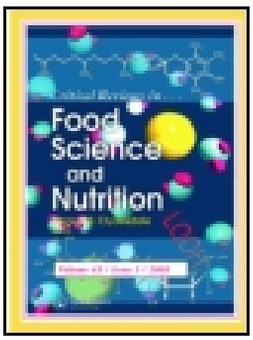

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REVIEW

Ensuring seafood safe to spoon: a brief review of biosensors for marine biotoxin monitoring

Qi Wang, Qingli Yang, and Wei Wu

College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, China

ABSTRACT

With harmful algal blooms, marine food poisoning caused by marine biotoxins frequently occurs and is life-threatening if severe. However, the conventional detection methods of marine toxins have a few limitations: low sensitivity and high-cost. Therefore, it is necessary to establish a fast and sensitive on-site detection method for real seafood sample. Biosensors based on aptamers, antibodies, and cells have been applied in marine toxins monitoring. This review presents the classification and toxic effects of marine toxins, and recent biosensor for marine toxin detection. In addition, we have compared the superiority and limitation of these biosensors. Finally, challenges and opportunities of biosensors in food safety detection were discussed. Considering the excellent results achieved by the aptasensor in the field of detection, it seems ready to be put into practical applications.

KEYWORDS

aptasensor; cell-based biosensor; food safety monitoring; immunosensor; marine toxins

Introduction

Marine toxins are a kind of toxic natural active micro-molecules existing in marine organisms, that possess high toxicity. The formation of marine toxins has a strong association with harmful algal blooms (HABs) that caused by water eutrophication (Fu, Tatters, and Hutchins 2012; Plumley 1997). In the process of food chain accumulation, marine toxins may undergo structural changes and increase toxicity (Yasumoto 2005). Marine toxins are non-protein low molecular toxic compounds in essence which can be classified as shellfish toxins, tetrodotoxins (TTX), ciguatoxins (CTX) depending on the carrier. They have strong toxicity and act on nervous or digestive system causing food poisoning symptoms such as diarrhea, paralysis and even death (Grasso et al. 2019). In recent decades, food poisoning caused by foodborne marine toxins spreads all over the world and has become a global public health hidden danger (Guardone et al. 2020; Jeremy and John 2005; Lehane 2001). In China, seafood poisoning often occurs in summer and autumn, among which shellfish toxins poisoning are the most common. Among the shellfish poisoning incidents, paralytic shellfish poison (PSP) and diarrhea shellfish poison (DSP) accounted for a high proportion (Wang et al. 2011). In May 2011, 57 cases of shellfish poisoning were reported in Zhejiang province, China (Chen et al. 2013). In 2013, 62 clinical illnesses of DSP were reported in British Columbia, Canada (Taylor et al., 2013). In the same year, 58 cases DSP poisoning incidents have been reported in Sabah, Malaysia (Suleiman et al. 2017). Puffer fish make a name for itself delicious but highly poisonous. Nevertheless, some people still eat puffer fish regardless of their lives. In Japan, eating puffer fish is popular and the poisoning of TTX happens every year (Madejska, Michalski, and Osek 2019). In tropical and subtropical areas, 50,000 people are poisoned by CTX every year (Solino and Costa 2020). Marine toxins not only cause negative impacts on water body, fishery, food safety, and human health but also suffer serious economic losses. Therefore, in order to prevent the occurrence of foodborne marine toxin poisoning, it is significant to diagnose and detect marine toxins early in seafood and sea water.

Until now, there is no effective antidote for marine toxin poisoning. Therefore, the early diagnosis of marine toxins is crucial to reduce food poisoning caused by marine toxins; however, due to its own limitations, the traditional methods cannot meet the requirements of rapid on-site detection. Mouse bio-assay is a classical method that used toxic symptoms and time of death of mouse to respond toxic dose. However, there are individual differences among mice, and they often suffer moral condemnation (Ganal et al. 1993). The instrument detection (e.g. high performance liquid chromatography (HPLC), HPLC tandem mass spectrometry and capillary electrophoresis) shows high precision and good stability, but its application is limited by time-consuming, high cost and unsuitable for in-field detection (Huang et al. 2020; Keyon et al. 2014; Wang et al., 2015). Although immunoassay (e.g. enzyme-linked immunosorbent assay (ELISA) and optical immunoassay) has the advantage of high sensitivity and simple operation, antibody is essentially a protein with poor stability and immunogenicity. Therefore, it is necessary to establish a rapid and sensitive method for on-site detection of marine biotoxins. In this

CONTACT Qingli Yang rice407@163.com; Wei Wu wuweiouc@126.com College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, China.

