

A brief review of novel nucleic acid test biosensors and their application prospects for salmonids viral diseases detection

Xiaofei Liu¹, Songyin Qiu¹, Haiping Fang², Lin Mei¹, Hongli Jing¹, Chunyan Feng¹, Shaoqiang Wu^{1,†}, and Xiangmei Lin^{1,†}

¹Institute of Animal Inspection and Quarantine, Chinese Academy of Inspection and Quarantine, Beijing 100176, China

²Research Center of GACC for international Inspection and Quarantine Standards and Technical Regulations, Beijing 100013, China

Abstract: Viral diseases represent one of the major threats for salmonids aquaculture. Early detection and identification of viral pathogens is the main prerequisite prior to undertaking effective prevention and control measures. Rapid, sensitive, efficient and portable detection method is highly essential for fish viral diseases detection. Biosensor strategies are highly prevalent and fulfill the expanding demands of on-site detection with fast response, cost-effectiveness, high sensitivity, and selectivity. With the development of material science, the nucleic acid biosensors fabricated by semiconductor have shown great potential in rapid and early detection or screening for diseases at salmonids fisheries. This paper reviews the current detection development of salmonids viral diseases. The present limitations and challenges of salmonids virus diseases surveillance and early detection are presented. Novel nucleic acid semiconductor biosensors are briefly reviewed. The perspective and potential application of biosensors in the on-site detection of salmonids diseases are discussed.

Key words: salmonids virus; detection; nucleic acid test; biosensors; semiconductor

Citation: X F Liu, S Y Qiu, H P Fang, L Mei, H L Jing, C Y Feng, S Q Wu, and X M Lin, A brief review of novel nucleic acid test biosensors and their application prospects for salmonids viral diseases detection[J]. *J. Semicond.*, 2023, 44(2), 023103. <https://doi.org/10.1088/1674-4926/44/2/023103>

1. Introduction

Salmonids is a typical cold-water fish with high economic value and promising market prospects. As one of the important economic fish in the world^[1], salmonids have been farmed in China for more than 50 years, and the current farmed species include more than 10 species such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), silver salmon (*Oncorhynchus kisutch*), golden trout (*Oncorhynchus mykiss*, albino phenotype) and Siberian Taimen (*Hucho taimen*). The scale of farmed salmonids in China is expanding year by year, but the occurrence of viral diseases remains a significant limited factor in aquaculture production^[2]. The major viral diseases of salmonids are viral hemorrhagic septicemia (VHS), infectious hematopoietic necrosis (IHN), infectious pancreatic necrosis (IPN) and infection with salmonid alphavirus (ISA)^[3]. Infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) are of particular concern for the farmed salmonids in China. Infection caused by each virus has features including rapid transmission, rapid onset, high mortality and difficulty in treatment. They have seriously affected the development of the farmed salmonids industry of China. It is significant that disease causing agents are rapidly identified and differentiated^[4]. For this reason, early and accurate nucleic acid detection is crucial and essential in preventing and controlling the spread of salmonids diseases^[5, 6].

The biosensors have attracted the researchers due to su-

perior performance in sensitivity, low sample, high throughput and potential for miniaturization^[7] and have been widely explored for disease detection, environmental monitoring, food quality monitoring and many other aspects^[8–10]. Biosensors consist of two main components: recognition element and transducer. According to the type of the recognition element, biosensors can be divided into nucleic acid sensors, immunosensors, cell sensors, enzyme sensors, etc. It can be categorized into optical, electrochemical, and piezoelectric sensors by the transducer^[11]. With the recent development of semiconductors, nanomaterials and microfabrication technologies, semiconductor materials are widely used in biosensing. A variety of novel semiconductor biosensors for pathogen detection with better performance were explored^[12]. The nucleic acid biosensors containing semiconductor materials are the most promising tools for early and accurate on-site detection of salmonids viral diseases.

Here, we give a brief description of the current development of salmonids viral diseases detection. And then the present limitations and challenges of early detection for salmonids viral diseases are proposed. Finally, advances of nucleic acid semiconductor biosensors which have potential to apply for on-site early detection of salmonids viral diseases are introduced and the prospects are put forwarded.

2. Salmonids viral diseases detection developments

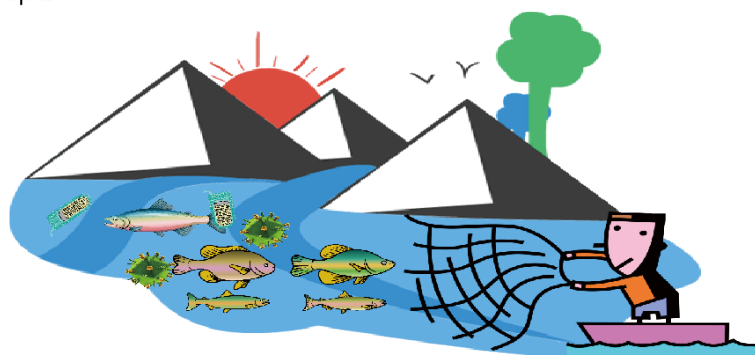
In 2021, the economic losses caused by diseases in China's aquaculture were about 53.9 billion RMB. Due to the rapid transmission, high mortality and difficulty in treatment of salmonids viruses, the viral diseases outbreaks have resul-

Correspondence to: S Q Wu, sqwu@sina.com; X M Lin, linxm@caiq.org.cn

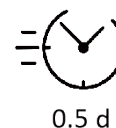
Received 18 DECEMBER 2022; Revised 18 JANUARY 2023.

