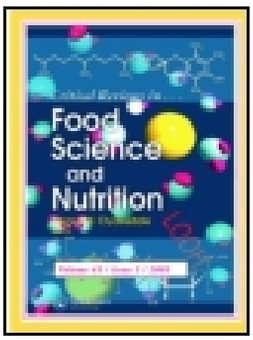

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REVIEW

Recent progress of personal glucose meters integrated methods in food safety hazards detection

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ABSTRACT

Development of personal glucose meters (PGMs) for blood glucose monitoring and management by the diabetic patients has been a long history since its first invention in 1968 and commercial application in 1975. The main reasons for its wide acceptance and popularity can be attributed mainly to the easy operation, test-to-result model, low cost, and small volume of sample required for blood glucose concentration test. During past decades, advances in analytical techniques have repurposed the use of PGMs into a general point-of-care testing platform for a variety of non-glucose targets, especially the food hazards. In this review, we summarized the recent published research using PGMs to detect the food safety hazards of mycotoxins, illegal additives, pathogen bacteria, and pesticide and veterinary drug residues detection with PGMs. The progress on PGM-based detection achieved in food safety have been carefully compared and analyzed. Furthermore, the current bottlenecks and challenges for practical applications of PGM for hazards detection in food safety have also been proposed.

KEYWORDS

Food hazards; food safety; glucose sensing principle; illegal additives; mycotoxins; pathogen bacterial; personal glucose meter; pesticides and veterinary drug residues; point-of-care testing

Introduction

Food safety is a basic required human right that essentially affecting public health and the well-being of society. In a long history, documented human tragedies and economic disasters due to consuming contaminated food strongly remind us at every moment the importance of food safety and how the severity of food safety issues they are (Fritsche 2018; Lin et al. 2018). To date, billions of people in the world are still at a high risk of unsafe food in modern food industry every day since all kinds of food from farm to fork are easily exposed with various food hazards, such as mycotoxins, illegal additives, pathogens, and pesticides and veterinary drug residues (Bernard et al. 2002; Brambilla and Loizzo 1997; Jackson 2009; Knowles and Samuel 2003; Pennings, Wansink, and Meulenberg 2002; Puoci et al. 2005; Lin, Zhou, and Tang 2017; Lai et al. 2017). The Centers for Disease Control and Prevention (CDC) of United States estimated that about 9.4 million people were adversely affected by foodborne illnesses every year (E. Scallan et al. 2011). Moreover, application of the new food process technologies, economic globalization, and expansion of international food commerce also caused series of unintentional and unpredictable personal conducts, making the concerns over food safety and potential contaminants continually increased. A recent example is that the pathogenic COVID-19 can spread worldwide by cold-chain food transportations. Such situations and challenges prompt us to devote much endeavor on the development of reliable and accurate analytical tools to monitor food hazards, avoid health risks, and maintain a sustainable food industry (Su, Song, et al. 2019).

In general, high performance liquid chromatography (HPLC), mass spectrometry (MS), liquid chromatography tandem mass spectrometry (LC-MS/MS), ultraviolet and visible spectrum (UV-Vis), infrared spectroscopy (IR), surface-enhanced Raman scattering (SERS) spectrometry, and atomic absorption/emission spectrum (AAS/AES) are the common analytical tools that have been widely used in detecting a variety of food hazards (Bracker and Brockmeyer 2018; Chaibun et al. 2018; Lee et al. 2014; Robbins et al. 2015). Immunoassays, such as colorimetric immunoassays, photoelectrochemical immunoassays, electrochemical immunoassays, and potentiometric immunoassays also been developed in food hazards detection (Lai, Zhuang, and Tang 2015; Lai et al. 2016; Lin, Zhou, Lin, Tang, Chen, et al. 2015; Lin, Liu, et al. 2016). Despite their impressive achievements, the requirement of specialized/expensive instruments,

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professional technicians, and labor-intensive operations hindered their applications in the field of point-of-care testing (Yu, Cai, et al. 2019; Chen, Xue, et al. 2020). Although user-friendly methods such as enzyme-linked immunosorbent assays (ELISA) and lateral immuno-chromatographic test strips (LFS) are able to timely and simply report detection results (Bolarinwa, Orfila, and Morgan 2014; Fritsche 2018; Mercader and Abad-Fuentes 2009; Song et al. 2011; Wang et al. 2013; Zhou et al. 2011) most of them can only be used to qualitative or semi quantitative detection of harmful substances in food, ruling them out for the cases wherein accurate measurements are imperative.