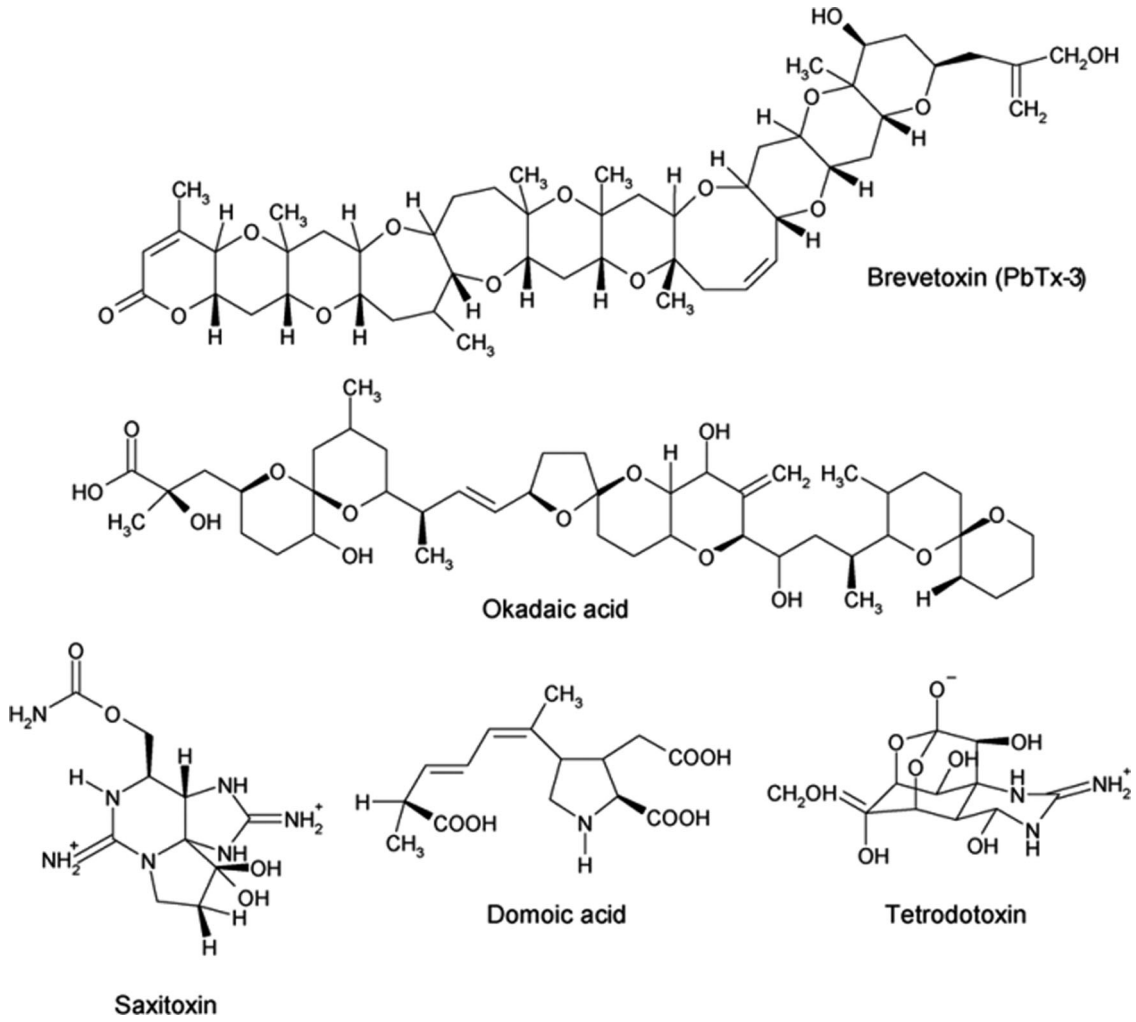

Figure 1. The chemical structure of representative marine toxins.

review, we mainly introduced the application of sensitive and portable biosensors such as aptasensor, immunosensor, and cell-based biosensor in marine toxins detection.

Classification and toxicity of marine toxins

There are many kinds of marine toxins, which can be divided into eight categories (saxitoxin (STX), okadaic acid (OA), domoic acid (DA), brevetoxins (BTX), azaspiracid toxins (AZT), TTX, CTX, and palytoxin (PLTX)) according to their chemical structures (Workshop 2004). STX can induce paralytic shellfish poisoning and cause sodium ion transmission trouble in the nervous system. In addition, STX TTX both are strong inhibitor of sodium ion channel and have specific binding ability to the receptor of voltage-gated sodium channels (Ahmed et al. 2006). OA, the active components of diarrhea shellfish toxin, combines with protein phosphatase receptor and inhibits its activity, leading to diarrhea and tumor promotion (Vale and Botana 2008). DA is an amino acid analogue that is the main components of amnesic shellfish toxin (AST). It affects on glutamate receptor of hippocamupus region in brain which associates with memory coding and storing (Costa, Giordano, and Faustman 2010). BTX is typical neurotoxin which is generated by harmful algal such as *ptychodisusbrevis* and *gambierdiscums*. Moreover, BTX and CTX also work on sodium ion channel but them make sodium channel in an open state, which leads to the internal flow of sodium ions and depolarization of nerve cell membrane (Lombet, Bidard, and Lazdunski 1987). PLTX is a kind of polyether toxin, which selectively acts on sodium ion channel to make it open. In addition, the mechanism of action of AZT is not clear and needs to be explored further. The representative marine toxins structure was shown in Figure 1 and the representative, source, carriers, toxin effect and toxic symptoms of marine toxins was summarized in Table 1.

Marine toxins basically come from toxins emerged by harmful algal. With predation, algal toxins can transfer and accumulate in organisms and finally enter the human body via food chain (Figure 2). With the development of marine fishery, a variety of seafood (e.g. mussels, oysters, scallops, shrimps, crabs, and lobsters) enters people's tables and the seafood may be carriers of marine toxins. Accordingly, marine toxins poisoning gradually become a potential public health problem in the world. The contamination of marine toxins has posed a grave threat to water environmental quality, seafood quality and human health. Therefore, early diagnosis and detection of marine toxins have great significance to preventing food poisoning and economic losses.

Table 1. The representative, source, carriers, toxin effect and toxic symptoms of marine toxins.

Representative	Source	Carrier	Toxic effect	Toxic symptoms
STX	*A. catenella*, *Gonyaulax catenella*, and *A. tamarense*	Purple clam	Strong inhibitor of sodium ion channel	Paralysis, headache and respiratory arrest
OA	*Dinophysis* and *Prorocentrum*	Mussels, oysters, scallops, clams, shrimps, crabs and lobsters	Combine with protein phosphatase receptor	Diarrhea, hepatotoxicity and tumor promoter
DA	*Nitzschiapungen* and *Nitzschia pseudodelicatissima*	Mussels, oysters, scallops and clams	Works on glutamate receptor	Memory loss
BTX	*Ptychodisusbrevis* and *Gambierdiscums tox-incus*	Oysters, clams and mussels	Open sodium channel	Nerve palsy
TTX	Liver and ovary of puffer fish	Puffer fish, takifugu coronoidus and takifugu fasciatus	Strong inhibitor of sodium ion channel	Nerve center paralysis
CTX	*Gambierdiscus toxicus*, *Prorocentrum* and *Pyrocystis*	coral reef fish	Cholinesterase inhibition and open sodium channel	Vomiting, diarrhea, numbness and depression
PLTX	*Palythoa, Zoanthus, Ostreopsis* and *Trichodesmium*	Rock anemone and soft coral	Acts on sodium ion channel to make it open	Fever, cough, dyspnea, visceral hemorrhage, renal failure
AZT	*Protoperidinium*	Mytilusedulis	Not clear	Gastrointestinal disorders

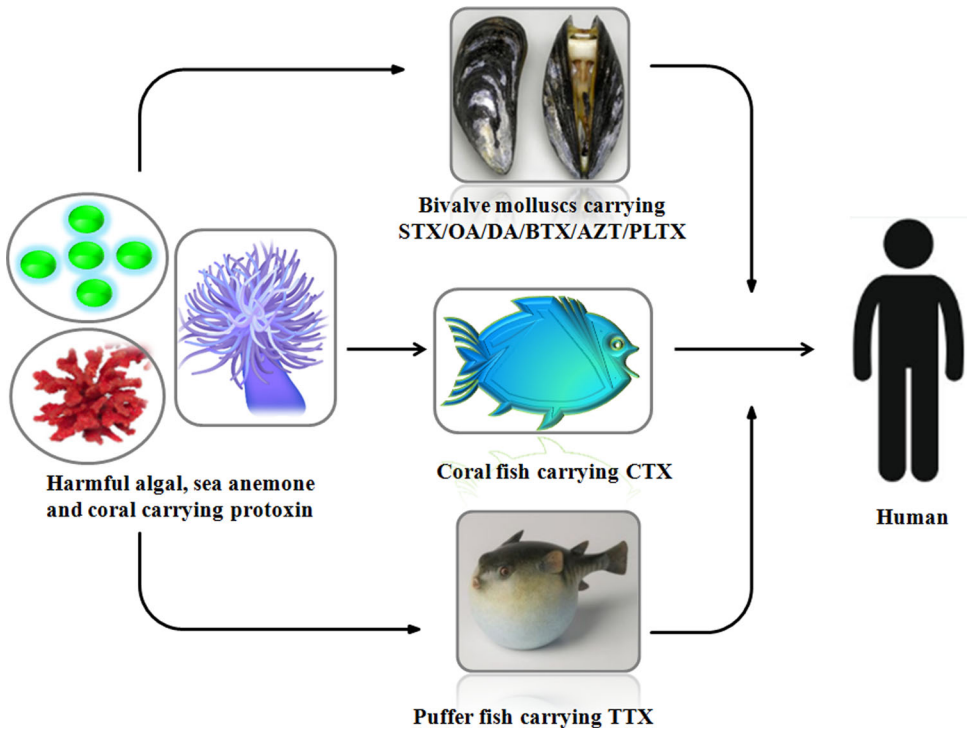

Figure 2. The transfer pathway of marine toxins in food chain.

