was uncertain at low
sample concentrations. Ling-Zhang and et al. [110] coworkers improved the sensitivity by developing a SERRS-based Hg\(^{2+}\) sensor with a detection sensitivity of 1 pM. In their study, nanoporous gold (NPG) is introduced as a plasmonic element, and a thymine-rich ssDNA probe labeled with Cy5 is attached to NPG via SH functional groups (Fig. 12a). When there are no Hg\(^{2+}\) ions in the sample, the distance between Cy5 and NPG is short, resulting in increased Raman signal intensity from Cy5. Upon the addition of Hg\(^{2+}\) ions, thymine residues are linked via T-\(\equiv\)Hg\(^{2+}\)-T interaction. Because strong and specific binding is formed between ssDNA aptamers, a strict duplex structure is observed. Unlike the flexible ssDNA aptamer, the distance between Cy5 and NPG increases when a strict duplex structure is formed. As a result, the local surface plasmon resonance effect of NPG is reduced as well as the SERRS signal of Cy5. Figure 12b shows that the Raman intensity increases as the concentrations of Apt8@NPG decreases. The peak of the Raman intensity graph is obtained at 1365 cm\(^{-1}\). Since the distance between Cy5 and NPG is an important factor for the intensity of the SERRS signal, an appropriate thymine-containing aptamer is needed for appropriate sensitivity. It is reported that fewer thymine residues containing aptamer require fewer Hg\(^{2+}\) ions to form a rigid duplex structure. Therefore, aptamers containing a few thymine bases were used for highly sensitive detection. In this study, two kinds of aptamer, one containing 15 thymine bases (Apt15) and one containing eight thymine bases (Apt8), were used to recognize the effect of aptamer length. Figure 12c shows the Apt8@NPG sensor is more sensitive than the Apt15@NPG sensor. It is noteworthy that the device is easily reusable after addition of 100 mM ascorbic solution for 1 h and washing for 15 min [109]. According to the reproducibility test result, the SERRS signal intensity varies within the range of 10% for ten cycles [110].

3.2. Antibiotics: Tetracycline

Tetracycline is a commonly prescribed antibiotic that inhibits protein synthesis for a broad spectrum of diseases. However, extensive use of tetracycline is a global issue as tetracycline residues remain in food products such as raw milk, meat, and honey. The residues can cause lethal side effects and allergic reactions in humans. Emerging bacterial resistance to tetracycline is also considered a big problem. For these reasons, sensitive and selective analysis of tetracycline concentrations in food products has been studied.
Mohammad-Ramezani et al. [117] have designed a biosensor to detect tetracycline in milk that is based on a triple-helix molecular switch (THMS) system. The colorimetric THMS system has been used to detect other species, such as materials like potassium ions, and this was the first time it was used for detection of tetracycline with AuNPs and aptamer [111]. The THMS system has remarkable features compared to the double-helix DNA aptamer in that it is much more stable and sensitive, with similar binding affinity [112]. In this study, the ssDNA aptamer 5’-CTCTCTCGGTGGTGTCTCTC-3’ was used for high-affinity binding to tetracycline. Triple-helix structures consisting of aptamers and signal transduction probes were employed for the detection of tetracycline. In the absence of tetracycline, the AuNPs become unstable and aggregate. Because since the color of the AuNPs changed from red to blue, when the AuNPs formed aggregates, colorimetric detection of tetracycline was achieved. In the presence of tetracycline, aptamers bind to tetracycline instead of the signal transduction probe. The remaining signal transduction probes react with AuNPs and attach to the surface of AuNPs. As a result, AuNPs remain stable, avoiding aggregation. The THMS system-based sensors based on the THMS system have the ability to detect tetracycline in samples containing the antibiotic at a concentration of at least 266 pM (0.127μg/L) of the antibiotic. In other studies, the limit of detection was 0.5 μg/L for high-performance liquid chromatography with UV detection [113], 2 μg/L for microbiological tests [114], 0.19 μg/L for immunoassays [115], and 2.4 μg/L for electrochemical aptasensors [116]. As the absorbance of AuNPs increases depending on the concentration of tetracycline, quantitative analysis of tetracycline is possible. The sensor shows a wide range of linearity from 0.3 nM to 10 nM for tetracycline, and can specifically react with tetracycline in mixtures with other antibiotics such as clindamycin, amoxicillin, and ciprofloxacin. Furthermore, the designed aptasensors can be directly applied to detect tetracyclines in real samples such as milk and serum. The maximum allowed concentration for tetracyclines in milk is 0.1 mg/L, which is much higher than the limit of detection for THMS system-based aptasensors based on the THMS system [117].