©2023 Chinese Institute of Electronics

Step 1



Live fish collection

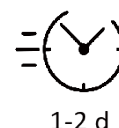


0.5 d

Step 2

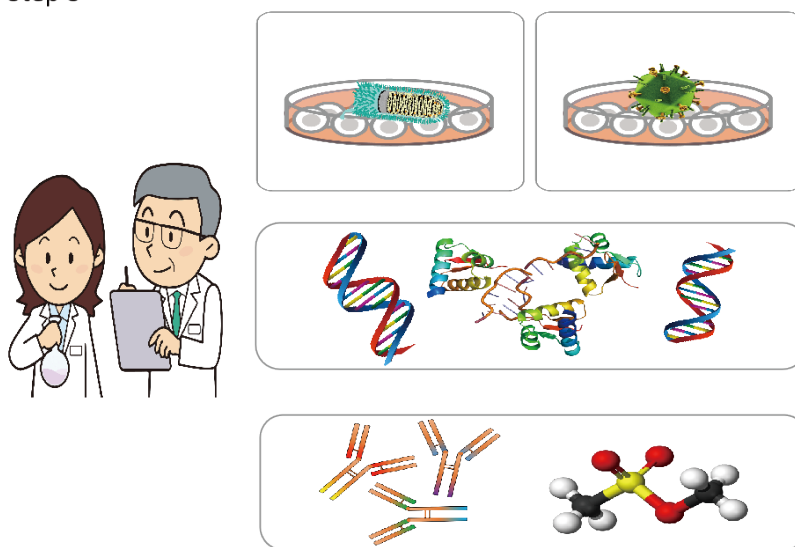


Live fish transportation

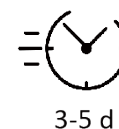


1-2 d

Step 3

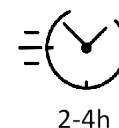


Cell culture



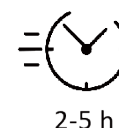
3-5 d

Nucleic acid tests



2-4 h

Immunological methods



2-5 h

Fig. 1. (Color online) The traditional workflow of salmonids diseases detection and surveillance.

ted in significant economic losses for salmonid fishes. IHN and IPN are the most prevalent two viral diseases of farmed salmonids in China. IHN caused by IHNV is one of the most contagious viral diseases in salmonids that should be reported to the World Organization for Animal Health (WOAH), which can lead to significant mortality rates and huge economic losses on the salmonid farming industry^[13–15]. The natural host range of IHNV includes many species of wild and farmed salmon and trout^[16, 17]. Mortality caused by IHNV can be substantial, even approaching 100%. IPN is a highly contagious disease that affects salmonids reared in intensive culture systems whose etiological agent is IPNV^[18]. It was first isolated in

China in 1984^[19] and can cause higher than 90% mortality in fry and juvenile trout and salmon and lower mortality in adult fish^[20]. Survived adult fish can become asymptomatic carriers of the virus, eventually shedding the virus particles to the environment as a source of horizontal transmission^[2, 3]. Early detection and depopulation of the infected fishes are the most common practices to control the spread of IHNV and IPNV^[21].

Detection and surveillance on farmed salmonids diseases in China mainly follows the traditional workflow which transport live fish to the testing laboratory (Fig. 1). The first step is to collect samples. Live symptomatic or healthy fishes

are caught from salmonids farming sites such as ponds, reservoirs, cages and other aquatic animal breeding sites. The second step is transportation. The samples need to be transported to the testing laboratory within 48 h. The sampled fishes need to be preserved in oxygenated devices to ensure survival and need to be kept at 0–10 °C throughout. Finally, after the samples arrived at the laboratories, pathogen screening and testing are carried out.

The tests of salmonids viruses used in the laboratory mainly are the traditional methods including virological, immunological and nucleic acid test methods. The viral culture needs too much time for isolating and authenticating. Moreover, there is no sensitive cell lines for some viruses of aquatic animals^[2, 4]. Enzyme-linked immunosorbent assay (ELISA) is the most typical immunological method, which could detect virus antigen or antibody. But for fish virus, ELISA is not suitable for early detection because virus load is low and antibody is not available in the early stage of fish virus infection^[11]. Thereby, the immunological method is hysteretic for fish virus detection. In contrast, the nucleic acid test is a timely and sensitive method since nucleic acid can be detected within 24 h of infection^[22]. It can also provide faster diagnosis and multiplexing options for the detection of several viruses at the same time^[23]. The reverse transcription-polymerase chain reaction (RT-PCR), RT-quantitative PCR (RT-qPCR) and RT-droplet digital PCR (RT-ddPCR) assays have been developed to qualitative or quantitative detection of IHNV or IPNV^[5, 18, 24–32]. Pinheiro *et al.* developed a multiplex RT-PCR for simultaneous detection of major viruses that infect rainbow trout, including IHNV, IPNV and VHSV^[3]. And RT-qPCR is recommended for IHNV detection and surveillance as the validated method by WOAHI^[5, 15, 26, 33], Suebsing^[34, 35] and Gunimaladevi *et al.*^[36] developed the reverse transcription loop-mediated isothermal amplification (RT-LAMP) to detect IHNV, with sensitivity 10-fold higher than nested PCR. The RT-LAMP methods were also successfully developed to detect the IPNV^[20, 34, 37]. Rodriguez *et al.*^[4] evaluated the five serological methods and RT-PCR and found that RT-PCR were the most appropriate and sensitive methods for the routine detection of IPNV from affected fish. Therefore, the nucleic acid test is the most commonly used detection method in the diagnosis of aquaculture viral pathogens.

3. Salmonids viral diseases detection limits and challenges

The current salmonids virus detection and monitoring mode are time-consuming, high cost and limited to laboratory. Firstly, the traditional monitoring mode consumes lengthy time and financial costs during the transportation of samples because aquaculture farms are usually located in lakes, reservoirs and sea areas far from towns and transportation arteries. And the temperature, dissolved oxygen, pH and other conditions of the water during transportation are difficult to control, which are distinct from the living environment of fish and virus, leading to the inaccurate results. This will affect the fish farmers take timely and effective prevention and control measures. In addition, as stated, the widely applied detection method in practice concentrated on the traditional nucleic acid-based methods. However, their detection is too traditional and cumbersome to detect on field. Sophisticated instruments, high power condition, long time and profes-

sional staff cause traditional RT-PCR or RT-qPCR non-amenable for on-site and real-time detection^[11].

