Chapter

Nanomaterials-Based Biosensors against *Aspergillus* and Aspergillosis: Control and Diagnostic Perspectives

Xiaodong Guo, Mengke Zhang, Mengzhi Wang, Jiaqi Wang and Marie-Laure Fauconnier

Abstract

Aspergillosis is the name given to the spectrum of diseases caused by the genus *Aspergillus*. Research on aspergillosis has shown a progressive expansion over the past decades, largely due to the rise in the number of immunocompromised individuals who are at risk for the infection. Nanotechnology provides innovative tools in the medicine, diagnosis, and treatment. The unique properties of nanomaterials like small size in the nanoscale have attracted researchers to explore their potential, especially in medical diagnostics. Aptamers, considered as chemical antibody, are short, single-stranded oligonucleotide molecules with high affinity and specificity to interact with target molecules even superior to antibody. Accordingly, development of nanomaterials-based biosensors technology such as immunosensors and aptasensors against *Aspergillus* and Aspergillosis is of great significance and urgency. In this book chapter, we comprehensively introduce and analyze the recent progress of nanomaterials-based biosensors against *Aspergillus* and Aspergillosis. In addition, we reveal the challenges and provide our opinion in future opportunities for such sensing platform development. Ultimately, conclusion and future prospects are highlighted and summarized.

Keywords: nanomaterials, biosensor, *Aspergillus*, Aspergillosis, biomarker

1. Introduction

Biosensors are integrated analytical devices that are capable of transferring the binding events between bioreceptor and target into detectable optical and electric signals. Therefore, bioreceptors, regarded as the recognition elements, play a crucial role for constructing advanced biosensors. Conventional bioreceptors like antibodies have been extensively prepared and developed for immunosensors' establishment. The barriers such as high cost, complicated procedures, and lack of stability limited the wide applications of such sensing approaches [1–3]. Fortunately, nucleic acid aptamers are short and single-stranded oligonucleotides, selected and identified by

SELEX (Systematic Evolution of Ligands Exponential Enrichment) *in vitro* selection process [4, 5]. Aptamer, considered as chemical antibody and even superior to antibody, can recognize the target molecule to form unique 3D configuration with high affinity and selectivity [6, 7]. Accordingly, aptasensors have attracted increasing attention in recent years toward a variety of target molecules including proteins, cells, viruses, bacteria, metal ions, as well as the disease biomarkers [8, 9].

It is worth noting that the current biosensors generally suffer from the concerns such as lack of biocompatibility, low stability, as well as the poor detection sensitivity. Various advanced nanomaterials can be integrated into the sensing systems for signal transduction and improved analytical performance [10–12]. The commonly used nanomaterials for biosensors mainly include fluorophores, quantum dots (QDs), graphene oxide (GO), gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), metal-organic frameworks (MOFs), upconversion nanoparticles (UCNPs), zinc oxide (ZnO) and other semiconducting nanomaterials, and so on [13–15]. Fascinatingly, bioreceptors can be universally designed and modified with these nanomaterials and are available for optical and electrochemical signal transduction strategies, which further are measured by portable detectors such as fluorimeter, naked eyes, strip readout, as well as electrochemical detectors [16, 17]. For instance, in our recent

Figure 1.
Comprehensive overview of aptamer- and antibody-based biosensors toward Aspergillus *and Aspergillosis based on advanced nanomaterials.*

study, we have introduced a fluorescent aptasensor toward mycotoxin fumonisin B1 (FB1) based on the aptamer recognition. In this effort, GO was embedded for fluorescent quenching and acted as a protectant of the specific aptamer from nuclease cleavage. The target cycling was then triggered for signal amplification and improved detection sensitivity [9].

At the present time, great advances have been achieved for immunosensors and aptasensors in numerous hazard control. Nevertheless, in this book chapter, we only outlined and highlighted the recent progress toward *Aspergillus* and Aspergillosis (**Figure 1**). *Aspergillus* is an extraordinary fungus that occurs naturally all around the world. Sixteen of the 200 known species of *Aspergillus* are isolated and identified due to their severe hazards to animals and humans. Prolonged exposure to high levels of *Aspergillus* can induce allergic symptoms, toxic symptoms, and infection. In particular, for instance, *Aspergillus fumigatus* is the most common and predominate one that can cause invasive aspergillosis (IA) [18, 19]. Conventional approaches for the detection of *Aspergillus* detection like biopsies of cerebral lesions and extraction of cerebrospinal fluid are usually not appropriate for immunocompromised patients. Therefore, simple, rapid, and accurate analytical techniques for *Aspergillus* and Aspergillosis diagnosis are of great importance for human health.

Immunosensors and aptasensors have witnessed a remarkable progress over the past decade. To the best of our knowledge, the comprehensive discussion on immunosensors and aptasensors toward *Aspergillus* and Aspergillosis diagnosis has not yet been reported. Inspired by this status, we proposed an overview of the biosensor construction and their improved performance (**Figure 1**). Moreover, we highlighted the challenges and new opportunities of advanced biosensors for early diagnosis of Aspergillosis infection.