Sensing strategies of biosensors

Biosensors are a kind of detector that convert target signal into detectable signal such as optical, electrical, quality signal. Biosensors are usually made up of bio-recognition component, signal converter and signal measurement system (Piro et al. 2016; Wang, Yang, and Wu 2020b). The sensitivity of biosensors largely depends on specificity and selectivity of bio-recognition element to targets. Aptamer, antibody and cell are common bio-materials as recognition component for marine toxins which have high selectivity and specificity for specific target substances (Fu, Chen, and Choo 2017; Gupta et al. 2019; Wu et al. 2020). The features of aptamer, antibody and cell were compared in Table 2. The conversion from bio-signal of targets to physical signals is a critical step. In biosensor, optical and electrochemical signal are common signal conversion technology. Materials that produce physical signals are often applied as converters and labeled on bio-recognition component. Nanomaterials such as gold/silver nanomaterials (Au/AgNPs) are employed as signal label owing to excellent optical and electrochemical properties (Pandey, Datta, and Malhotra 2008; Wongkaew et al. 2019). The duty of signal measurement system is quantized physical signals by testing equipment.

Here, we briefly discuss recognition elements of biosensor for micro-molecule marine toxins detection. Aptamers are short single-strand oligonucleotides that are screened from random library by systematic evolution of ligands by exponential enrichment (SELEX) technique (Stoltenburg, Reinemann, and Strehlitz 2007; Wu et al. 2020; Yan et al. 2019) in vitro. Due to few binding sites of small molecule targets, researchers have developed a variety of screening

Table 2. The comparison of bio-recognition component.

Feature	Cell	Antibody	Aptamer
Element	Reporter element	Recognition element	Recognition element
Target	Macro-molecule and micro-molecular	Macro-molecule	Macro-molecule and micro-molecular
Screening	Cell culture in vitro	In vivo	In vitro
Cost	Relatively high	Relatively high	Low
Storage	Short	Short	Long
Stability	Poor	Poor	Good
Modify	Difficult	Difficult	Easy
Sensitivity	Relatively high	High	High
Specificity	Low	High	High
Reusability	Low	Low	High

methods for small molecules by improving the traditional SELEX technology. The SELEX screening method of fixed targets was first applied to screen aptamer which was a separation method for aptamer by fixed targets on the surface of agarose granules or magnetic beads (Chao et al. 2015; Lato et al. 2002). However, the low coupling efficiency of micro-molecule targets and agarose granules or magnetic beads caused low screening efficiency owing to few functional groups for coupling on the surface of small molecular targets. Therefore, non-immobilized SELEX technologies such as capillary electrophoresis SELEX, capture SELEX and graphene oxide (GO) SELEX were gradually applied in screening aptamer for micromolecuar targets (Ozer, Pagano, and Lis 2014; Paniel et al. 2017; Zhuo et al. 2017). In particular, GO SELEX technique has accelerated the selection of aptamers crediting to the feature of GO which showed effective separation of single and double stranded nucleotides (Xing, Zhang, and Yang 2019). The recognition and binding of aptamers with their targets similar to the interaction of antibody–antigen. Unlike antibody, aptamers not only can be easily synthesized and modified in vitro but also have excellent stability to temperature and pH. In addition, aptasensors have been successfully applied in micro-molecules (e.g. mycotoxins, marine toxins, pesticides, and peptides) and macro-molecules (e.g. cell, protein, pathogen, and DNA) detection (Alizadeh et al. 2018; Hou et al. 2020; Piro et al. 2016; Torres-Chavolla and Alocilja 2009; Wang, Yang, and Wu 2020a).

Compare with antibody, aptamers as single-stranded oligonucleotides (10–100 bases) show high stability and are suitable for small molecule target. In addition, high affinity of aptamer to targets can match with antibody. Moreover, aptasensor is a very promising detection method and is expected to become the second most popular biosensor after immunosensor. At present, antibody-based kits have been widely used, but aptamers are mostly in the scientific research stage. There are few reports about the commercial application of biosensors in seafood safety. However, in other aspects, the application of biosensors is mature, such as blood glucose meter (http://www.bioscan.com). The first aptamer drug "Macugen" was approved by the US FDA in 2005 (Valigra 2005). Aptamers have successfully achieved targeted drug delivery in complex human environment (Yoshihiro et al. 2018), so aptamers and aptasensors may be competent for the detection task in food environment. Overall, we believe that biosensor can be used in seafood detection in the near future, as we envisioned in Figure 3.

Antibody occupies an important position in immunoassay which relies on specific recognition of antigen and antibody (Jin et al. 2019). Owing to high sensitivity and specificity, immunosensors as a mature detection method not only applied in food security, environmental detection and clinical medicine field but also have been put into commercial application on a large scale in recent decades (Felix and Angnes 2018; Zhu et al. 2019). Nevertheless, the preparation of antibody is time-consuming and expensive. Moreover, antibody as a protein has some characteristic of instabilities and poor storage qualities.

Cell as a living organism has high sensitivity, high-throughput, rapid response and role of information transmission and cell-based biosensor has been applied in detection of different targets (Banerjee, Kintzios, and Prabhakarpandian 2013). For example, there are transmembrane channels in cardiomyocyte which can produce transmembrane potential and excitation. Many marine toxins can combine with the receptors on ion channels and influence generation of action potential and conduction of excitation (Ferreira et al. 2018). Therefore, biosensors based on cell as recognition element are applicable for monitoring electric potential change caused by marine toxins. There is no denying that cell culture is time-consuming and tedious. In addition, it is very difficult to maintain cell activity for a long time. In particular, cell-based biosensor cannot achieve qualitative determination of toxins and easy to confuse results.

Aptasensor for marine toxins

Optical aptasensor for marine toxins

Due to intuitive reaction and fast response speed, optical sensing strategy has always been popular. Because of high sensitivity and quick response, fluorescence analysis has drawn great attention in recent decades and it is believed that this trend will be the same in the coming decades (Jeong et al. 2018). Fluorescent dyes (such as FAM, Cy3, and Cy5), quantum dots (QDs) and upconversion nanomaterials (UCNPs) are ordinary fluorescent sources. Fluorescence resonance energy transfer (FRET) and fluorescence signal switch-on strategies are applied commonly in biosensor for marine toxins monitoring.