Alternatively, as one of the most rapid and simple portable device invented by Tom Clemens in 1968 and finally manufactured by Bayer company (Clarke and Foster 2012) personal glucose meters (PGMs) have been utilized in careful personal glucose monitoring and management by the diabetic patients. The properties of easy operation, test-to-result model, low cost, and small volume of sample required for blood glucose concentration test lay the strong foundation of the popularity and wide acceptance of PGMs (Clark and Lyons 1962) and highlight its role in the field of point-of-care testing.

Currently, tremendous effort has significantly contributed to strip a PGM from its preconceived blood glucose monitoring function and exploit its simple detection principle to generate well-defined sensing device for almost analyzing any non-glucose targets at their sampling sites (Que-Gewirth and Sullenger 2007; Xiang, Lan, and Lu 2014; Xiang and Lu 2011; Xiang and Lu 2012a, 2012b; Xiang and Lu 2013). Compared to professional laboratory testing, the PGM-based point-of-care testing maximally retains the core advantages of sample collection, sample analysis, and results readout in a shorten time and reduced sample volume. On this basis, the fundamental and applied research of PGMs have become a hotspot of many analytical scientists. Hundreds of studies related to the employment of PGMs have been published for the screening of food hazards in a short period (Fu et al. 2013; Qiu et al. 2016).

In this review, we plan to deliver an overview of the basic feature of PGM, and critically discuss recent representative works using PGM-based sensing device for different types of food hazards detection. We also summarized the advances to gain insight into the trajectory of the field. Finally, the challenges and outlook of PGM-based sensing device are considered.

Fundamentals of PGMs for non-glucose targets sensing

Basic features of PGMs

Commonly, the PGM is a smart device composed by a glucose meter and a glucose test trip. The former is used to determine the glucose concentration based on the current or voltage change. The latter is actually an enzyme electrode containing glucose oxidase or dehydrogenase in dehydrated form. Presence of glucose is expected to react with the enzyme electrode, causing a defined electrochemical reaction to make the change of current or voltage that is correlated well with the tested glucose concentration. When used for detection, only a small amount of blood sample adopted from any fingers is required to be added onto the test strip, causing an enzymatic reaction that can be reported immediately by the PGM. Its popularity and wide acceptance inspire us to consider that how we can replace it to detect non-glucose targets (Burritt 1990; Clarke and Foster 2012; Z. Wang, Xie, et al. 2015).

PGMs for non-glucose targets sensing

To broaden the application of PGMs toward non-glucose targets rather than glucose, the first challenge is to find a mediator with close link to the glucose concentration. Researchers have found that invertase is a highly efficient enzyme that can catalyze the hydrolysis of sucrose to fructose and glucose. Even nanomolar invertase can convert millimolar sucrose to glucose under harsh environmental conditions, making it an ideal mediator candidate to be related with the glucose (Chen, Gan, et al. 2015; Hun, Xu, and Luo 2015; Xiang and Lu 2011; Xiang and Lu 2012a, 2012b; Xu, Liang, and Zeng 2015b). Besides, glucose oxidase and glucoamylase can also play the similar function as that of invertase and sever as alternative mediators (Fu et al. 2015; Lin, Liu, et al. 2016; Taebi, Keyhanfar, and Noorbakhsh 2018). However, using enzymes as signal transfer mediators often requires the enzymes to be functionalized by chemical crosslinking. The enzyme activity is often weakened during the modification process. To solve this issue, some non-enzymatic detection systems based on encapsulating glucose in nano-containers (lipid spheres, core-shell nanoparticles, etc.) were developed to avoid the use of enzymes (Yan et al. 2013; J. Tang, Huang, et al. 2016; Wang et al. 2019).

The second challenge is to find another mediator that can combine the mediator, such as invertase, with interested non-glucose targets. To fulfill this purpose, the bio-macromolecules that can execute the connection function were the common choice in most published reports, which can be mainly categorized into two types. The first type is based on the connection function of oligonucleotide probes. Advances in synthesis chemistry enable batches synthesis of versatile oligonucleotide probes easier and faster than ever before. Representative functional oligonucleotide probes including aptamers, also known as "chemical antibody," and DNAzymes can be used as high-affinity recognition molecules for small molecules, proteins, and even whole pathogens. Numerous food hazards such as aflatoxin B_1 (Xia et al. 2018), ochratoxin (Xu et al. 2019), kanamycin (Zhang et al. 2020), cocaine (Sachan et al. 2016), melamine (Liu et al. 2020) have been screened with dozens of specific aptamers or DNAzymes via an in vitro systematic evolution of ligands by exponential enrichment (SELEX) selection. Our group also recently reported a CAJ_1 aptamer that can specifically interact with *Campylobacter jejuni* (Chen, Xue, et al. 2020). Easy labeling of functional oligonucleotide probe with invertase can thus build the relationship of non-glucose