The limitations and problems existing related to the above-mentioned encourage researchers to discover and develop more robust and accurate detection systems for efficient on-site detection and screening^[38, 39]. To achieve the goal of early detection and timely control, rapid, low-cost, portable with high sensitivity and specificity, nucleic acid detection methods are highly demanded for on-site detection of virus infection early stage. The biosensors are seen as suitable candidates which have fast response, cost-effectiveness, high sensitivity and selectivity for early detection and surveillance of salmonids viral diseases. Therefore, the nucleic acid test combined with sensing technology are more preferred for accurate and on-site detection. Semiconducting materials make the biosensors more accurate and sensitive due to their advantageous properties such as chemical and mechanical stability, high biocompatibility, unique photoelectric properties and sensitive response^[40]. The following section will focus on introducing the developed nucleic acid test-based biosensors which were fabricated by semiconducting materials.

4. Nucleic acid semiconductor biosensors

Semiconductor materials such as silicon (Si), TiO₂, quantum dots (QDs) and indium tin oxide (ITO) are widely used in biosensors. Researchers have developed highly sensitive nucleic acid amplification biosensors and amplification-free biosensors based on the properties of semiconductor materials. In addition, microfluidics researches on on-site detection highlights integrated sample-to-detection chip for nucleic acid test assays. They can realize nucleic acid tests implemented on a portable, miniaturized, and integrated device with rapid and sensitive results readout^[41, 42]. The integration of microfluidic technology and biosensors can also become a promising tool to shorten detection time and achieve high-throughput detection^[43–45].

4.1. Nucleic acid amplification biosensors

In combination with classical PCR amplification and isothermal amplification technology such as LAMP, helicase dependent amplification (HDA) which are more suitable for on-site detection^[46], many biosensors based on nucleic acid amplification were developed.

Si is the most commonly used material in large-scale semiconductor biosensors manufacturing, which can greatly reduce the fabricating costs. A portable and battery-powered PCR device which integrated a PCR thermal control system, a PCR reaction chip and the end-point fluorescence detection system was developed by Jie *et al.* The disposable reaction chips were fabricated with a silicon substrate due to its excellent thermal conductivity, silicone rubber and a quartz plate. The PCR thermal control system was developed using Al thin-film heaters and RTDs to provide fast and precise temperature conditions with an average heating rate of 32 °C/s and an average cooling rate of 7.5 °C/s. The time consumption of the 40 cycles of PCR was less than 1 h^[47]. Sun *et al.* realized to detect five equine respiratory diseases pathogens simultaneously on a silicon microfluidic chip using digital LAMP. Silicon chips are highly stable, have no auto-fluorescence and can be manufactured easily and efficiently. The chips contained ten parallel flow channels with a volume of 1 μL each.

The chip surface was thermally oxidized to grow a 200-nm layer of SiO₂ that used to reduce the potential for bare silicon to inhibit the amplification process. The detection took place within 30 min with LOD of 18 copies per reaction^[48]. Powell and colleagues developed rapid and sensitive nucleic acid amplification tests for HCV, HIV, Zika, HPV16 and HPV18 on the same silicon microchip platform integrated nucleic acid extraction and PCR. These on-chip assays were performed in under 25 min and the efficiency was similar to the assay performed on a bench scale real-time PCR instrument^[49]. Nunez-Bajo and co-workers reported a disposable, ultra-low-cost integrated silicon-based on-site transducer (TriSilix) for pathogen nucleic acid amplification and real-time detection. Si was selected as the substrate material to make itself can be used both as an electrical heater and temperature sensor without adding any additional components for temperature transduction. Both isothermal and cyclic nucleic acid amplification can be performed and it integrated temperature control and DNA detection on the same device, which was label-free, requires minimal sample handling. And the TriSilix chips did not need cleanroom for fabrication and can be produced in a standard wet-lab with easily accessible laboratory equipment worldwide. TriSilix was an ideally low-cost, high-performance, on-site nucleic acid detection technology^[50] (Fig. 2).

ITO is a conductive and transparent oxide that has advantages of high stability, ease of combination with microfabrication processes, which is used as an electrode material for biosensors^[51]. Barreda-Garcia and co-workers utilized the advantages of ITO to develop an optimized and nucleic acid amplification-based biosensor for detecting salmonella. They designed an electrochemical DNA sensor based on a sandwich hybridization assay on the surface of ITO electrodes, which realizes monitoring both PCR and HDA amplicons. ITO was used as a thin film heating element and the reaction surface^[52, 53].

Molybdenum disulfide (MoS₂) is a typical transition-metal dichalcogenide semiconductor which has unique optical, electronic and catalytic properties. Zang and coauthors developed a CdS/MoS₂ heterojunction-based photoelectrochemical DNA biosensor. The CdS/MoS₂ photocathode was produced by the stepwise assembly of MoS₂ and CdS QDs on ITO. The dual hemin-labeled DNA probe was introduced on high-efficiency CdS/MoS₂ electrode via catalytic hairpin assembly (CHA) cycling reaction. This ultrasensitive biosensor achieved a detection limit of 0.39 fM^[54] (Fig. 2).

4.2. Nucleic acid amplification-free biosensors

The principle of nucleic acid amplification-free biosensors depends on the hybridization through spontaneous hydrogen bonding between the target nucleic acid and its complementary strand^[55]. The biosensors are usually utilized by immobilizing the single-stranded DNA onto a suitable surface. Au nanorods, CdTe QDs and metallic oxide and so on are growing frequently used to fabricate sensor surface as a promising semiconductor material.