Gu et al. constructed a fluorescence aptasensor based on rolling circle amplification (RCA) for OA detection (Gu et al. 2017; Figure 4). Biotin modified aptamers were immobilized on the streptavidin coating 96 microplate wells by

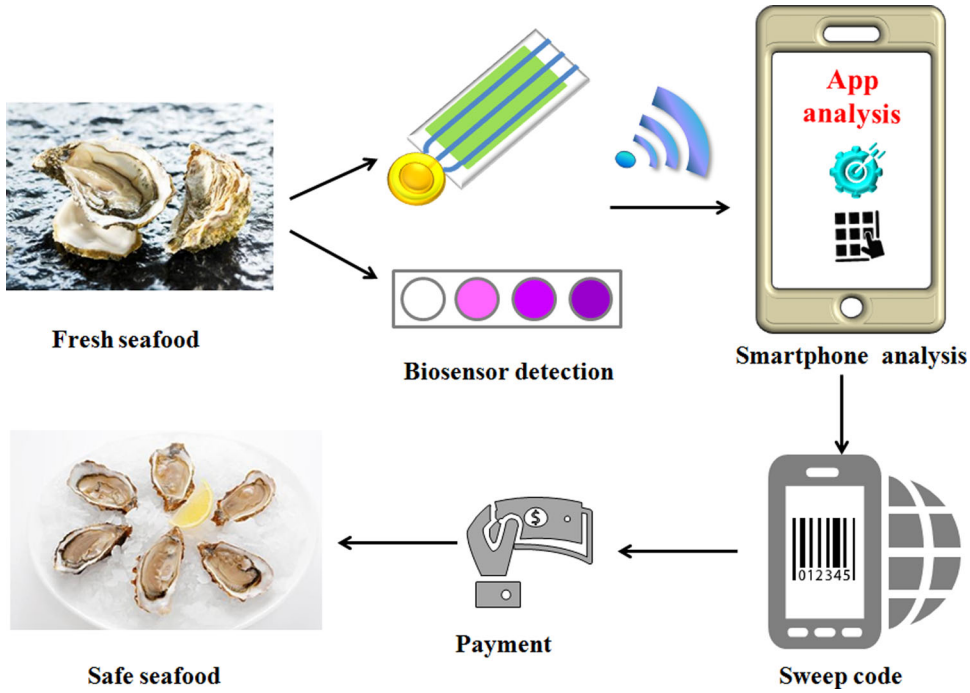

Figure 3. The prospect of ensuring seafood safe to spoon. Fresh seafood such as oysters, shrimps, crabs, puffer fish and so on may carry marine toxins which could cause seafood poisoning in different degrees. The combination of biosensors and smartphone can achieve in situ and rapid detection and portable devices. The test results are uploaded to network and showed in the form of two-dimensional code or bar code via smartphone App. People can scan the code with their smartphones to check the results of marine toxins detection in seafood and purchase qualified seafood from market.

streptavidin-biotin. A part of complementary chain of aptamer (cDNA) form double strands with aptamer, and another free part was stretched by RCA. A lot of FAM modified signal probes hybridized with the stretched strand, forming fluorescent double strands. In presence of target OA, OA would combine with aptamer to displace cDNA because of higher affinity of aptamer and OA than that of aptamer and cDNA. The fluorescent signal probe released from the microplate wells and produced intense fluorescence signal in displacement reaction. The aptamer for OA exhibited high sensitivity with linear detection range from 1 pg/mL to 100 ng/mL and LOD with 1 pg/mL under optimal conditions. In general, it was a good strategy to amplify the detection signal by RCA, but the stability and repeatability of the system may be poor due to the introduction of catalytic enzymes.

There are invalid binding sequences on the aptamers screened by SELEX technology. Some studies showed that the invalid binding sequences reduced the sensitivity and tissue penetration (Chinnappan et al. 2017; Manimala et al. 2004; Vu et al. 2017). Chinnappan et al. optimized the effective binding sequence of aptamers by truncating invalid sequence and fabricated a switch-on fluorescence aptasensor for OA detection (Chinnappan et al. 2019) (Figure 5). Here, the dissociation constant value (Kd) of truncated aptamers is lower than complete aptamer which means higher affinity of truncated aptamers. In this aptasensor, truncated aptamers hybridized with two short complementary DNA, one modified fluorescent dye (FAM) at 5′ terminal, another one modified quenching group (BHQ1) at 3′ terminal. With the formation of dsDNA, the fluorescence of FAM was quenched by BHQ1. After the presence of OA, the fluorescence of FAM stayed switch-on state because the distance between fluorescence probe and quencher probe separated from aptamers became longer. This aptasensor could monitor OA at concentrations as low as 39 pg/mL and showed the superiority of simple operation.

The multiple detection is the future development of food harmful micro-molecules detection technology and have advantage of simple operation, saving-time and low costs. For instance, Wu et al. published an aptasensor based on fluorescence resonance energy transfer (FRET) strategy for detection of dual targets, microcysin-LR (MC-LR) and OA (Wu et al. 2015). In FRET strategy, the green and red upconversion nanoparticles (UCNP) were recognized as fluorescence donor, and the quencher BHQ1 was used as fluorescence receptor. The poly acrylic acid capped UCNP was modified with avidin. Biotin labeled aptamers were further immobilized on the surface of UCNP via avidin-biotin. With the addition of BHQ1-complementary chain of aptamer (cDNA), BHQ1 could respectively quench the fluorescence of UCNP1 and UCNP2 due to the close distance. In the presence of dual targets, targets and cDNA could compete with anti-MC-LR aptamers and combine with aptamer. Then, cDNA was released and UCNP recovered the remarkable fluorescence. The LOD of MC-LR was 0.025 ng/mL and the LOD of OA was 0.05 ng/mL.

In addition to modifying fluorescent dyes, other luminescent materials can be introduced into DNA. For example, Cheng et al. developed a surface enhanced Raman scattering (SERS) aptasensor for detection STX using crystal violet as the Raman reporter (Cheng et al. 2019). Precious metals such gold and silver possess high SERS activity after surface roughening. In this method, anti-saxitoxin aptamers were

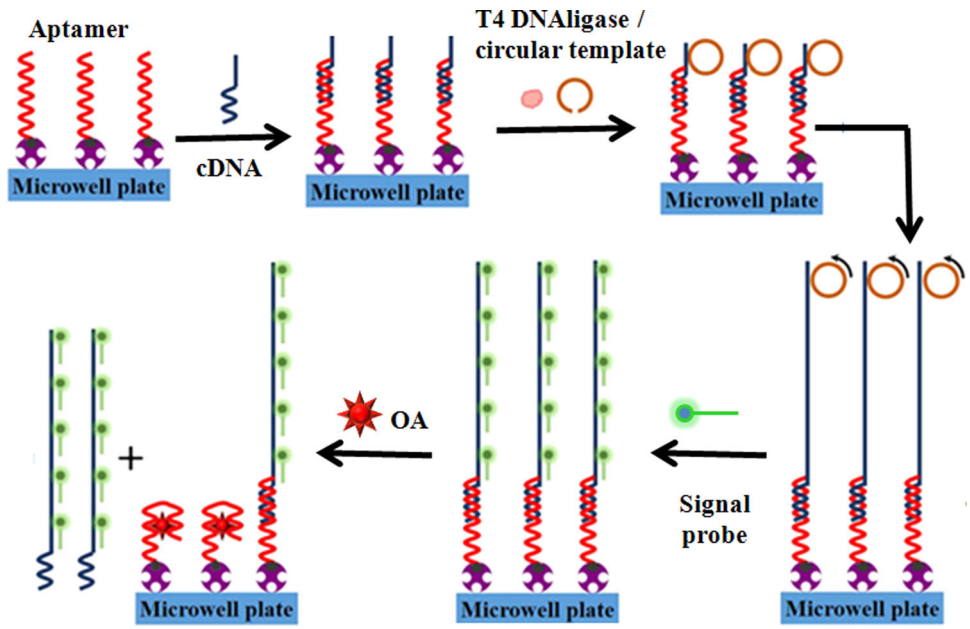

Figure 4. The scheme of fluorescence aptasensor based on RCA for OA detection (modified from Gu et al. 2017).