2. Recent advances of biosensors

In the past three decades, antibody-based immunoassays have been well established from scientists in medical and biotechnology fields, including biosensing, therapy, environment, and so on, and gradually extend to disease diagnosis for various targets and biomarker identification and determination. Classic immunoassays like ELISA (enzyme-linked immunosorbent assays) and LFIA (lateral flow immunoassay) are frequently realized for screening purposes and commercial application in the market [20–22]. In particular, ELISA kit, one of the most widely available products, is suitable for qualitative and quantitative assessment of target biomolecule [23]. The ELISA-based rapid screening methods exhibit desirable sensitivity and selectivity, as well as ease of operation [24, 25]. However, high cost in antibody production and antibody stability issue in complicated environment restrict their extended applications [26, 27]. More importantly, antibody preparation against small molecule remains a rigorous challenge since its non-immunogenic and antibody generation are not significant in the process of animal immune [28–30].

Fortunately, nucleic acid aptamer, considered as "chemical antibody," is short ssDNA or RNA that shows excellent affinity and specificity against its target biomolecule even superior to antibody. Noteworthy, the aptamer selection is performed *in vitro* by SELEX technique instead of the complicated animal experiments *in vivo* [10]. Therefore, compared to antibody, the aptamer possesses obvious advantages like convenient production, low cost, high stability, non-immunogenicity, ease of modification, as well as various targets (proteins, cell, tissue, even small molecules) [31, 32].

Correspondingly, the past decade witnessed a remarkable progress on the development of novel aptasensors against *Aspergillus* and Aspergillosis and mycotoxin contamination. However, it is worth noting that there were few studies focusing on the comparison of analytical performance between antibody and aptamer. Stimulated by this status, we noticed that previous works on aptasensors demonstrated the more excellent analytical performance than that of immunosensors [33, 34]. Therefore, the discovery of specific aptamer and its further research opened up a new horizon for rapid and accurate determination of various hazards with high sensitivity and selectivity.

3. Applications of biosensing strategies for *Aspergillus*

Aspergillus has received global concern due to its hazards on food spoilage and food safety [35]. In particular, *Aspergillus flavus* contamination can produce aflatoxins and cause Aspergillosis [36]. *AflD* gene, a potential biomarker for *Aspergillus flavus* pollution, is a structural gene in aflatoxins gene cluster of *Aspergillus* species like *Aspergillus flavus* [37]. Nevertheless, the analytical strategies for *Aspergillus* control are relatively less reported. Biosensors are thus emerged as advanced techniques for the detection of DNA attributed to their portability, high sensitivity, as well as ease of operation [38]. Sedighi-Khavidak et al. firstly fabricated a novel biosensor toward *aflD* gene analyses of *Aspergillus flavus* based on impedimetric electrochemical signal detection and Au NPs [39]. The Au NPs modified with specific DNA probe were immobilized on the glassy carbon electrode. The presence of target DNA induced the formation of double-stranded DNA *via* hybridization reaction, disrupting the reduction of $[Fe(CN)_6]^{3-}$ and enhancing the electrochemical signal (**Figure 2A**). The electrochemical biosensor exhibited a dynamic response of *aflD* gene that ranged from 1 nM to 10 μM with an LOD of 0.55 nM. Furthermore, the feasibility of this sensing protocol was confirmed for the detection of *aflD* gene in real pistachio samples.

Very recently, Liang et al. have synthesized Cu-anchored PDA (polydopamine) nanomaterials, which exhibited photothermal property and catalytic ability for colorimetric signal [40]. In this regard, the nanomaterials were further embedded into LFIA for multimodal sensing of *Aspergillus flavus* based on a sandwich design (**Figure 2B**). Consequently, compared to traditional colorimetric method, the novel LFIA platform showed a significantly sensitive detection of *Aspergillus flavus* with LODs of 0.45 and 0.22 ng/mL, respectively. The feasibility of the sensing strategy was further investigated to monitor *Aspergillus flavus* in peanut and maize samples. On the other hand, apart from the detection of *Aspergillus flavus*, volatile organic compounds (VOCs), the representative metabolites of *Aspergillus flavus*, could be identified and detected for *Aspergillus flavus* contamination [43]. In order to improve the detection efficiency, Lin et al. incorporated three types of nanomaterials into nanocomposites for colorimetric sensing of VOCs [41]. MOF (metal-organic framework), PSA (poly styrene-co-acrylic acid), and PSN (porous silica nanoparticles) were employed and integrated for improved performance due to their ultra-large surface area, excellent catalytic property, and numerous binding sites (**Figure 2C**). The developed sensing method showed high sensitivity and stability for the detection of VOCs and great promise for wheat mildew monitoring. From another viewpoint, the identification and detection of VOCs is significantly correlated with food spoilage event [44]. Traditional analytical technologies like gas chromatography-ion mobility spectrometry (GC-IMS) [45] and electronic nose [46] require expensive instruments, professional personnel, and complicated procedures. Fortunately, whole-cell biosensor