A biosensor for the Cauliflower Mosaic Virus 35S (CaMV35S) promoter detection was reported to distinguish transgenic soybean from non-transgenic samples. The Au nanoparticles-reduced graphene oxide acted as a nanocarrier to immobilize the thiol-functional probe, and the SiO₂@CdTe QDs core-shell nanoparticles tagged with the amino-functional probe acted as signal indicators, respectively. In the pres-

ence of target DNA of CaMV35S, the binding of tDNA with two probes through the high specific DNA hybridization led to the fabrication of sandwich structure, and thus the high loading of the signal indicators SiO₂@CdTe QDs at the electrode surface, which increased the PEC signal. The increased signal depended on the concentration of tDNA and the low detection limit of 0.05 pM was obtained^[56] (Fig. 3). Zhang *et al.* found that monolayer MoS₂ has high fluorescence quenching efficiency and shows different affinity towards the single-stranded DNA and double stranded DNA. Therefore, a thin-layer MoS₂ nanosheets based and label-free DNA sensor was constructed, with a detection limit of 1.9×10^{-17} M^[58, 59] (Fig. 3).

TiO₂ is a classical n-type semiconductor, its production method is simple. The raw materials are cheap and easily available. More importantly, it has good biocompatibility, excellent photostability, fast charge conduction rate and excellent photoelectric chemical activity. Many biosensors have been achieved high-performance based on TiO₂^[60]. A photoelectrochemical DNA sensor based on FTO/TiO₂/Au electrode was successfully developed by Liu and coworkers. Combining the small band gap of CdSe QDs with the large band gap of TiO₂, the photoelectric conversion efficiency was enhanced. The limit of detection was 3 fM^[61]. Tang's research group modified the electrode surface with the defect-engineered TiO_{2-x} nanorods as photoelectric materials. A photoelectrochemical biosensor platform was constructed for sensitive analysis of target DNA with the surface plasmon resonance enhancement technology of Au nanoparticles. The introduction of Au NPs can not only further enhance the photoelectric signal response but also improve the analytical performance of the sensor on the target DNA. This signaling format combined with exonuclease III-assisted target recycling amplification shows excellent analytical performance^[57] (Fig. 3).

Chen and co-workers developed a nanostructured impedance biosensor to detect hepatitis B virus (HBV) DNA. The anodic aluminum oxide membrane having a uniform nano hemisphere array was selected as the substrate of the sensing electrode. A 30-nm gold film was sputtered onto the Al₂O₃ layer surface as the electrode, followed by electrochemical deposition of Au nanoparticles on each hemisphere surface. Target HBV DNA was captured by hybridization with the specific probe which immobilized on the nanostructured electrode. The LOD was measured to be 111 copy/mL^[62]. Zhu *et al.* developed a DNA sensor to detect HBV with a Cu₂O hollow microsphere that can be rapidly synthesized at room temperature. The device has a sensitive detection response and good selectivity for hybridization detection with a detection limit of 1.0×10^{-10} M. This is mainly due to the high specific surface area of Cu₂O nanoparticles and the high probe affinity of the particle surface to ssDNA^[63]. Shariati *et al.* reported an ITO nanowires based DNA biosensor compatible with metal-oxide-semiconductor (CMOS) technology to detect HBV. The immobilization of thiolated HBV single-strand DNA is supported with the employment of ITO nanowires reaching the detection limit of 1 fM^[64] (Fig. 3). Shariati's group also developed the HBV detection biosensor whose heterojunction optical electrodes based on WO₃/In₂O₃ nanowires doped with ITO with high sensitivity and stability^[65]. Manzano and co-workers reported a label-free user-friendly electrochemical DNA biosensor for hepatitis A virus (HAV). The hybridization reaction occurs on the disposable electrode made of gold functional-