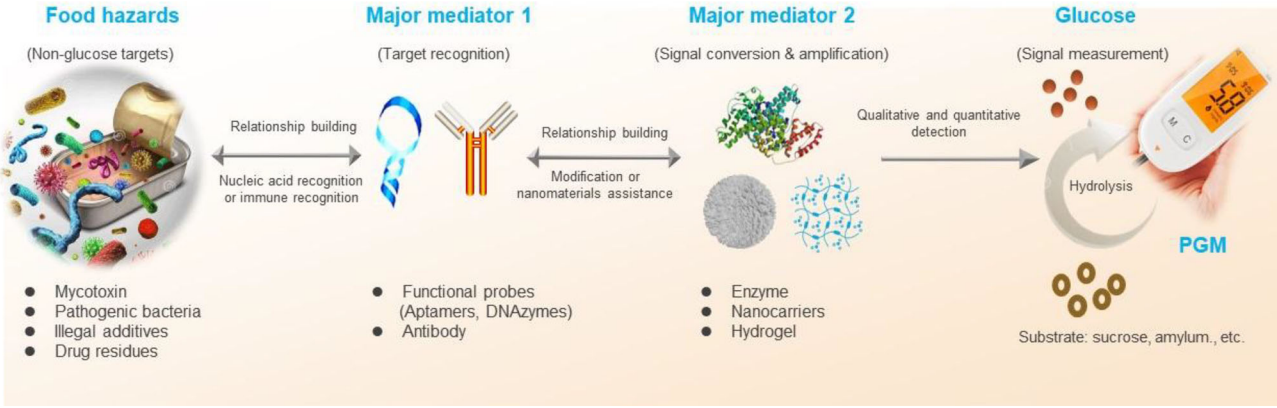

Figure 1. Schematically illustration of mediator promoted non-glucose targets detection using a typical PGM.

targets with invertase, in turn rendering the PGM-based sensing device with recognition ability to identify and quantify non-glucose targets. Based on this design, most of these methods possess impressive sensitivity and specificity (Deng, Png, and Gao 2016; Fang et al. 2018; Xu, Liang, and Zeng 2015a). Moreover, predictability, programmability, and controllability of oligonucleotide probes have led to its coupling with organic or inorganic materials for assembly purposes, allowing the construction of diverse PGM-based target recognition and signal conversion and amplification processes for on-line and point-of-care testing of food hazards (Hou et al. 2014; Xiang and Lu 2012a, 2012b; Xu et al. 2012). The second type is based on the immune-recognition couple of antibody-antigen (Hage 1999). The technique for antibody preparation allows the exploring of any antibodies toward a wide spectrum of food hazards. As mentioned above, the ELISA is often used for the determination of sample components but suffered from insufficient sensitivity and semi quantitative ability. However, by taking its sandwich and competition detection models, we can fabricate non-glucose responsive PGMs by simply chemical crosslinking of antibodies with invertase. This coupling facilely widens the application field of PGMs and, at the same time, improve the quantitative ability of ELISA with the using of glucometer readout system. Also, alternative is to synthesize invertase and antibody coated nanomaterials (e.g., gold nanoparticles, magnetic nanoparticles) to bridge them well. The immuno-recognition based PGMs have also undergone tremendous development during the last few decades. Overview of the PGMs with mediator's assistance to determine non-glucose can be seen in Figure 1.

Taken together, the combination of nucleic acid recognition or immuno-recognition with PGMs has definitely aroused the interest in developing essential analytical tools for food safety monitoring because the ever-increasing concerns of food safety. A trend has been increasing for new PGM-based sensing devices for protecting a continuing supply of safe food and ensuring the health and wellness of people (Gao et al. 2014). The main food hazards including mycotoxins, illegal additives, pathogen bacteria, and pesticide and veterinary drug residues can all be quickly, easily, cheaply, and effectively screened and summarized as follows.