For analysis of trace metal ions and small biological elements such as enzymes, proteins, and DNA, resonance scattering (RS)-spectral analysis has been reported to exhibit high selectivity and sensitivity. Tao et al. [118] demonstrated that the resonance scattering RS spectral signal could be improved with the addition of an inorganic catalyst,
which indicates the enhancement of sensitivity [118]. For example, inorganic catalysts such as AuNP-gold nanoparticles have characteristics suitable for biosensors: high conductivity, catalytic activity, and biocompatibility. Single-stranded DNA (ssDNA) aptamers can be immobilized on the surface of AuNP-gold nanoparticles via van der Waals forces and stabilize the nanoparticles. Unlike aptamer-coated nano-gold (ACNG), uncoated AuNP-gold nanoparticles aggregate and lose catalytic activity. Enhanced catalytic activity via the Fehling reaction rarely occurs before the temperature reaches 100 °C. However, the addition of ACNG induces the Fehling reaction following the reduction of Cu²⁺ and glucose to produce cubic Cu₂O cubic at 60 °C.

In addition, Luo and coworkers et al. [119] designed an aptasensor using the resonance scattering RS spectral detection method with catalytic nanogold particles-[119]. Nanogold particles were modified and coated with tetracycline-binding single-strandss DNA aptamers. Without tetracycline, the aptamer binds to the surface of the nanogold particles, which stabilizes the nanogold particles (Fig. 13a). The stabilized nanogold particles catalyze the Fehling reaction and generate a large amount of cubic Cu₂O-cubic, resulting in increased resonance scattering RS signal level. With tetracycline, tetracycline binds to the aptamer and naked nanogold particles aggregate, reducing the magnitude of the Fehling reaction observed. As a small amount of cubic Cu₂O cubic is produced, the resonance scattering RS signal level decreases. In conclusion, the relationship between the amount of tetracycline and the resonance scattering RS signal level is inversely proportional. The calibration curve for the ACNG resonance scattering RS method was obtained at 620nm, at which cubic Cu₂O cubic shows a significant peak. To observe the linearity range of the aptasensor, samples containing 0–to-2500 nM of tetracycline were examined. The resonance scattering RS signal level at 620 nm decreased consistently as more tetracycline was added, up to 625 nM. Above a concentration of 625 nM, the calibration curve rarely increased, indicating that the aptamers were saturated with tetracycline (Fig. 13b). The limit of detection was 11.6 nM, low enough for use in milk samples. The selectivity of the aptasensor has also been tested with using antibiotics such as thiamphenine[A9], kanamycin, and aztreonam and small molecules such as tyrosine, valine, and lysine. When 0.5 mM of other molecules at 0.5 mM are individually added, the changes in the resonance scattering RS signal level are all below 200. In comparison, tetracycline samples result in a change of about 900 |A11| in
the resonance scattering RS signal.

## 3.3. Fungal toxin: ochratoxin A

Food safety has drawn global awareness attention, and food intoxication in particular is an increasing problem. Ochratoxin can have irreversible effects on human health, such as nephrotoxic, immunotoxic, teratogenic, and carcinogenic effects. Ochratoxin is categorized into three types: ochratoxin A (OTA), ochratoxin B, and ochratoxin C. The types have slightly different chemical structures. Among the three subtypes, ochratoxin A is often found in food products, including cereals, wheat, coffee beans, beer, and grape juice. The maximum permitted level of OTA in various food products was recently established: cereals (3.0 ppb in cereals), and 2.0 ppb in beer and grape juice (2.0 ppb). To confirm and verify the permitted level, sensitive and selective detection methods are required without the use of complex and time-consuming instruments are required.