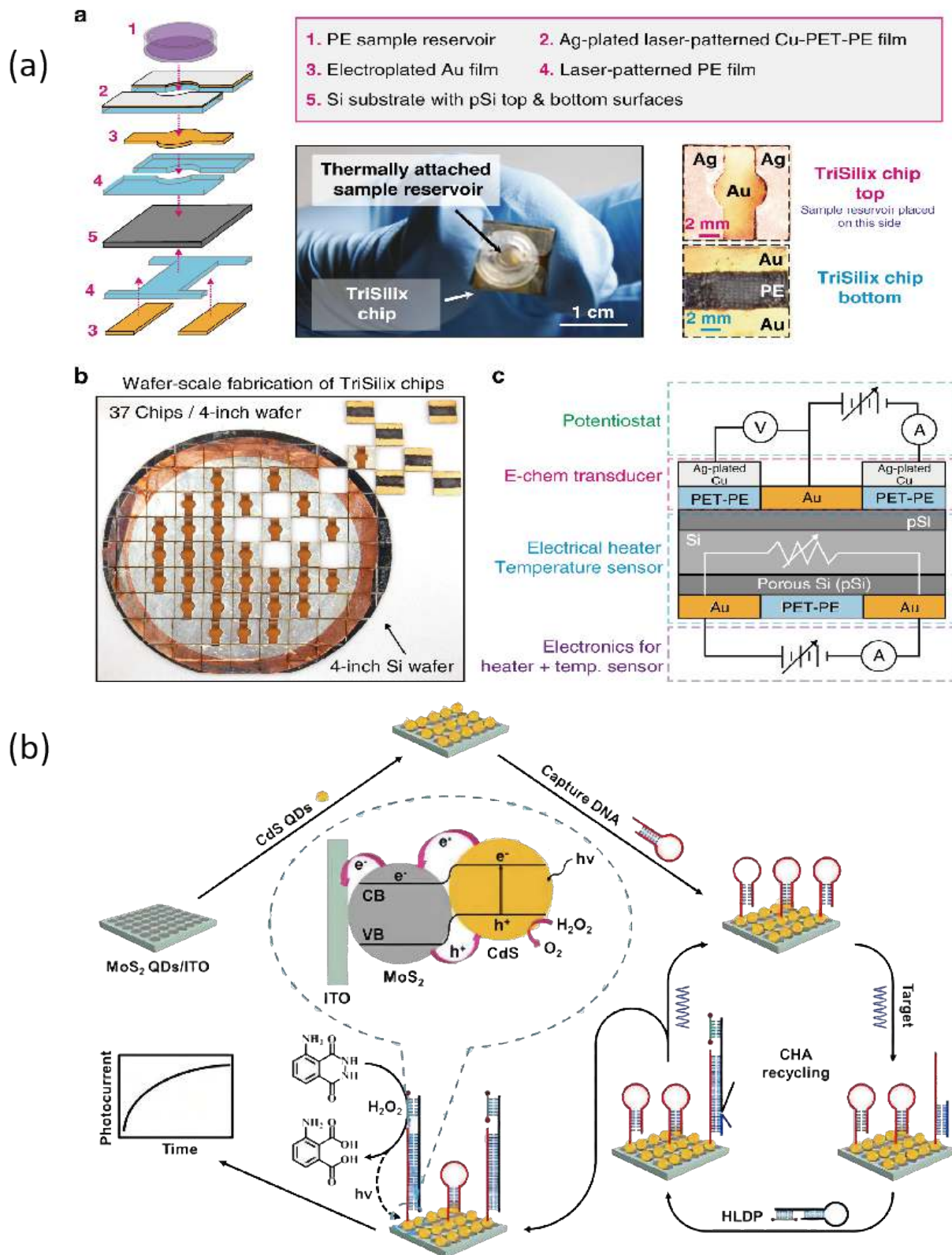


Fig. 2. (Color online) (a) Schematic illustration of a TriSilix chip and photographs of the actual device^[50]. Reproduced with permission. Copyright 2020, Springer Nature. (b) Schematic illustration for CdS/MoS₂ heterojunction-enhanced photoelectrochemical sensing strategy coupling with CHA and hemin-mediated chemiluminescence^[54]. Reproduced with permission. Copyright 2016, Elsevier.

ized with specific capture probes. The results are monitored through the oxidative peak potential by using cyclic voltammetry^[66].

5. Perspectives and conclusions

Considerable efforts of researches demonstrate the utility of robust nucleic acid tests biosensors with semiconduct-

or for development of a flexible nucleic acid diagnostic platform. With the advantages of semiconductor materials, these biosensors have their own characteristics in rapidity, low cost, high sensitivity and specificity. Accurate and sensitive bioanalysis of nucleic acids is crucial for earlier detection of salmonids viral diseases. Currently, there are no practical applications of semiconductor-based biosensors for salmonids virus

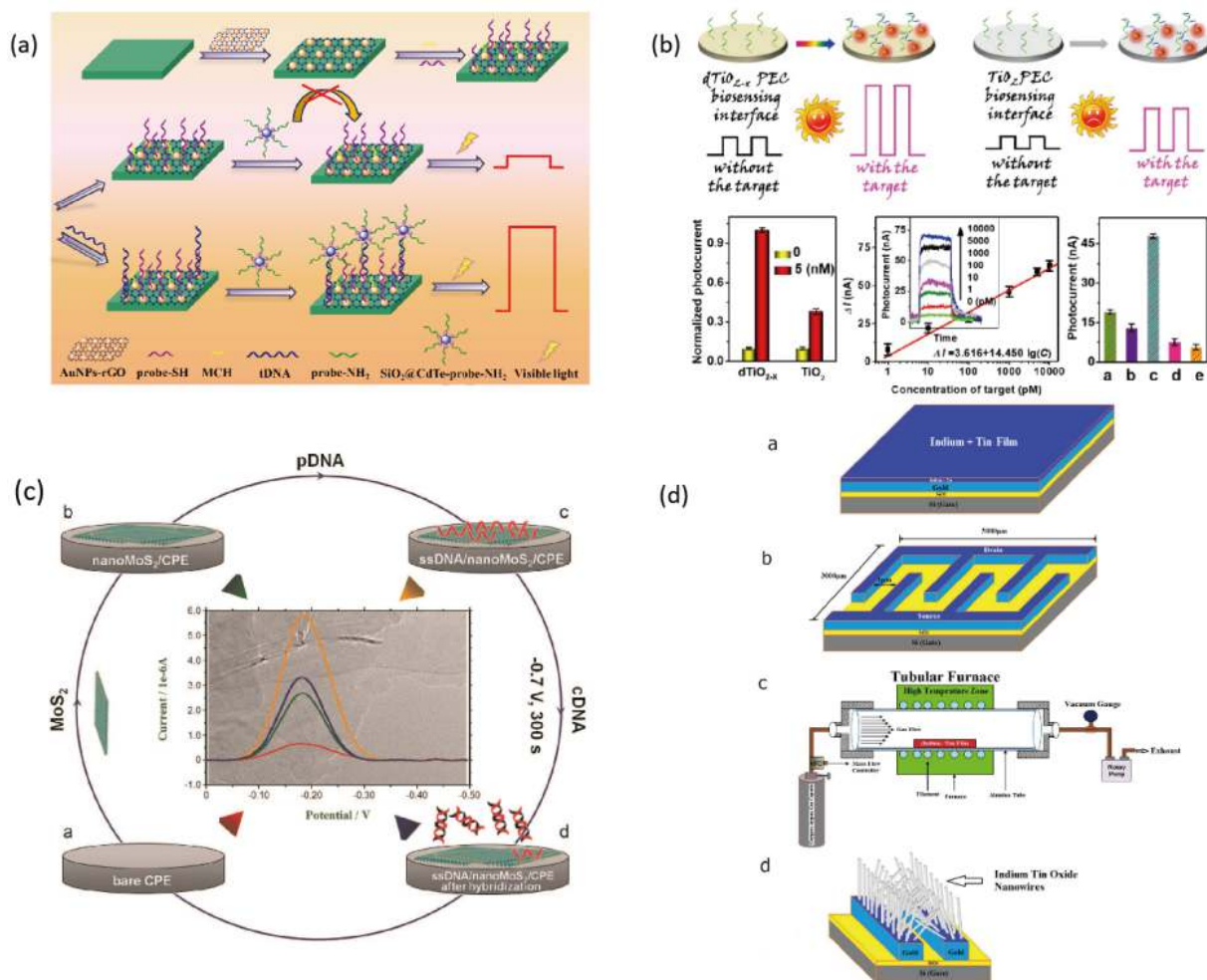


Fig. 3. (Color online) (a) Schematic illustration of the fabrication procedure of the CaMV35S promoter detection biosensor based on the application of SiO₂@CdTe QDs^[56]. Reproduced with permission. Copyright 2016, Elsevier. (b) Schematic illustration of biosensor strategies based on the application of dTiO_{2-x}^[57]. Reproduced with permission. Copyright 2018, ACS. (c) Schematic illustration of the label-free electrochemical DNA assay based on nanoMoS₂ modified electrode (nanoMoS₂/CPE)^[59]. Reproduced with permission. Copyright 2015, Elsevier. (d) Schematic illustration of the fabrication of ITO nanowires device^[64]. Reproduced with permission. Copyright 2018, Elsevier.