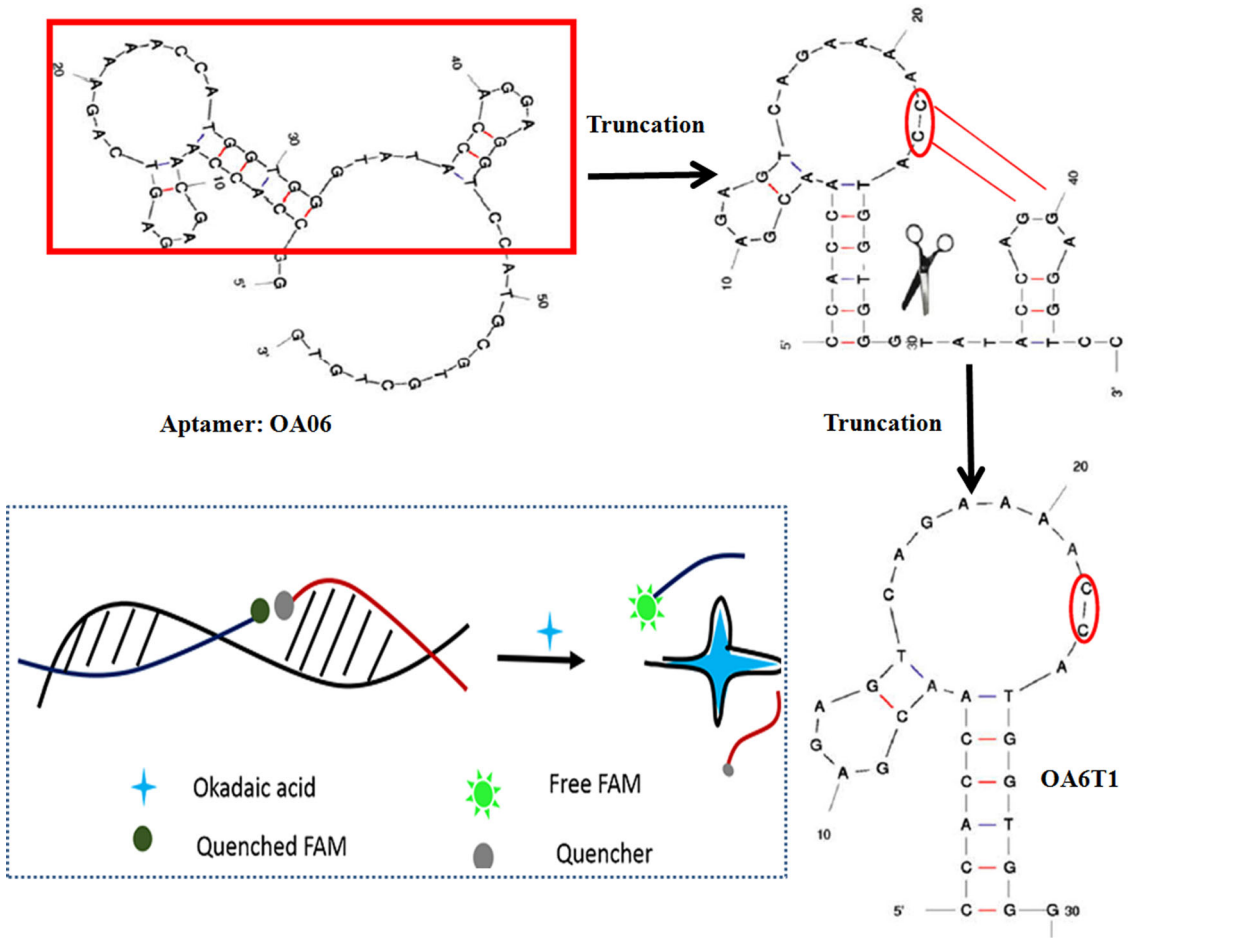

Figure 5. The truncating optimization process of full-length aptamer and the scheme of switch-on fluorescence aptasensor based on truncating aptamer for OA detection (modified from Chinnappan et al. 2019).

employed as capture probe and immobilized on AuNPs with Au–S interaction. Previous studies showed that crystal violet has the property of selective binding ability with the G-quadruplex (Guo et al. 2009). Therefore, crystal violet would combine with G-quadruplex structure aptamers and enhance Raman signal of AuNPs. As a result, this SERS-aptasensor has high sensitivity for saxitoxin with monitoring limitation of 11.7 nM. Lan et al. illustrated a label-free fluorescence

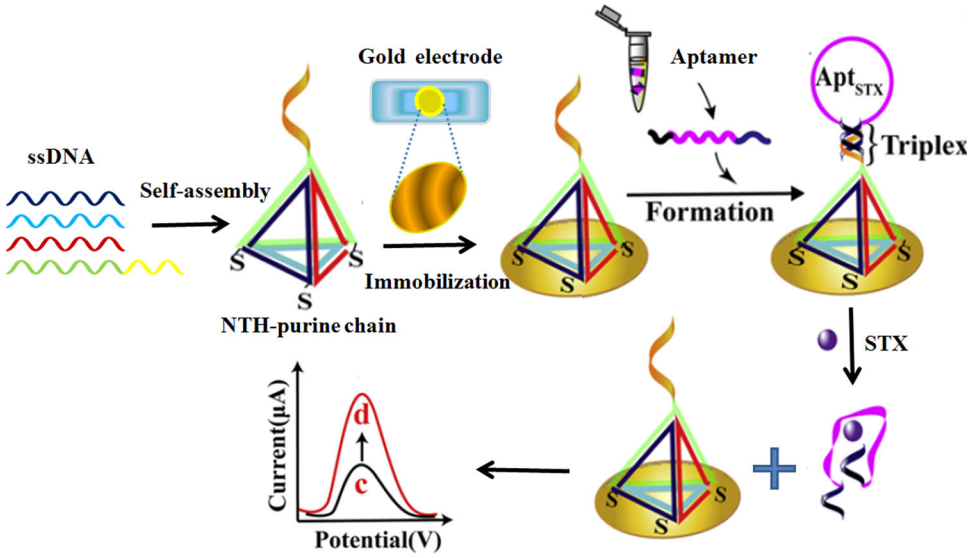

Figure 6. The scheme of electrochemical aptasensor based DNA nano-tetrahedron and DNA triplex helix for detection saxitoxin (modified from Qi et al. 2020).

aptasensor using berberine (BBR) as fluorescence source for TTX monitoring (Lan et al. 2019). BBR is an isoquinoline alkaloid that is a weak fluorescent substance and shows good binding ability with DNA. In this work, there was significant linear relationship between the fluorescence intensity and the concentration of TTX with working range of 0.1–500 nM and LOD was 0.074 nM.