For this purpose, DNA hydrogels composed of DNA and aptamers are designed as biosensors and drug delivery systems [120, 121]. Hydrogels have several advantages, including stability, portability, low cost, and flexibility similar to natural tissue and exhibit a unique property in that they can swell and disintegrate based depending on the surrounding environment. Because of these properties of hydrogels, Rudi et al. [122] chose target-responsive DNA hydrogels as the base for an OTA sensor [122]. In their novel biosensor, an OTA aptamer is used as a linker strand (Apt-linker in Fig. 14a) and two kinds of DNA strands are attached to a linear polyacrylamide chain (Fig. 14a). The DNA strands contain sequences that are complementary to some parts of the aptamer used as a linker strand Apt-linker. For qualitative analysis, AuNPs are added to the hydrogel as an indicator material. With OTA, DNA hydrogels dissociate because OTA binds with the aptamer used as a linker strand Apt-linker. As cross-linking between hydrogels collapses, preloaded AuNPs are released, which can be recognized easily by the naked eye. Figure 14b shows the reaction of the colorimetric OTA-detecting hydrogels with different concentrations of OTA. The color of the supernatant gradually increases with an increasing concentration of OTA. From analysis of the absorbance of AuNPs at 520nm, a calibration curve can be obtained, with the an upper limit of OTA concentration of at 2.5 μM. The absorbance increases with up to 2.5 μM of OTA if the sample contains more OTA [A12].
This result corresponds well with the proposed detection mechanism, which assumes that decomposition of the hydrogel will release more free AuNPs. Quantitative detection as well as qualitative detection of OTA is achieved using a volumetric bar-chart chip (V-Chip) [123]. Instead of AuNPs, nanoparticles consisting of a gold Au-core and a platinum Pt shell (Au@PtNPs) are loaded. Au@PtNPs have high catalytic activity for decomposing H₂O₂ to O₂, which can change the height of the ink bar. As more O₂ is produced by Au@PtNPs, the indicator on the ink bar goes up on the V-Chip. Figure 14c shows the V-Chip readout constructed with two glass slides and six parallel channels. Due to the scale marks on the parallel channels, quantitative detection is easy and simple. The supernatant including Au@PtNPs is loaded in the bottom lane and is unable to move into other lanes because only horizontal channels are connected. For the same reason, H₂O₂ is in the middle lane and the red ink is in the top lane. After loading, horizontal channels are disconnected and vertical channels are connected to make the sample react with H₂O₂. Catalytic Au@PtNPs decompose hydrogen peroxide H₂O₂ to produce O₂. As the concentration of OTA increases, the distance moved by the red ink is greater. The linear relationship between the concentration of OTA and the distance moved by the red ink is shown in Fig. 14d. With the V-Chip, the sample containing OTA at a concentration ranging from 0 to 1μM shows linearity with the height of the ink bar. The limit of detection with the V-Chip is 10.8 nM. This result shows that the sensitivity is highly improved by use of a V-Chip as compared to the previous detection method using AuNPs (0.24 μM). The DNA-hydrogel-based aptasensor is very promising because it is easy to handle, detection is simple, and it is able to distinguish between OTA and ochratoxin OTB.

In another study, a localized surface plasmon resonance (LSPR) aptasensor was designed to detect OTA [124]. The LSPR-based aptasensor offers an easy, quick, and sensitive detection method based on the principle that a red-shift of the LSPR band occurs when molecules are absorbed on the surfaces of nanoparticles, changing the refractive index. The LSPR aptasensor is comprised of glass, gold nanorods, and OTA-specific aptamers (Fig. 15a). An OTA-specific aptamer is tightly linked to the surface of the gold nanorod through thiol–gold interaction. When immobilized aptamers encounter OTA, the structure of the aptamer is changed, forming a G₄-quadruplex structure. Due to the stable G₄-quadruplex structure, the LSPR aptasensor can resist relatively high temperatures, and the aptasensor
can be reused several times if the temperature is increased to 70 °C to remove bound OTA. The G-quadruplex structure also has the advantage of increasing the local density of the aptamer on the gold nanorod (GNR), enhancing the sensitivity of the LSPR aptasensor. The changed structure of the OTA aptamer leads to the red-shift of the LSPR band, as the refractive index on the surface of the gold nanorod is changed. As a result, a red-shift from 712.91 to 716.71 nm occurs with 1 μM concentration of OTA in the sample (Fig. 15b). To evaluate the linear relationship between the OTA concentration and the red shift of the LSPR band, different concentrations of OTA were added and analyzed. The calibration curve exhibits linearity in the range from 0.1 nM to 10 μM (Fig. 15c).