detection, but these biosensors have great potential application prospects for the diagnosis of salmonids virus infections in the field. Their portable, rapid and sensitive prosperities could meet the needs of salmonids diseases detection and achieve the goal of early detection of salmonids virus infections.

In spite of wide application prospects of biosensors in salmonids virus detection, there are still some challenges in on-site virus detection and surveillance in salmonids aquaculture. The development trends of on-site virus detection biosensors are mainly in these aspects: (1) Miniaturization and integration of the detection system. It will accord with the diagnostic needs of point-of-care (POC) in resource limited settings such as fish farms. (2) High availability and low cost. Fabricating biosensors can use the lower-cost and easily available semiconductor materials. (3) Multiplexed detection. This is highly desirable in early salmonids virus detection because virus has genetic variability such as IPNV. (4) Application effect evaluation. All biosensors need to be applied in the practical environment to evaluate their detection effect and performance. These goals will encourage researchers to make great progress in more accurate, portable, ultracompact reliable and smart on-site virus detection^[67, 68].

Acknowledgements

The authors thank anonymous reviewers for their constructive comments on the resenatation of this article. This research was supported by the National Key Research and Development Program of China (2022YFC2601304) and National Key Research and Development Program of China (2022YFC2602100)

References

- [1] Bianchi M C, Chopin F, Farme T, et al. FAO: the state of world fisheries and aquaculture. Rome, Italy: Food and Agriculture Organization of the United Nations, 2014
- [2] Crane M, Hyatt A. Viruses of fish: An overview of significant pathogens. *Viruses*, 2011, 3, 2025
- [3] Pinheiro A C A S, Volpe E, Principi D, et al. Development of a multiplex RT-PCR assay for simultaneous detection of the major viruses that affect rainbow trout (*Oncorhynchus mykiss*). *Aquacult Int*, 2016, 24, 115
- [4] Rodriguez Saint-Jean S, Borrego J J, Perez-Prieto S I. Comparative evaluation of five serological methods and RT-PCR assay for the detection of IPNV in fish. *J Virol Methods*, 2001, 97, 23
- [5] Hoferer M, Akimkin V, Skrypski J, et al. Improvement of a diagnostic procedure in surveillance of the listed fish diseases IHN and