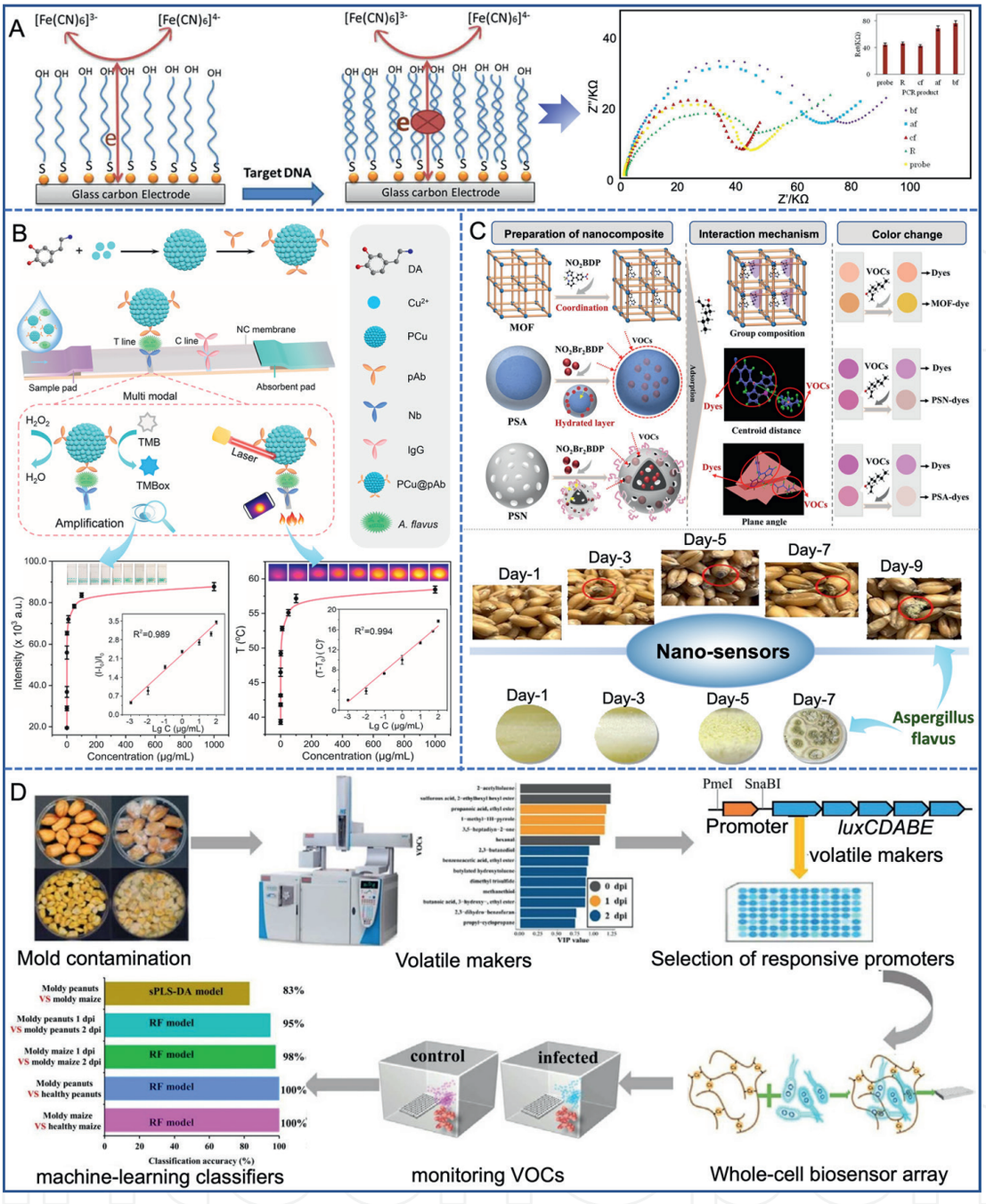

Figure 2.
(A) Schematic representation of impedimetric electrochemical DNA sensor for sensitive detection of aflD *gene of* Aspergillus flavus *based on the Au NPs modification on electrode. The illustration was recreated according to ref. [39]. Copyright 2017: Taylor & Francis Group, LLC. (B) Schematic diagram of lateral flow immunoassay for colorimetric and photothermal detection of* Aspergillus flavus via *the functionalization of Cu-anchored PDA. The illustration was recreated according to ref. [40]. Copyright 2022: Elsevier. (C) Schematic illustration of the colorimetric biosensor toward* Aspergillus flavus *attributed to the analysis of VOCs by integrating nanocomposites. The illustration was recreated according to ref. [41]. Copyright 2022: Elsevier. (D) Mechanism illustration of a novel whole-cell biosensor for identification and monitoring of VOCs and* Aspergillus *contamination by integrating machine-learning models. The illustration was recreated according to ref. [42]. Copyright 2023: Elsevier.*

possesses a great promise for rapid, portable, highly efficient, and sensitive control of VOCs [47]. Herein, Ma et al. developed a novel whole-cell biosensing platform for VOCs finding and *Aspergillus* infection monitoring by engineering machine learning