3.4. Endocrine disruptors

Endocrine disrupting chemicals (EDCs) exist in everyday items such as plastic cups or dishes. EDCs such as bisphenol A (BPA) act similarly to the biological hormone estrogen. It can provoke precocious puberty and induce infertility [125]. Therefore, there is need for rapid and facile detection of EDCs for production and regulatory practices. However, there are various limitations to the detection of EDCs. In reality, the environmental concentration of EDCs is too low for accurate detection. In addition, EDCs exist mainly in complex mixtures with other materials, such as detergents and organic substances. To resolve these issues, multiple approaches have been adopted for the development of environmental biosensors for BPA [127]. BPA is an artificial organic material that is present in common plastic goods [128]. BPA is included in polycarbonate plastics as a plasticizer, which is an additive to increase flexibility and elasticity of plastics. There are various conventional analysis methods for the detection of BPA in portable water, such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC/Mass), and liquid chromatography–mass spectrometry (LC/Mass) [128]. However, these existing methods have long detection times and analytical quantification is not accurate at a low concentration of BPA. In contrast to old methods, there is a novel sensor for detecting BPA in portable water. This assay is based on surface-enhanced Raman scattering (SERS) using Au/Ag–silver core–shell nanoparticles labeled with double-stranded DNA. The double-stranded DNA consists of Cy3-labeled BPA aptamer and thiolated probe DNA. In the presence of BPA, BPA binds with BPA aptamer instead of probe...
DNA, inducing Cy3 signal reduction. This analytical method is highly sensitive, having a limit of detection (LOD) of 10 fM and a detection range from 100 nM to 10 fM. It is 100–1000 times more sensitive than conventional methods.

4. Conclusion

This review has highlighted various oligonucleotide-based biosensors for medical diagnosis, crime detection, food analysis, and environmental field monitoring. Current molecular diagnostic methods such as enzyme-linked sandwich immunoassay (ELISA) and PCR have many limitations because of complicated, expensive, and time-consuming procedures. Therefore, there remains a need for the development of novel biosensors with facile, rapid, and sensitive detection. As an alternative approach, oligonucleotide-based biosensors have drawn tremendous attention due to their unique advantages of having the dual role of simultaneous detection and amplification of target analytes. However, there are also inevitable drawbacks of oligonucleotide-based biosensors. First, many of these biosensors need temperature control for the detection and amplification of the target analyte. This requires the equipment for temperature control such as a thermal cycler. Second, many of their detection methods are based on fluorescence detection. This requires the use of nucleic acid binding dyes and additional equipment for image analysis. Conventional PCR analysis is a good example, showing the aforementioned disadvantages.