Electrochemical aptasensor for marine toxins

Electrochemical biosensors show miniaturization, easy to operation and high sensitivity property that make them an outstanding candidate for the design of biosensor (Moro, De Wael, and Moretto 2019). Electrochemical techniques such as electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were applied to detect electric signal. EIS is a ratio of alternating current (AC) potential to current signal when an AC potential wave with different frequency is applied on system. CV is the recorded current-potential curve that the oxidation wave and reduction wave are obtained by applying the pulse voltage to the working electrode.

Wang's group reported that triple helix-based terminal fixation contributed to improve the affinity of aptamers to targets by reducing excessive flexibility of aptamers (Zhao et al. 2019). On this basis, this team member Qi et al. developed a electrochemical aptasensor based DNA nano-tetrahedron and DNA triplex helix for monitoring saxitoxin (STX) (Qi et al. 2020; Figure 6). In this work, the purine chain and aptamer-pyrimidine arms were assembled into hairpin structure of DNA triplex helix on the nano-tetrahedron that was fabricated by four ssDNA. The DNA nano-tetrahedrons were immobilized on the gold electrode. When STX was added, aptamer recognized and combined STX releasing from the gold electrode. The current signals of electrochemical aptasensor detection were measured by CV. The limit of detection for STX was 0.92 nM, and the detection range was 1–400 nM. The introduction of tetrahedral rigid structure greatly improves the performance of the aptasensor, and can be used to detect other targets by replacing aptamers.

Pan et al. developed an electrochemical aptasensor based on self-growth of AuNPs for detection OA (Pan et al. 2017). In this work, AuNPs were evenly distributed on the surface of microelectrode in interval state. AuNPs was blocked by negatively charged aptamer that absorbed on the surface of AuNPs. When added in OA, aptamers could combine with OA and released from AuNPs, then self-growth of AuNPs were catalyzed by reduction reaction. The amount of targets is proportional to the electrical signal generated by the self-growth of AuNPs. The LOD of this interesting aptasensor was as low as 1 ppb. Ramalingama et al. established microfluidic electrochemical aptasensor for detection of OA (Ramalingam et al. 2019). In presence of target OA, aptamer could capture and combine with targets on the surface of screen-printed carbon electrodes. Differential pulse voltammetry and CV were used for detecting the change of electronic signal. This aptasensor showed a low detection limit of 8 pM with working linear of 10–250 nM.

Immunosensor for marine toxins

In immunosensor, an interesting note was that peroxidase (e.g. horseradish peroxidase (HRP) and g-C3N4-PdNPs peroxidase) played a pivotal role in process of signal conversion. Here, colorimetric sensing strategies rely on the obvious color change based on variation of aggregation state of AuNPs, enzyme (e.g. HRPand alkaline phosphatase) catalysis and acid alkali indicator colorimetry (Chen et al. 2016; Leonardo et al. 2017; Xu et al. 2020). The results of colorimetric detection can be directly observed by naked eyes without any instruments. In addition, HRP is commonly used in colorimetric sensor for marine toxins detection. 3,3′-5,5′-tetramethylbenzidine (TMB) is a multifunctional substrate of peroxidase that could be used as hydrogen supply substrate and chromogenic substrate. The catalytic reaction of peroxidase not only released electrons

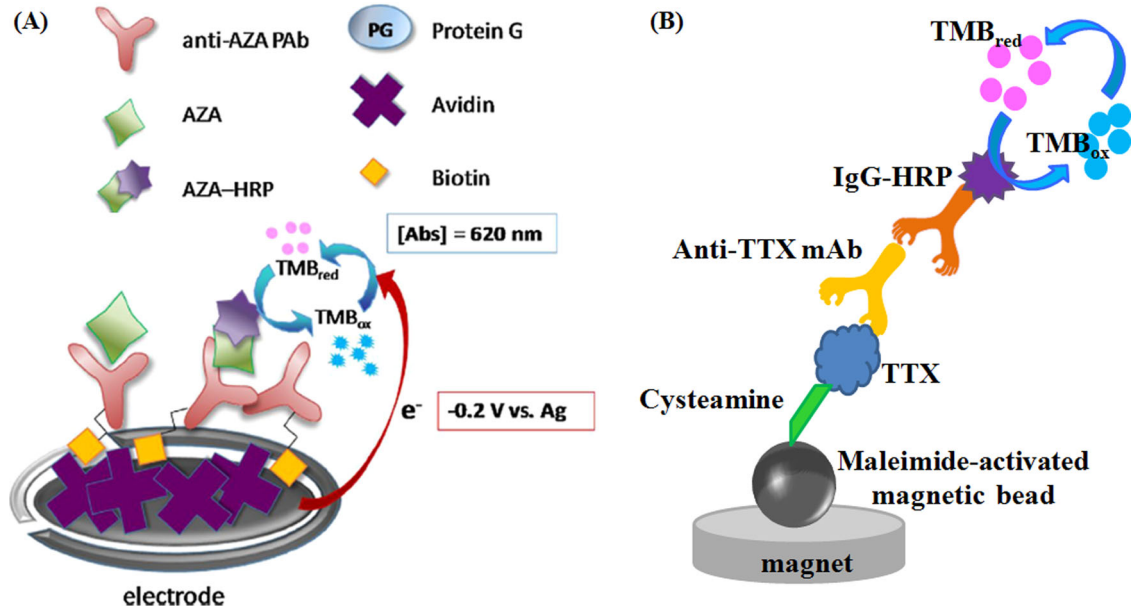

Figure 7. (A) The scheme of electrochemical and colorimetric immunosensor based on HRP for AZA monitoring. (B) The scheme of electrochemical immunosensor based on self-assembled monolayersu for TTX detection using magnetic bead as solid support (modified from Leonardo et al. 2018 and Reverte et al. 2017).

but also changed color from red to blue, realizing double signal conversion (Chen et al. 2018; Yun et al. 2019).

Leonardo et al. demonstrated an immunosensor employing protein G-coated magnetic beads (MBs) as holders for azaspiracid (AZA) detection (Leonardo et al. 2017). Here, HRP labeled AZAs were added in MBs with anti-AZA antibody. Then, colorimetric measurements and electrochemical measurements were performed after added TMB. Under optimal conditions, the LOD of AZA was found to be as low as 63 µg/kg with a linear from 120 to 2875 µg/kg. The sensitivity of the biosensor depends not only on the specificity of the antibody to the antigen, but also on the way the antibody is linked on the solid support. A good immobilization of the antibody on a solid support as far as possible to ensure that the conformation of the antibody is not changed. Furthermore, Leonardo et al. reported another immunosensor based on colorimetric and electrochemical immunoassays for AZA monitoring (Leonardo et al. 2018; Figure 7A). In this study, biotin modified antibody was immobilized on streptavidin labeled electrodes by avidin–-biotin. In this connection mode, this immunosensor showed more sensitivity AZA detection with a linear range from 46 ± 2 to 3079 ± 358 µg/kg.