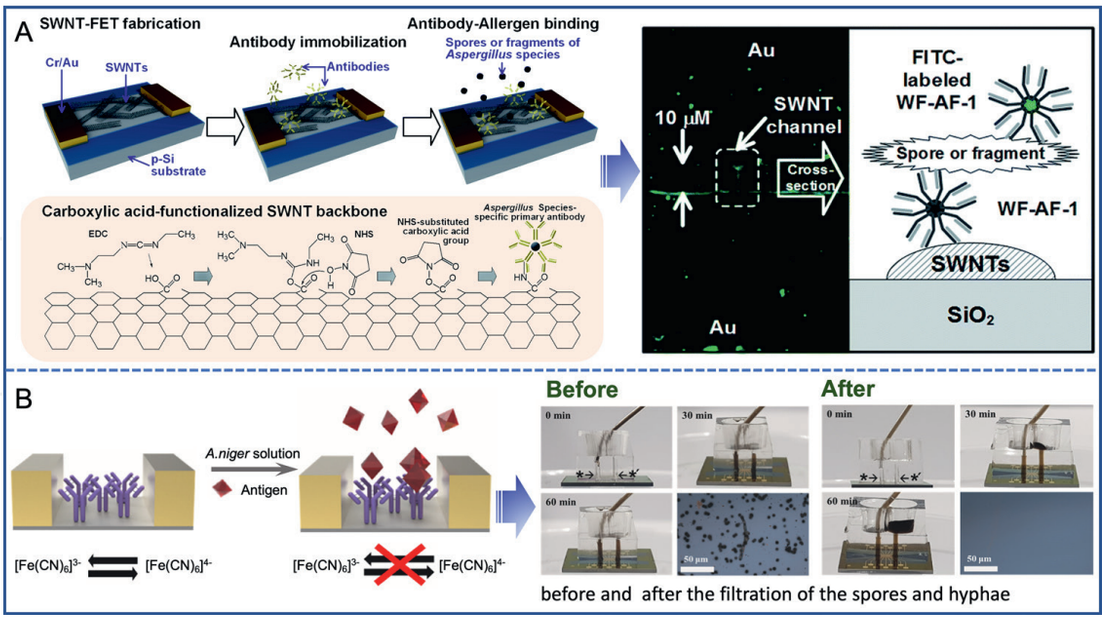

Figure 3.
(A) Schematic illustration of the novel immunosensor for highly sensitive detection of Aspergillus Niger *based on the functionalization of single-walled carbon nanotube (SWNT). The illustration was recreated according to ref. [51]. Copyright 2015: Royal Society of Chemistry. (B) Schematic diagram of electrochemical immunosensor for selective quantification of the* Aspergillus Niger *based on the extracellular proteins monitoring and antibody–antigen reaction. The illustration was recreated according to ref. [52]. Copyright 2021: Elsevier.*

models [42]. Three VOCs' markers were identified in peanut by *Aspergillus flavus* infection and further realized for the validation of various response modes and the construction of biosensor (**Figure 2D**). Moreover, the proposed biosensor coupled with machine-learning models exhibited excellent prediction accuracy in both infected matrices and pre-mold stages. Hence, this novel biosensing strategy opened a new avenue for *Aspergillus* infection prediction and food control.

Conventional *Aspergillus* analytical methods generally involve sampling and culturing of *Aspergillus* spores for immunoassay signal detection, followed by DNA sequencing and quantitative analysis. These protocols suffer from the drawbacks like high cost, complicated procedures, and time-consuming [48–50]. To overcome the barrier, advanced nanomaterials-based biosensors are attracting increasing attention for improved performance. For instance, Jin et al. developed a nanoscale immunosensor for real-time monitoring of *Aspergillus niger via* single-walled carbon nanotube (SWNT) and antigen-antibody recognition (**Figure 3A**) [51]. Encouragingly, the integrating of carbon nanomaterials significantly increased the antibody immobilization sites for enhanced detection signal and high sensitivity at sub-picomolar levels. On the basis of similar antigen-antibody recognition, Lee et al. proposed an electrochemical immunosensor for selectively quantification of *Aspergillus niger via* detecting the extracellular proteins (**Figure 3B**), which relied on the immobilization of the extracellular proteins and its interference of redox cycling in interdigitated electrodes [52]. The utilization of secretion promoter at the sampling stage realized a highly sensitive response by 200-fold improvement for the *Aspergillus niger* monitoring in their previous study [53], which might attribute to the specific antibody recognition rather than to the amplified oxidation oxygen reduction reaction.