Recently, there have been various efforts to overcome these limitations. One of them is the nucleic acid detection system that uses an isothermal amplification method such as rolling circle amplification (RCA). This isothermal amplification does not require any equipment to control the reaction temperature. In addition, due to the repeated amplification or generation of the designed template, long chains of oligonucleotide can be produced, and their self-assembly and self-entanglement can induce the formation of a highly swollen network structure such as hydrogel. This allows non-fluorescence-based optical detection without the help of any equipment [70]. Such progress in oligonucleotide-based biosensors offers future promise for in the design of ideal diagnostic devices. Nonetheless, clinical applications are still rare, since there are many limitations in adapting the laboratory devices to real clinical diagnostic applications.
For overcoming this bottleneck, the following requirements are necessary for clinical applications. First, it is very important to develop highly sensitive biosensors. Oligonucleotide-based biosensor can be a suitable candidate in this regard due to its high recognition ability and high binding affinity. Second, high selectivity is required for many in vitro diagnostics. This is a major obstacle in the clinical applications of biosensors. Although many biosensors work very well in a laboratory set-up, many of them fail to analyze real serum samples from patients because of the lack of specificity. Therefore, it is important to develop highly specific and selective biosensors in which can differentiate the small differences among tested samples. It is well known that oligonucleotide-based biosensors have clear advantage for sequence-specific detection of genes. However, the specificity of aptamer-based biosensors still remains to be improved with higher specificity. Lastly, multiplexing of detection is a key for saving the analysis time. Especially, it is critical for clinical and environmental applications dealing with many samples with different target markers. To this end, we believe the oligonucleotide-based biosensors can be an excellent platform technology for in vitro diagnostic applications and for the detection of environmentally hazardous materials. Overall, we have shown that the oligonucleotide-based biosensors have enormous potential to ensure the new era of facile, accurate, and cost-effective in vitro diagnostics.

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Conflict of Interest
The Authors declare that they are have no conflict of interest related with this work.
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**Figure 1.** (a) The long products with multiple biotin labels and multiple branched arms were synthesized by multibranched hybridization chain reaction (mHCR) reaction. The cancer cells conjugated with mHCR products can be attached to the DNA nanostructured surface through multivalent binding. (b) Electrocatalytic current was collected from 1000 MCF-7
cells and no MCF-7 cells, respectively. The electrocatalytic current without amplification was significantly higher than that without mHCR amplification (top). The catalytic signal was increased proportionally to the number of cancer cells. The detection limit was 24 cells without amplification and four cells with mHCR amplification, respectively. (bottom).

Figure 2. (a) The Schematic diagram depicting the strategy for a of aptamer-based fluorescence biosensor for detection of Cellular prion protein PrPc detection. (b) Fluorescence intensity of the tetramethylrhodamine (TAMRA)-containing aptamer in the neutral state (black line) and the thermally-denatured state (red line), and that resulting from the hybridization with its complementary single-stranded DNA sequence (cp-DNA; green line) (c) Circular dichroism spectrum of the TAMRA-containing aptamer (black line), its complementary single-stranded DNA sequence (cp-DNA; red), and its hybrid with its complementary single-stranded DNA sequence cp-DNA (green line).

Figure 3. (a) Amyloid β derived diffusible ligand (Schematic representation of ADDL) detection using by the bio-barcode amplification assay. (b) Schematic illustration of the scanometric detection assay. (c) A normalized intensity curve for a series of serial ADDL concentrations. (d) Scatter plot of ADDL concentrations from the scanometric detection assay for AD diagnosed subjects-patients with Alzheimer’s disease (AD; positive control) and control subjects (negative control). Ab antibody, ds-DNA double-stranded DNA, NPs nanoparticles.

Figure 4. (a) The schematic representation of circular template hybridization with a target strand and a single-mismatch strand, respectively. After hybridization and ligation of the circular probe, rolling circle amplification (RCA) reaction was initiated. (b) The RCA product was immobilized on the gold electrode surface through hybridization with the capture probe. (c) The relationship between the frequency shift and target concentration (log scale) in the range from 10^3 to 10^9 copies per milliliter. HBV hepatitis B virus.

Figure 5. The detection limit test of the DNA hydrogel formation by isothermal amplification of complementary target in fluidic channels (DhITACT) system (a)—by the dye solution method (a), and (b)—by the fluorescence detection method (b).

Figure 6. Detection of cocaine with quantum dots labeled with cocaine aptamer. (a)
Schematic design of a biosensor using quantum dots QDs labeled with cocaine aptamer. (b) Fluorescence signals from 605QD–aptamer–Cy5 complexes in various conditions: A—Cy5 fluorescence signals from the 605QD–aptamer–Cy5 complex in the presence of cocaine (A); B—signal off in the absence of Cy5 linked to the aptamer (B); C—correlation of the Cy5 fluorescence signal depending on the ratio of Cy5 and 605QD (C); D—Cy5 fluorescence signal reduction depending on the concentration of cocaine (D). FRET fluorescence resonance energy transfer, QD quantum dot.