In addition, small molecule targets were indirectly immobilized on solid base in the construction of biosensor. Reverte et al. developed an immunosensor based on self-assembled monolayer (SAM) for TTX detection (Reverte et al. 2017; Figure 7B). Herein, SAM was assembled by carboxylatedithiol and ethanolamine on screen-printed gold electrodes (SPGE) through Au–S bond. Then, anti-TTX antibody could capture TTX which was cross-linked with ethanolamine by formaldehyde cross-linking. IgG-HRP as secondary antibody was used as signal label and specifically combined with anti-TTX antibody. CV and colorimetric measurements were employed in monitoring signal convert. This method could sensitively monitored TTX with the LOD as lower as 0.07 mg/kg. Another example, SAM strategy as connecting bracket also used in immunosensor for TTX detection. The difference is that maleimide activated magnetic beads (MB) and cysteamine were assembled SAM. However, the LOD of TTX was 1.2 ng/mL with detection linear of 1.2–52.7 ng/mL.

Jin et al. developed an electrochemical immunosensor based on signal on strategy for the monitoring of STX (Jin et al. 2019). In this work, biotin labeled secondary antibody was fixed on the surface of avidin tagged MBs through avidin–biotin bond. Anti-STX antibody would capture and immobilize on MBs. When added in STX, antibody would combine with target STX. The g-C3N4-PdNPs peroxidase that possesses negative charges would adsorb by MBs-antibody-STX complex via electrostatic interaction. This peroxidase could catalyze oxidation of TMB mediated by H_2O_2 and released electric signal. The electric signal was detected by Zeta-potential measurements and electrochemical impedance spectra (EIS) measurements. Using this approach, the immunosensor could ultra-sensitively detect STX with LOD of 1.2 pg/mL in real sample. Antunes et al. fabricated an electrochemical immunosensor based on graphene for OA detection in seawater sample (Antunes et al. 2018). The graphene nanoplatelets have excellent electrochemical property and were dropped on the surface of field effect transistors (FET) forming Gr-FET complex. Next anti-OA antibody was mixed and cross-linked with Gr-FET. In the presence of OA, OA was captured by immobilized antibody blocking the electric signal. Compare to traditional methodology (ELISA), this immunosensor for OA showed a wide work linear range of 0.05–300 ng/mL and the LOD with 0.05 ng/mL.

Liu et al. established an isothermal cycling amplification strategy to monitor MC-LR using electrochemical immunosensor based on target-induced proximity reaction for DNA hybridization (Liu et al. 2015; Figure 8). Upon addition of target, the combination of three-way DNA structure was

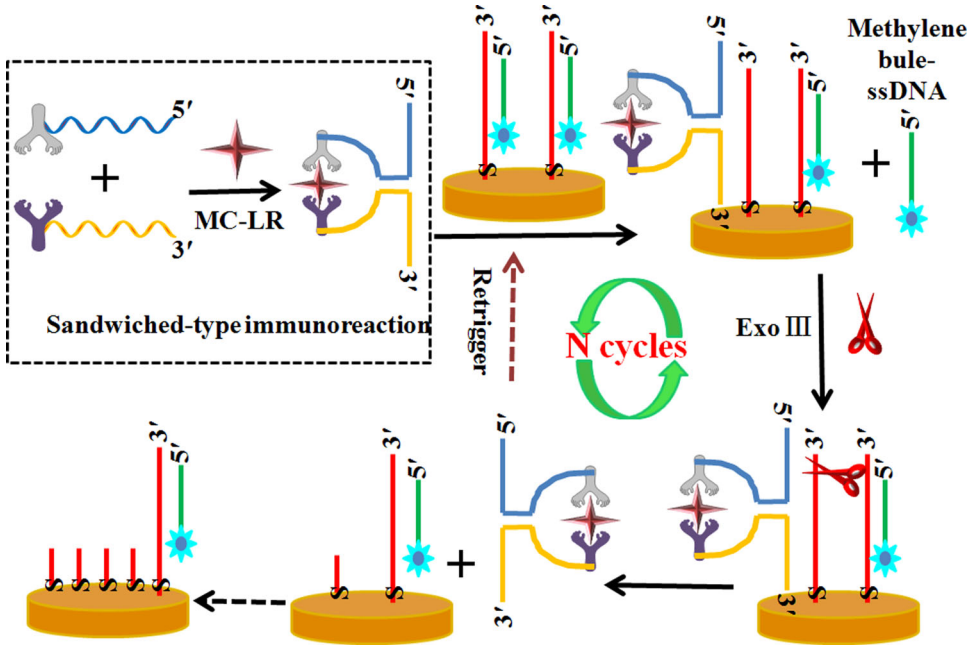

Figure 8. The scheme of electrochemical immunosensor based on exonuclease III-induced signal amplification strategy for detection MC-LR (modified from Liu et al. 2015).

triggered by the sandwiched-type immunoreaction of antibody and antigen complex (mAb_1-DNA_1/MC-LR/mAb_2-DNA_2). The hybrid chain formed by athiolated DNA (capture probe) and amethylene blue-labeled DNA (signal probe) is immobilized on the gold electrode surface via Au–S bond. The three-way DNA structure could combine with capture probe to displace signal probe because the binding of the former two is stronger than capture probe-signal probe. Then, exonuclease III mediated isothermal cycling amplification was triggered by the hybridization of three-way DNA and capture probe. The three-way complex could be freed and recombine with capture, realizing signal amplification. In this system, the LOD of MC-LR was as low as 1.0 ng/kg.

Zou et al. discussed a competitive electrochemical immunosensor using Love wave sensor as detection platform for OA detection (Zou et al. 2017). BSA-antibody was cross-linked on chip by staphylococcal protein A (SPA). When added in free OA and anti-OA antibody, free OA and immobilized BSA-OA would compete for antibody. Then the AuNPs-labeled IgG secondary antibody (AuNPs-IgG) was dropped in solution and capture the anti-OA antibody. The more free-OA was added, the less AuNPs was introduced. Finally, CV and EIS methods were applied in detecting electric signal. Using this approach, the detection limit for OA was as low as 5.5 ng/mL with linear range of 10–150 ng/mL. Similarly, Reverté et al. reported a novel competitive wave-guide immunosensor for TTX detection in puffer fish (Reverté et al. 2017). Firstly, TTX was conjugated with jeffamine-keyhole limpet hemocyanin (KLH) forming TTX complex. TTX complexes cross-linked with BSA and immobilized on planar wave-guide nanoarray. When added free TTX to solution, antibody would bind with free TTX instead of immobilized TTX. Therefore, the fluorescence signal of antibody immobilized on wave-guide nanoarray would be reduced. This immunosensor could selectively detect TTX in a linear range of 0.5–3.29 μg/g.

Cell-based biosensor

Cells are used as response elements coupling on signal converter in cell-based biosensor. Higher eukaryotic cells such as cardiomyocyte, hepatic (HepG2) cells, THP-1 cells and neuronal (Neuro2a) cells are commonly applied to detect marine toxins. In general, cells are cultured at 3 °C in humidified air with 5.0% CO_2 in an incubator. Cytotoxicity of marine toxins can be detected by the change of cell growth, cell morphology and beating status.