More importantly, current diagnosing methods for *Aspergillus* infection are not appropriate in clinical POC testing. To solve this concern, Yu et al. developed a novel simple and rapid analytical technique for DNA amplification based on loop-mediated

isothermal amplification (LAMP) [54]. The LAMP approach was employed to detect the target gene TR34, a biomarker for *Aspergillus fumigatus* infection in patients. Assisted by the primer design of LAMP, this protocol contributed to rapid and selective identification of TR34, as well as the high detection sensitivity with 10 genomic copies per reaction. It was demonstrated that the TR34-LAMP platform can be considered as a POC diagnosis strategy for the screening of clinical *Aspergillus fumigatus* infection.

4. Applications of biosensing strategies for Aspergillosis

4.1 Gliotoxin-related biomarker

Genus *Aspergillus* is a group of fungi that can induce a majority of *Aspergillus* infection from allergic reaction to invasive diseases. Invasive aspergillosis (IA), one of the most severe and devastating *Aspergillus* infections, is defined as a rapid, acute, and life-threatening invasive disease with mortality rate as high as 90%. Of the various *Aspergillus* (such as *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*), *Aspergillus fumigatus* is the most common and predominate one that causes IA. Gliotoxin, considered as the most toxic metabolites occurred by *Aspergillus fumigatus*, poses great hazards to immunosuppressed individuals. Therefore, the development of simple, rapid, sensitive, and point-of-care strategies for gliotoxin control is of high significance and urgency for early diagnosis of IA.

In the direction, assisted by the immobilization-free and GO (graphene oxide)-SELEX technique, Gao et al. proposed the pioneer work for the isolation and selection of the specific aptamer toward gliotoxin (**Figure 4A**) [55]. After the eighth selection cycle, fortunately, the ssDNA was enriched, and the aptamer APT8 was obtained and sequenced with a dissociated constant (K_D) of 376 nM. Then, the APT8 was further truncated into a shorter sequence consisting of only 24 nucleotides according to the mfold structure prediction. More encouragingly, the truncated aptamer APT8T1 was confirmed to recognize the gliotoxin with higher specificity (K_D = 196 nM) and could be further designed to APT8T1M with 18-fold improvement of K_D value. Accordingly, to validate the feasibility and selectivity of the aptamer, a simple fluorescent aptasensor was established for the detection of gliotoxin based on base pairing between the aptamer and its complementary DNA. The specific recognition of aptamer against gliotoxin caused the release of the aptamer/gliotoxin complex from the microplate and the fluorescent signal enhancement. The fluorescent signal was observed to be in linear relationship with levels of target gliotoxin in the range of 0.1–100 nM. The LOD was estimated to be 0.05 nM, which is significantly lower than the previous instrument methods like HPLC-MS/MS. Moreover, the successful application of the fluorescent aptasensor in human serum and urine samples demonstrated that this developed aptasensing platform offered a promising value in gliotoxin control. Inspired by this pioneer finding, combining the unique superiorities of MXene (Ti_3C_2) and TDNs (tetrahedral DNA nanostructures), Wang et al. developed a novel electrochemical aptasensor for highly efficient detection of gliotoxin based on nanomaterial functionalization (**Figure 4B**) [56]. Ti_3C_2 nanosheets, exhibiting large surface area, were modified with TDNs *via* the coordination interaction. The prepared nanocomposites allowed outstanding conductivity and molecule recognition toward the target for signal amplification. After the binding events between the aptamer and target, the cDNA was released and bound to the aptamer-modified nanocomposites, leading to