Figure 7. (a) Schematic design of cocaine detection using gold nanoparticles (AuNPs) with magnetic microbeads inside MB-AuNPs labeled with cocaine aptamer. (b) Chemiluminescence (CL) intensity depending on concentration of cocaine. MB magnetic microbead, p-IP p-iodophenol.

Figure 8. Platelet-derived growth factor (PDGF) detection by rolling-circle amplification RCA. (a) General design principles for and design of the conformation-switching aptamer. (b) Fluorescence intensity units depending on RCA time and protein concentration.

Figure 9. Lead detection by a photoelectrochemical aptasensor. (a) General design scheme of the aptasensor with CdS QDs and and AuNP-labeled aptamer based on the principle of resonance energy transfer (RET) between CdS QDs and AuNPs. (b) Increasing photocurrent for the aptasensor analyzed at different concentrations of lead ions: from 0 to 1 (a), 0.05 (b), 0.1 (c), 0.5 (d), 1.0 (e), 5.0 (f), 10 (g), 50 (h), and 100 nM (i). (c) Calibration curve of photocurrent versus the concentration of the lead ions. PEC photoelectric current.

Figure 10. Lead ion detection using an optofluidics–based–aptasensor. (a) Scheme of the optofluidical aptasensor with a fluorescence-labeled aptamer. (b) Fluorescence signal of the aptasensor at different concentrations of lead ions ranging from 0 nM to 1 M. (c) Calibration curve of fluorescence signal versus the concentration of the lead ions. cDNA complementary DNA, G4 G-quadruplex.

Figure 11. (a) Scheme of a mercury ion detecting aptasensor using a T–Hg2+–T complex. (b) Fluorescence polarization (FP) of the aptasensor at different concentrations of mercury ions ranging from 2.0 nM to 20.0 μM. MNPs magnetic nanoparticles.
**Figure 12** (a) Scheme of a mercury ion detecting aptasensor based on the surface-enhanced resonance Raman scattering (SERRS) method. (b) Raman intensity of the aptasensor at different concentrations of mercury ions. (c) Raman intensity of the $1365 \text{-cm}^{-1}$ peak; the plot for the Apt15@NPG SERRS is shown obtained for comparison. *NPG* nanoporous gold, *PBS* phosphate-buffered saline.

**Figure 13** (a) Scheme of Aptamer-coated nanogold (ACNG) catalytic resonance scattering (RS)RS assay for detection of tetracycline (*TET*). (b) The calibration curve of the aptamer-coated nanogold ACNG–tetracycline sensor shows the intensity of the resonance scattering signal level decreases with increasing tetracycline concentration. The curve was obtained with a correlation of 0.998.

**Figure 14** Ochatoxin A (*OTA*) detection using aptamer-cross-linked–hydrogel. (a) Scheme of basic The principle behind the aptamer-cross-linked–hydrogel for visual detection of OTA. With OTA, DNA hydrogels dissociate and preloaded AuNPs are released, which changes the color of the supernatant from colorless to red. (b) Quantitative detection of OTA with [HV-Chip][A15]–aptamer-cross-linked–hydrogel modified with volumetric a bar-chart chip for visual quantitative detection. (c) Images showing the decomposition of hydrogel in 120 min with different concentrations of OTA. (d) Images showing the linear relationship between ink movement and the concentration of OTA in the range from 0 to 1000 nM in 30 min. *PtNPs* platinum nanoparticles.

**Figure 15** (a) Localized surface plasmon resonance (–LSPR) OTA sensing system with a gold nanorod (GNR) and OTA-specific aptasensor. (b) Red-shift of the absorbance obtained curve analyzed with different concentrations of OTA. (c) Relative LSPR peak shift for the OTA aptasensor with different concentrations of OTA from 0.1 nM to 10 μM.