- VHS. *J Fish Dis*, 2019, 42, 559
- [6] Shao Y Z, Zhao J Z, Ren G M, et al. Early or simultaneous infection with infectious pancreatic necrosis virus inhibits infectious hematopoietic necrosis virus replication and induces a stronger antiviral response during Co-infection in rainbow trout (*Oncorhynchus mykiss*). *Viruses*, 2022, 14, 1732
- [7] Menon S, Mathew M R, Sam S, et al. Recent advances and challenges in electrochemical biosensors for emerging and re-emerging infectious diseases. *J Electroanal Chem*, 2020, 878, 114596
- [8] Bhalla N, Jolly P, Formisano N, et al. Introduction to biosensors. *Esays Biochem*, 2016, 60, 1
- [9] Kim U, Ghanbari S, Ravikumar A, et al. Rapid, affordable, and point-of-care water monitoring via a microfluidic DNA sensor and a mobile interface for global health. *IEEE J Transl Eng Health Med*, 2013, 1, 3700207
- [10] Gavrilescu M, Demnerová K, Aamand J, et al. Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnol*, 2015, 32, 147
- [11] Chen Y, Wang Z Z, Liu Y X, et al. Recent advances in rapid pathogen detection method based on biosensors. *Eur J Clin Microbiol Infect Dis*, 2018, 37, 1021
- [12] Pandey C M, Augustine S, Kumar S, et al. Microfluidics based point-of-care diagnostics. *Biotechnol J*, 2018, 13, 1700047
- [13] Amar E C, Kiron V, Akutsu T, et al. Resistance of rainbow trout *Oncorhynchus mykiss* to infectious hematopoietic necrosis virus (IHNV) experimental infection following ingestion of natural and synthetic carotenoids. *Aquaculture*, 2012, 330, 148
- [14] Ahmadvand S, Soltani M, Mardani K, et al. Infectious hematopoietic necrosis virus (IHNV) outbreak in farmed rainbow trout in Iran: Viral isolation, pathological findings, molecular confirmation, and genetic analysis. *Virus Res*, 2017, 229, 17
- [15] WOA. Infection with infectious haematopoietic necrosis virus. Manual of Diagnostic Tests for Aquatic Animals, 2021, chapter 2.3.5
- [16] Ziafati Kafi Z, Ghalyanchilangeroudi A, Nikaein D, et al. Phylogenetic analysis and genotyping of Iranian infectious haematopoietic necrosis virus (IHNV) of rainbow trout (*Oncorhynchus mykiss*) based on the glycoprotein gene. *Vet Med Sci*, 2022, 8, 2411
- [17] Kurath G, Winton J R, Dale O B, et al. Atlantic salmon, *Salmo salar* L. are broadly susceptible to isolates representing the North American genogroups of infectious hematopoietic necrosis virus. *J Fish Dis*, 2016, 39, 55
- [18] Tapia D, Eissler Y, Torres P, et al. Detection and phylogenetic analysis of infectious pancreatic necrosis virus in Chile. *Dis Aquat Organ*, 2015, 116, 173
- [19] Zhu L, Wang X L, Wang K Y, et al. Outbreak of infectious pancreatic necrosis virus (IPNV) in farmed rainbow trout in China. *Acta Trop*, 2017, 170, 63
- [20] Suebsing R, Oh M J, Kim J H. Evaluation of rapid and sensitive reverse transcription loop-mediated isothermal amplification method for detecting infectious pancreatic necrosis virus in chum salmon (*Oncorhynchus keta*). *J Vet Diagn Invest*, 2011, 23, 704
- [21] Yong C Y, Ong H K, Tang H C, et al. Infectious hematopoietic necrosis virus: Advances in diagnosis and vaccine development. *PeerJ*, 2019, 7, e7151
- [22] Ning B, Huang Z, Youngquist B M, et al. Liposome-mediated detection of SARS-CoV-2 RNA-positive extracellular vesicles in plasma. *Nat Nanotechnol*, 2021, 16, 1039
- [23] Cella L N, Blackstock D, Yates M A, et al. Detection of RNA viruses: Current technologies and future perspectives. *Crit Rev Eukaryot Gene Expr*, 2013, 23, 125
- [24] Dhar A K, Bowers R M, Licon K S, et al. Detection and quantification of infectious hematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*) by SYBR Green real-time reverse transcriptase-polymerase chain reaction. *J Virol Methods*, 2008, 147, 157
- [25] Liu Z X, Teng Y, Liu H, et al. Simultaneous detection of three fish rhabdoviruses using multiplex real-time quantitative RT-PCR assay. *J Virol Methods*, 2008, 149, 103
- [26] Purcell M K, Thompson R L, Garver K A, et al. Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV). *Dis Aquat Organ*, 2013, 106, 103
- [27] Kerr C C, Cunningham C O. Moving molecular diagnostics from laboratory to clinical application: A case study using infectious pancreatic necrosis virus serotype A. *Lett Appl Microbiol*, 2006, 43, 98
- [28] Alonso M, Rodríguez S, Pérez-Prieto S I. Viral coinfection in salmonids: Infectious pancreatic necrosis virus interferes with infectious hematopoietic necrosis virus. *Arch Virol*, 1999, 144, 657
- [29] Hoferer M, Braun A, Skrypski J, et al. One-step cross-genogroup multiplex RT-qPCR with an internal control system for the detection of infectious pancreatic necrosis virus (IPNV). *J Virol Methods*, 2017, 247, 68
- [30] Orpetveit I, Mikalsen A B, Sindre H, et al. Detection of infectious pancreatic necrosis virus in subclinically infected Atlantic salmon by virus isolation in cell culture or real-time reverse transcription polymerase chain reaction: Influence of sample preservation and storage. *J Vet Diagn Invest*, 2010, 22, 886
- [31] Bowers R M, Lapatra S E, Dhar A K. Detection and quantitation of infectious pancreatic necrosis virus by real-time reverse transcriptase-polymerase chain reaction using lethal and non-lethal tissue sampling. *J Virol Methods*, 2008, 147, 226
- [32] Jia P, Purcell M K, Pan G, et al. Analytical validation of a reverse transcriptase droplet digital PCR (RT-ddPCR) for quantitative detection of infectious hematopoietic necrosis virus. *J Virol Methods*, 2017, 245, 73
- [33] Cuenca A, Vendramin N, Olesen N J. Analytical validation of one-step realtime RT-PCR for detection of infectious hematopoietic necrosis virus (IHNV). *Bull Eur Ass Fish Pathol*, 2020, 40, 261
- [34] Suebsing R, Kim J H, Kim S R, et al. Detection of viruses in farmed rainbow trout (*Oncorhynchus mykiss*) in Korea by RT-LAMP assay. *J Microbiol*, 2011, 49, 741
- [35] Suebsing R, Jeon C H, Oh M J, et al. Reverse transcriptase loop-mediated isothermal amplification assay for infectious hematopoietic necrosis virus in *Oncorhynchus keta*. *Dis Aquat Organ*, 2011, 94, 1
- [36] Gunimaladevi I, Kono T, Lapatra S E, et al. A loop mediated isothermal amplification (LAMP) method for detection of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (*Oncorhynchus mykiss*). *Arch Virol*, 2005, 150, 899
- [37] Soliman H, Midtlyng P J, El-Matbouli M. Sensitive and rapid detection of infectious pancreatic necrosis virus by reverse transcription loop mediated isothermal amplification. *J Virol Methods*, 2009, 158, 77
- [38] Zambry N S, Obande G A, Khalid M F, et al. Utilizing electrochemical-based sensing approaches for the detection of SARS-CoV-2 in clinical samples: A review. *Biosensors*, 2022, 12, 473
- [39] Chen H, Liu K K, Li Z, et al. Point of care testing for infectious diseases. *Clin Chimica Acta*, 2019, 493, 138
- [40] Zang Y, Fan J, Ju Y, et al. Current advances in semiconductor nanomaterial-based photoelectrochemical biosensing. *Chemistry*, 2018, 24, 14010
- [41] Tay A, Pavesi A, Yazdi S R, et al. Advances in microfluidics in combating infectious diseases. *Biotechnol Adv*, 2016, 34, 404
- [42] Escobar A, Chiu P, Qu J X, et al. Integrated microfluidic-based platforms for on-site detection and quantification of infectious pathogens: Towards on-site medical translation of SARS-CoV-2 diagnostic platforms. *Micromachines*, 2021, 12, 1079
- [43] Zhang L, Ding B Z, Chen Q H, et al. Point-of-care-testing of nucleic acids by microfluidics. *Trac Trends Anal Chem*, 2017, 94, 106
- [44] Jagannath A, Cong H J, Hassan J, et al. Pathogen detection on microfluidic platforms: Recent advances, challenges, and prospects.