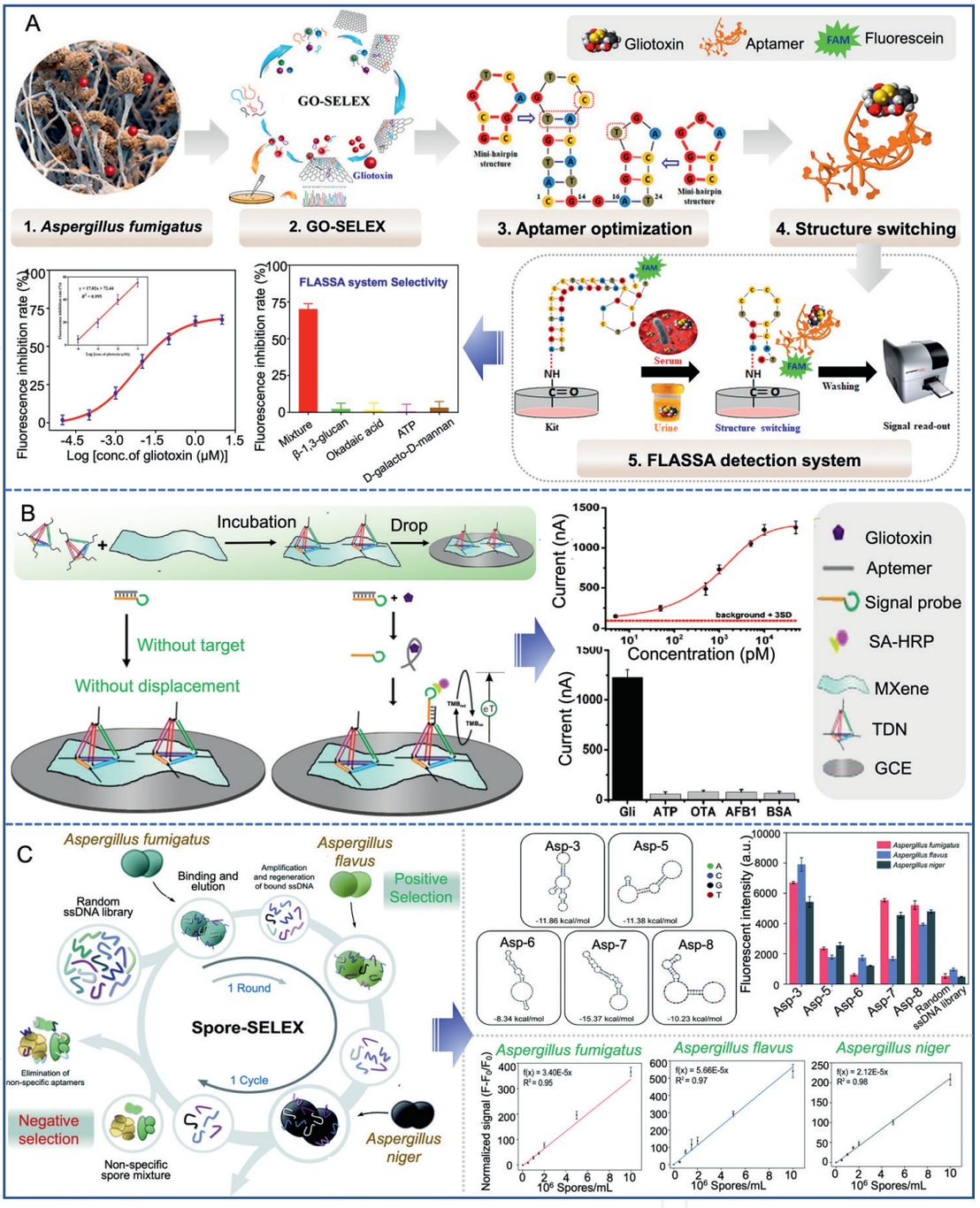

Figure 4.
(A) Working principle of the fluorescent aptasensor against gliotoxin detection for early diagnosis of invasive aspergillosis based on the specific aptamer selection. The illustration was recreated according to ref. [55]. Copyright 2018: American Chemical Society. (B) Schematic representation of electrochemical biosensor for label-free detection of gliotoxin incorporating of tetrahedral DNA nanostructures (TDNs) and MXene nanocomposites. The illustration was recreated according to ref. [56]. Copyright 2019: Elsevier. (C) Schematic diagram of isolation and characterization of the specific aptamer against spores of three representative Aspergillus *species. The illustration was recreated according to ref. [57]. Copyright 2021: Royal Society of Chemistry.*

the streptavidin-decorated HRP (horseradish peroxidase) catalysis and current signal generation. As a consequence, a dynamic response was achieved between the electrochemical signal and concentrations of gliotoxin ranged from 5 pM to 10 nM with an LOD of 5 pM. Accordingly, the detection sensitivity of this electrochemical aptasensor was improved by an order of magnitude than that of the previous fluorescent

detection. Notably, the practicality of this method was excellent for gliotoxin detection in human serum samples. It was demonstrated that, from this viewpoint, advanced aptasensor technology possessed a significant potential against gliotoxin for early diagnosis of IA.

On the other hand, considering the obvious superiorities of aptamer-based biosensors, Seo et al. isolated and identified the specific aptamers that can specifically recognize *Aspergillus* spore by using cell-SELEX technique for the first time [57]. In this effort, the aptamers against three *Aspergillus* spores including *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* were selected in the spore-SELEX process (**Figure 4C**). With 12 rounds of selection, the ssDNA was successfully enriched, and the specific aptamer Asp-3 was achieved and sequenced with dissociated constants (K_D) of 80.12, 35.17, and 101.19 nM versus *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger*, respectively. It was worth noting that the above aptamer also exhibited strong affinity to 1,3 β-D-glucans (BDGs) with K_D of 79.76–103.7 nM, demonstrating the excellent affinities and recognition potential toward *Aspergillus* spore surface molecules. However, the representative aptasensors have not been further developed. Hence, how to select and optimize the aptamer for *Aspergillus* spore control is of vital significance in the future. On the other hand, the biosynthesis of gliotoxin is related to gliP gene, and gliP gene-encoded enzyme further affects the occurrence of gliotoxin. In this direction, gliP gene can be considered as a potential biomarker for *Aspergillus* control, and it eventually contributed to the early diagnosis of IA. Encouragingly, Bhatnagar et al. fabricated an electrochemical biosensor toward gliP gene detection for the first time [58]. The gliP gene was immobilized onto chitosan-stabilized Au NPs on gold electrode. DNA hybridization reaction induced the formation of double-stranded DNA and its interaction with toluidine blue. The toluidine blue was acted as the electrochemical indicator for signal output. Upon the optimal conditions, the novel biosensor exhibited a dynamic response of the target in the range of 1×10^{-14}–1×10^{-2} M with an LOD of 0.32×10^{-14}. The proposed biosensor is stable and selective for detection of glip-T, demonstrating that it is useful for *Aspergillus* analysis and clinical diagnosis.