Cell-based impedance biosensor (CIB) is a technique that can achieve the monitoring of cell behavior in vitro. The attachment and growth of living cell on the surface of biosensor will vary the electric signal. Wang et al. established CIB for detection of STX and TTX (Wang et al. 2015a). In this work, cell was immobilized on the microtiter plate where gold electrode and circuit board were attached to the bottom of the plate. The sensitivity of biosensor was affected directly by the coupling rate of cells and electrode. The interdigitated electrodes could be used to detect the changes of cardiomyocyte morphology and beating status under the state of cell relaxation or contraction. In presence of targets, STX and TTX could block sodium channel and inhibit the generation of action potential and the transmission of electrical excitation of cardiomyocyte. The results showed that STX and TTX could slow down the beating rate of cardiomyocyte and STX has a stronger inhibitory effect. This method enables detection of STX and TTX at 0.087 and 89 ng/mL, respectively. OA combine with protein phosphatase receptor and inhibit its activity. Li et al. designed a CIB for OA detection (Li et al. 2015). The impedance biosensors were consisted of cell culture plate and circuit which

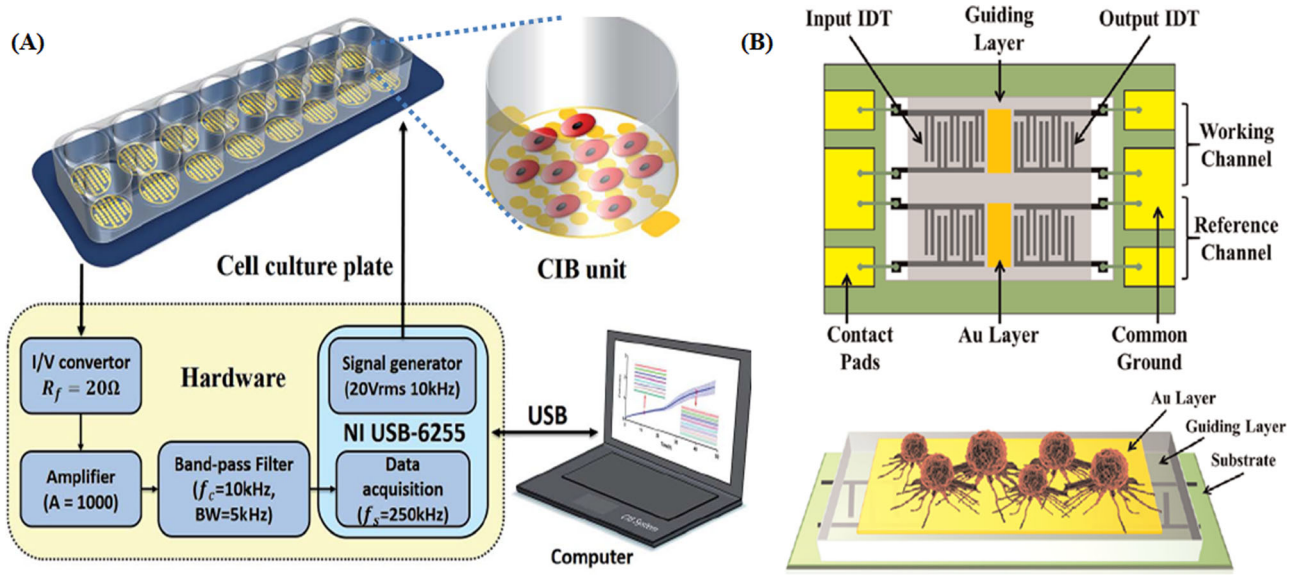

Figure 9. (A) The system structure of cell-based impedance biosensor for OA monitoring: cell culture plate, hardware system and computer process system (B) The structural representation of cell-based Love Wave sensor (modified from Li et al. 2015 and Zhang et al. 2016).

included glass substrate, Cr layer, Au layer and interdigital photoresist (Figure 9A). Neuro-2a cells was cultured on cell culture plate. Cellular growth was inhibited significantly by OA due to the inhibition of OA to protein phosphatase. This method provided a sensitive detection of OA with linear range of 20–100 μg/L.

Su et al. reported a cell viability biosensor (CVBS) based on smartphone for OA detection in situ (Su et al. 2018). Cell viability was used as signal response to OA. Within limits, the higher the concentration of OA, the lower the normalized cell viability index (NCVI). If the concentration of OA is too high, the cells would die. This method could selectively detect OA with LOD as low as 3.4083 μg/L.

Zhang et al. designed a cell-based on Love Wave biosensor for OA detection (Zhang et al. 2016). HepG2 cells were seeded and immobilized on 8-channel Love Wave sensor and this process would be monitored by Love Wave sensor via the change of acoustic waves (Figure 9B). When added in OA, it would be changed the cell morphology or forced cell to die. The insertion loss variations of cell had obviously positive correlation with the concentration of OA, and there was a good linear range of 10–100 μg/L. Deng et al. fabricated a cell based surface acoustic wave (SAW) sensor for OA detection (Deng et al. 2019). The mouse tongue isolated taste bud (MTITB) cells were seeded on electrode surface. In the presence of OA, MTITB cells had high sensitivity in carbamoyl toxin (CT) quantitative monitoring and cells fluctuating. In addition, the LOD of OA was 3×10^{-2} μg/100 g using this sensor.

Conclusion and outlook

With the expansion of fisheries and the changes of marine environment and global climate conditions, the outbreaks of harmful algae may produce a large of marine toxins. Therefore, strengthening the quality detection of seafood is a key step to ensure food safety. Herein, we have reviewed various biosensors for marine toxin detection and the prospect of ensuring seafood safe to spoon. The biosensor that used aptamer, antibody and cell as recognition or receptor component can sensitively detect micro-molecule targets in real sample. In the detection of marine toxins, biosensors show sensitive, rapid advantages compared to traditional detection methods, but biosensors still need to overcome drawbacks. Therefore, in order to ensure the safety of seafood, we need to improve all aspects of biosensors performance, and find a way to fabricate comprehensive aptasensors.

With the emergence of aptamer, aptasensors gradually occupy favorable place in the field of detection and diagnosis. However, there are three aspects should be improved. (1) The SELEX screening technology of aptamer to micro-molecule targets has large process room (more rapid and sensitive). (2) There are invalid binding sequences on the aptamers screened by SELEX technology. To optimized the effective binding sequence of full-length aptamers by truncating invalid sequence is contribute to improve the affinity of aptamer to target. We predict that the truncation of full-length aptamer will become a tendency after SELEX screening. (3) As for micro-molecule target, signal amplification strategy is an effective method to reduce the limitation of detection and improve the sensitivity of biosensor. Aptasensor combined with nucleic acid amplification strategies such as polymerase chain reaction (PCR), hybridization chain reaction (HCR), RCA, recombinase polymerase amplification (RPA) and DNA walker structure will amplify detection signals and enhance highly the sensitivity for detection marine toxins. In short, although there are some areas that need to be improved, the development potential of aptamers and aptasensor is undeniable.

Disclosure statement

The authors declare no conflicts of interest.

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ORCID

Wei Wu http://orcid.org/0000-0002-1772-3841

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