[Biosens Bioelectron X, 2022, 10, 100134](#)

- [45] Wu Q Y, Zhang Y Z, Yang Q, et al. Review of electrochemical DNA biosensors for detecting food borne pathogens. [Sensors, 2019, 19, 4916](#)
- [46] Maffert P, Reverchon S, Nasser W, et al. New nucleic acid testing devices to diagnose infectious diseases in resource-limited settings. [Eur J Clin Microbiol Infect Dis, 2017, 36, 1717](#)
- [47] Jie J Y, Hu S M, Liu W W, et al. Portable and battery-powered PCR device for DNA amplification and fluorescence detection. [Sensors, 2020, 20, 2627](#)
- [48] Sun F, Ganguli A, Nguyen J, et al. Smartphone-based multiplex 30-minute nucleic acid test of live virus from nasal swab extract. [Lab Chip, 2020, 20, 1621](#)
- [49] Powell L, Wiederkehr R S, Damascus P, et al. Rapid and sensitive detection of viral nucleic acids using silicon microchips. [Analyst, 2018, 143, 2596](#)
- [50] Nunez-Bajo E, Silva Pinto Collins A, Kasimatis M, et al. Disposable silicon-based all-in-one micro-qPCR for rapid on-site detection of pathogens. [Nat Commun, 2020, 11, 6176](#)
- [51] Blair E O, Corrigan D K. A review of microfabricated electrochemical biosensors for DNA detection. [Biosens Bioelectron, 2019, 134, 57](#)
- [52] Barreda-García S, Miranda-Castro R, de-Los-Santos-Álvarez N, et al. Solid-phase helicase dependent amplification and electrochemical detection of *Salmonella* on highly stable oligonucleotide-modified ITO electrodes. [Chem Commun, 2017, 53, 9721](#)
- [53] Barreda-García S, Miranda-Castro R, de-los-Santos-Álvarez N, et al. Sequence-specific electrochemical detection of enzymatic amplification products of *Salmonella* genome on ITO electrodes improves pathogen detection to the single copy level. [Sens Actu at B, 2018, 268, 438](#)
- [54] Zang Y, Lei J P, Hao Q, et al. CdS/MoS₂ heterojunction-based photoelectrochemical DNA biosensor via enhanced chemiluminescence excitation. [Biosens Bioelectron, 2016, 77, 557](#)
- [55] Asal M, Özen Ö, Şahinler M, et al. Recent developments in enzyme, DNA and immuno-based biosensors. [Sensors, 2018, 18, 1924](#)
- [56] Li Y Q, Sun L, Liu Q, et al. Photoelectrochemical CaMV35S biosensor for discriminating transgenic from non-transgenic soybean based on SiO₂@CdTe quantum dots core-shell nanoparticles as signal indicators. [Talanta, 2016, 161, 211](#)
- [57] Shu J, Qiu Z L, Lv S Z, et al. Plasmonic enhancement coupling with defect-engineered TiO_{2-x}: A mode for sensitive photoelectrochemical biosensing. [Anal Chem, 2018, 90, 2425](#)
- [58] Zhu C F, Zeng Z Y, Li H, et al. Single-layer MoS₂-based nanopores for homogeneous detection of biomolecules. [J Am Chem Soc, 2013, 135, 5998](#)
- [59] Wang X X, Nan F X, Zhao J L, et al. A label-free ultrasensitive electrochemical DNA sensor based on thin-layer MoS₂ nanosheets with high electrochemical activity. [Biosens Bioelectron, 2015, 64, 386](#)
- [60] Ma X G, Wang C, Wu F X, et al. TiO₂ nanomaterials in photoelectrochemical and electrochemiluminescent biosensing. [Top Curr Chem, 2020, 378, 28](#)
- [61] Liu X P, Chen J S, Mao C J, et al. Enhanced photoelectrochemical DNA sensor based on TiO₂/Au hybrid structure. [Biosens Bioelectron, 2018, 116, 23](#)
- [62] Chen C C, Lai Z L, Wang G J, et al. Polymerase chain reaction-free detection of hepatitis B virus DNA using a nanostructured impedance biosensor. [Biosens Bioelectron, 2016, 77, 603](#)
- [63] Zhu H T, Wang J X, Xu G Y. Fast synthesis of Cu₂O hollow microspheres and their application in DNA biosensor of hepatitis B virus. [Cryst Growth Des, 2009, 9, 633](#)
- [64] Shariati M. The field effect transistor DNA biosensor based on ITO nanowires in label-free hepatitis B virus detecting compatible with CMOS technology. [Biosens Bioelectron, 2018, 105, 58](#)
- [65] Shariati M, Sadeghi M. Ultrasensitive DNA biosensor for hepatitis B virus detection based on tin-doped WO₃/In₂O₃ heterojunction nanowire photoelectrode under laser amplification. [Anal Bioanal Chem, 2020, 412, 5367](#)
- [66] Manzano M, Viezzi S, Mazerat S, et al. Rapid and label-free electrochemical DNA biosensor for detecting hepatitis A virus. [Biosens Bioelectron, 2018, 100, 89](#)
- [67] Lim R R X, Bonanni A. The potential of electrochemistry for the detection of coronavirus-induced infections. [Trac Trends Anal Chem, 2020, 133, 116081](#)
- [68] Pumford E A, Lu J K, Spaczai I, et al. Developments in integrating nucleic acid isothermal amplification and detection systems for point-of-care diagnostics. [Biosens Bioelectron, 2020, 170, 112674](#)



Xiaofei Liu got her Master's Degree on Veterinary in 2014 at Jilin University. Her research interests include detection and monitoring technologies for animal diseases, animal disease risk analysis and genetically modified animals detection.



Shaoqiang Wu got his Doctor's Degree on Veterinary Parasitology in 2003 at China Agricultural University. His research interests include precision detection technology for biosecurity risk, on-site detection techniques for shellfish parasites and aquatic animal diseases.



Xiangmei Lin got her Doctor's Degree on Veterinary Pathology in 1998 at Nanjing Agricultural University. Her research interests include detection and monitoring technologies for animal diseases, zoonotic diseases, foreign animal diseases and monitoring technologies of genetically modified animals detection.