4.2 Galactomannan-related biomarker

GM (Galactomannan), regarded as a popular biomarker *Aspergillus* infection, is a heat-stable polysaccharide consisting of a linear mannan core with side chains of galactofuran. GM is metabolized and released in the blood and bronchoalveolar fluid (BALF) and is occurred as soluble antigen mainly in the cell wall of the genera *Aspergillus*. Ab (antibody)-based immunoassays such as ELISA (enzyme-linked immunosorbent assay), LFIA (lateral flow immunoassay), and immunosensor are the most frequently used protocols for the detection of GM in early infection of IA. Raval et al. firstly proposed an ELISA method to capture and detect GM based on the conjugation of polyclonal antibody to Au NPs [59]. The developed Au NPs immunoassay possessed simple and accurate detection of GM with low LOD at picomolar level (**Figure 5A**). Based on the similar mechanism, Guo et al. introduced a sandwich chemiluminescence immunoassay toward GM detection incorporated with luminescent nanomaterials (**Figure 5B**) [60]. In this attempt, the donor consisted of photosensitizer and phthalocyanine, and the acceptor contained the chemiluminescent dye, allowing the luminescent signal output under laser irradiation. The donor and the acceptor formed immunocomplex due to the close proximity less than 200 nm. The developed chemiluminescence ELISA platform displayed a linear response of GM in