Detection of food hazards

Detection of mycotoxins

Mycotoxins, the secondary metabolism of microbial, are a group of naturally occurring toxic products. At present, there are more than 500 kinds of mycotoxins including aflatoxin (Li et al. 2019), ochratoxin (Yu et al. 2011), deoxynivalenol (Zachariasova et al. 2012), patulin (Ritieni 2003), and zearalenone-2 (F-2) have been investigated with strong toxicities on people's health (Bellver Soto et al. 2014; Liu et al. 2015). Among them, aflatoxin B1 (AFB1) is the most toxic compound produced by *Aspergillus flavus* and *parasitic Aspergillus*, which has been classified as a Class I human carcinogen by the International Agency for Research on Cancer (Lin, Zhou, Lin, Tang, Chen, et al. 2015; Lin, Zhou, and Tang 2017, Su, Song, et al. 2019). It was found in many foods and food raw materials, such as corn, wheat, rice, peanut, various spices, olives, pistachios, and figs (Chen et al. 2020; Xia et al. 2019; Xiong, Liu, and Li 2012). Ochratoxin A (OTA) is also a kind of strong toxin produced by *Aspergillus* and *Penicillium*, which has been demonstrated with teratogenicity, carcinogenicity, and neurotoxicity via poisoning the kidney of human and animal (Bellver Soto et al. 2014). As a common natural pollutant, OTA exists in various foods and beverages (e.g., grains, beans, coffee, beverages, and beer). Although each country has set strict limits for the highest intake level of mycotoxins (Lee and Ryu 2017; Yu et al. 2011), pollution of mycotoxins in food still varies with different countries, nationalities, and regions. What's worse, it is evident that the incidences of mycotoxins poisoning may increase with rise in global temperatures even in countries regarded as safe. Aggressive public awareness has highlighted the importance of mycotoxin detection to safeguard consumers' health globally.

To face these challenges, Wang et al. first reported the usage of a "dual gates" locked, target-triggered nanodevice for point-of-care testing of AFB1 with a PGM readout system (Figure 2A) (Wang et al. 2019). The dual gates were actually an aptamer and dopamine sequentially coated layers on aminated magnetic mesoporous silica nanocomposites, so that it can block glucose in its pores and reduce the background. Introduction of AFB1 under acidic conditions (pH 5.5) would firstly induce the self-degradation of dopamine