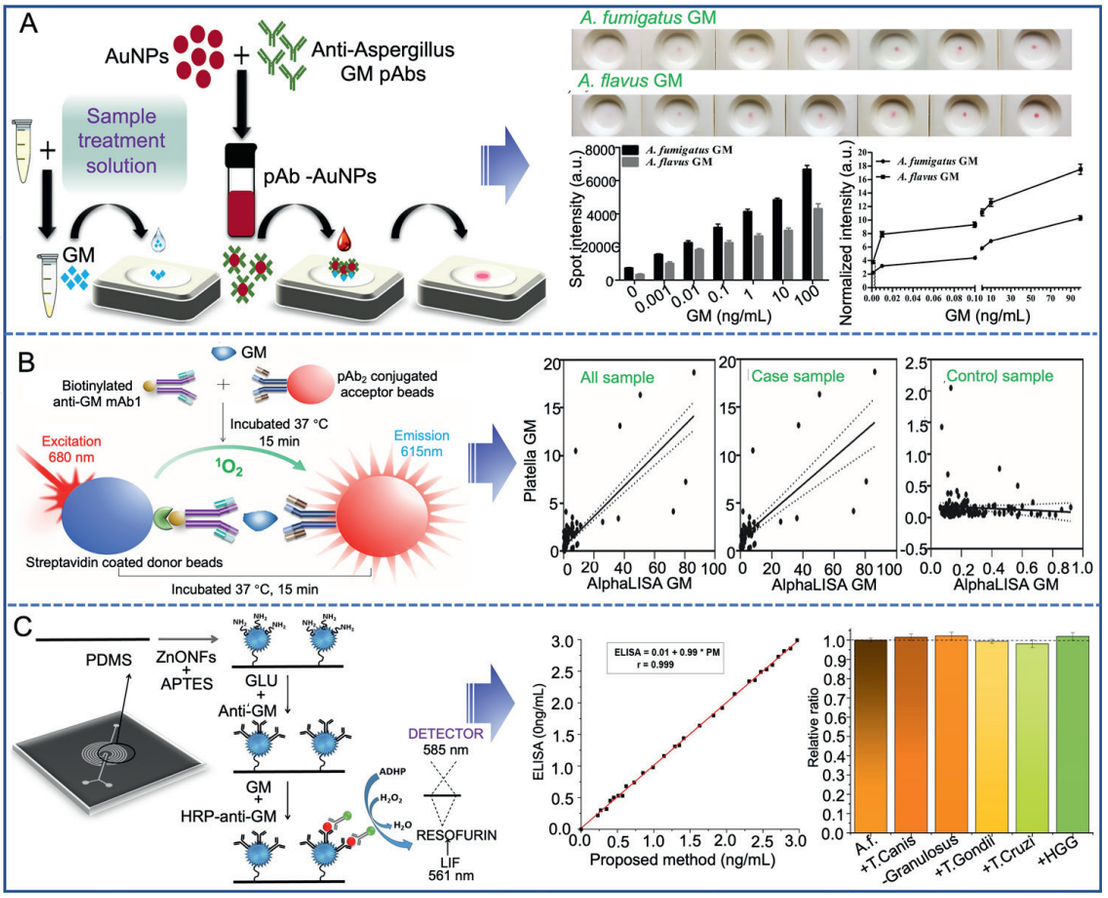

Figure 5.
(A) Schematic diagram of the ELISA-based immunoassay for the detection of galactomannan for early diagnosis of invasive aspergillosis based on the Au NPs conjugation. The illustration was recreated according to ref. [59]. Copyright 2019: Microbiology society. (B) Working principle of the chemiluminescence immunoassay toward galactomannan detection based on the donor and the acceptor. The illustration was recreated according to ref. [60]. Copyright 2022: Elsevier. (C) Schematic illustration of the fluorescence immunosensor to monitor galactomannan based on sandwich format and ZnO nanoflowers (ZnONFs). The illustration was recreated according to ref. [61]. Copyright 2020: Elsevier.

the range 0.05–100 ng/mL with an LOD of 0.032 ng/mL and was capable of highly efficient GM detection in serum and BALF. Besides, nanomaterials-based miniaturized biosensors possessed outstanding advantages in portable monitoring and high-throughput analytical manner [62–64]. Therefore, Piguillem et al. prepared the ZnO nanoflowers (ZnONFs), conjugated the nanomaterials to microfluidic channel, and employed the nanocomplex for antibody modification [61]. Correspondingly, a novel immunosensor was established for fluorescent detection of GM *via* the generation of fluorescent substance resorufin (**Figure 5C**). The immunoassay principle was designed by HRP-modified antibody for catalysis of 10-acetyl-3,7-dihydroxyphenoxacine oxidation to resorufin. Excitingly, the proposed immunosensor can realize a more sensitive and highly efficient detection of GM by 14-fold improvement in LOD over the commercial ELISA method.

5. Conclusions and outlook

Over the past decade, immunosensors and aptasensors have been well established for the detection and control of *Aspergillus* and Aspergillosis. Advanced

nanomaterials-integrated approaches possess great potential to improve the performance for early diagnosis of IA. Of them, optical and electrochemical responses (including fluorescent, colorimetric, electrochemical, and electrochemiluminescence systems) are the two major signal transduction mechanisms. Besides, several researches have also focused on the principles toward binding events between the aptamer and target, catalytic transformation of target, truncation of the known aptamer, as well as the selection of new aptamers, and so on. These efforts indicated that advanced biosensors possessed excellent dynamic response and high sensitivity for AFB1 detection, as well as the feasibility and accuracy in *Aspergillus* detection and Aspergillosis diagnosis. Accordingly, biosensors techniques should have fascinating potential in industrial applications for food safety and risk assessment in the future.

Even though nanomaterials-based biosensors toward *Aspergillus* and Aspergillosis have witnessed the remarkable achievement, there are still several vital scientific limitations and challenges that required to overcome. (i) Advanced material-integrated biosensors exhibited excellent and improved analytical performance. For instance, as mentioned in this article, combining GO with AuNCs can significantly improve the fluorescent quenching efficiency compared to single GO. Novel luminescent materials such as UCNPs, PLNPs, and AIE probes displayed unique superiorities in terms of photostability, anti-interference, and even label-free detection. Therefore, the exploration of new materials and their synergistic effects are of great significance. (ii) Numerous reports are rarely aimed at developing the novel biosensors for their detection analysis rather than at the mechanism research. It is worth noting that the configuration change, site modification, and binding kinetics are of vital importance to better understand the principles and signal response. (iii) The selectivity assay is not appropriate in some case studies; the structural analogues should be taken into consideration for the interferences. (iv) It can be seen that the novel aptamer and aptasensors were relatively less developed, which is mainly attributed to the barriers in precise selection of aptamers. The stability and folding characteristics may be influenced by the special conditions like temperature, pH, ionic strength, and so on, thereby affecting the detection performance. Thus, how to select and identify high-quality aptamers that can undergo various reaction systems is a pursuing work. (v) Most established aptasensing protocols are limited in the laboratory conditions; in particular, at the present time, no commercial kits integrated by aptamers are available in the market. Enormous endeavors should be exerted to design and develop portable sensors or miniaturized devices in POC testing of *Aspergillus* and Aspergillosis.

Acknowledgements

We thank the University of Liège-Gembloux Agro-Bio Tech and more specifically the research platform AgricultureIsLife for the funding of the scientific stay in Belgium that made this paper possible.

Funding

This work was supported by the National Natural Science Foundation of China (No. 32001682, 21305158), the Special Fund for Agro-scientific Research in the Public Interest (201403071), and the Agricultural Science and Technology Research system of the PR China (ASTIP-IAS12).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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