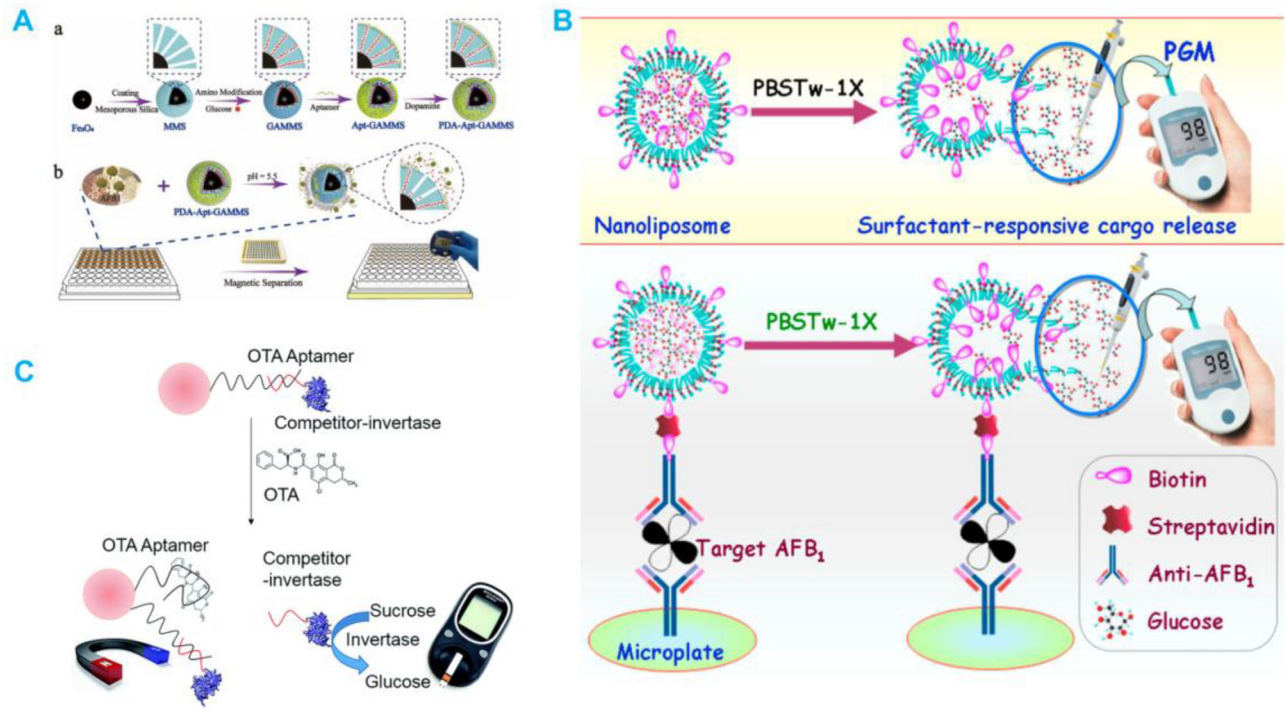

Figure 2. Mycotoxins detection. (A) Scheme of "Dual Gates" locked nanodevice synthesis and its application for the detection of AFB1. Reprinted with permission from Ref. (Wang et al. 2019) Copyright 2019 American Chemical Society. (B) Schematic diagram of glucose-encapsulated liposome-based immunosensing strategy with a PGM readout system. Reprinted with permission from Ref. (J. Tang, Huang, et al. 2016) Copyright 2016 Elsevier. (C) Principle of structures witching aptamer-based OTA detection with a PGM. Reprinted with permission from Ref. (Gu et al. 2016) Copyright 2016 Royal Society of Chemistry.

and then specially combine with its aptamer. This change led to the opening of the dual gates, and in turn release of the already blocked glucose to react with the enzyme on a PGM. Measured results matched well with those of HPLC but with unique features of portability, high sensitivity (0.02 ng/mL), and high throughput. By adjusting the type of aptamer or simultaneously employing several types of aptamers, the platform can also be used for detecting other targets or multiple targets in a 96-well plate. Detection of AFB1 can also be achieved by Tang's group on the basis of a nanomaterial-assisted glucometer-based immunosensing strategy (Figure 2B) (J. Tang, Huang, et al. 2016). They initially synthesized a glucose-encapsulated liposome nanocarriers. Next, a conventional sandwich immunoassay was applied to capture AFB1, and a classic streptavidin-biotin recognition was applied to bridge AFB1 concentration with the glucose-encapsulated liposome nanocarriers. Upon exposure to buffered surfactant (PBST), the immediate hydrolysis of liposomes allowed the release of glucose to be quantitatively determined by a PGM. Its simplicity, low cost, universality, and sensitivity provided a new horizon for point-of-care testing, and the behavior of encapsulating glucose in liposome reduced the process for enzyme functionalization. In addition, Shi and his colleagues realized the detection of OTA from red wine (Figure 2C) (Gu et al. 2016). In this method, a structure-switchable OTA aptamer was immobilized onto a magnetic bead to capture an invertase-labeled competitor DNA via base-pairing. Recognition of OTA by its aptamer strand can trigger the release of invertase-labeled competitor from the nanoconjugate, resulting in the invertase based catalysis hydrolysis of sucrose to glucose, and finally be measured by a PGM with detection limits of 3.31 and 3.66 μg/L in buffer and 2% red wine, respectively. Of note, the pairing bases between OTA aptamer and its complementary probe should be carefully considered to find a balance between the OTA aptamer/OTA recognition force and the OTA aptamer/complementary probe. Theoretically, all mycotoxins can be simply detected with these described representative examples. What needs to be considered is how much glucose needs to be generated or released to result in an identifiable change measured by the PGM.

Detection of illegal additives

Food additives refer to coloring agents, anticaking agents, antiseptic agents, swelling agents, certain sweeteners, and so on (Moore, Spink, and Lipp 2012; Tittlemier et al. 2009; Xiao, Jiang, and Bi 2018). Each of them is functionalized to be used such as to improve the food flavor, extend shelf-life, and give foods an appetizing appearance. They are safe below their tolerated amounts. However, incidents in foodstuffs due to the consumption of dairy products with food additives were reported. These additives such as melamine, clenbuterol (CLB), Sudan, and dioxin are not the traditional ones and being divided into illegal additives. Compared with those of permitted food additives, these illegal additives are very harmful to human bodies, which are strictly regulated by food safety supervision authorities to ensure foods are safe. For example, CLB is an adrenergic stimulant that can promote protein synthesis, fat transformation and decomposition. Some farms had illegally added CLB in animal meal to promote the growth of animals and increase the

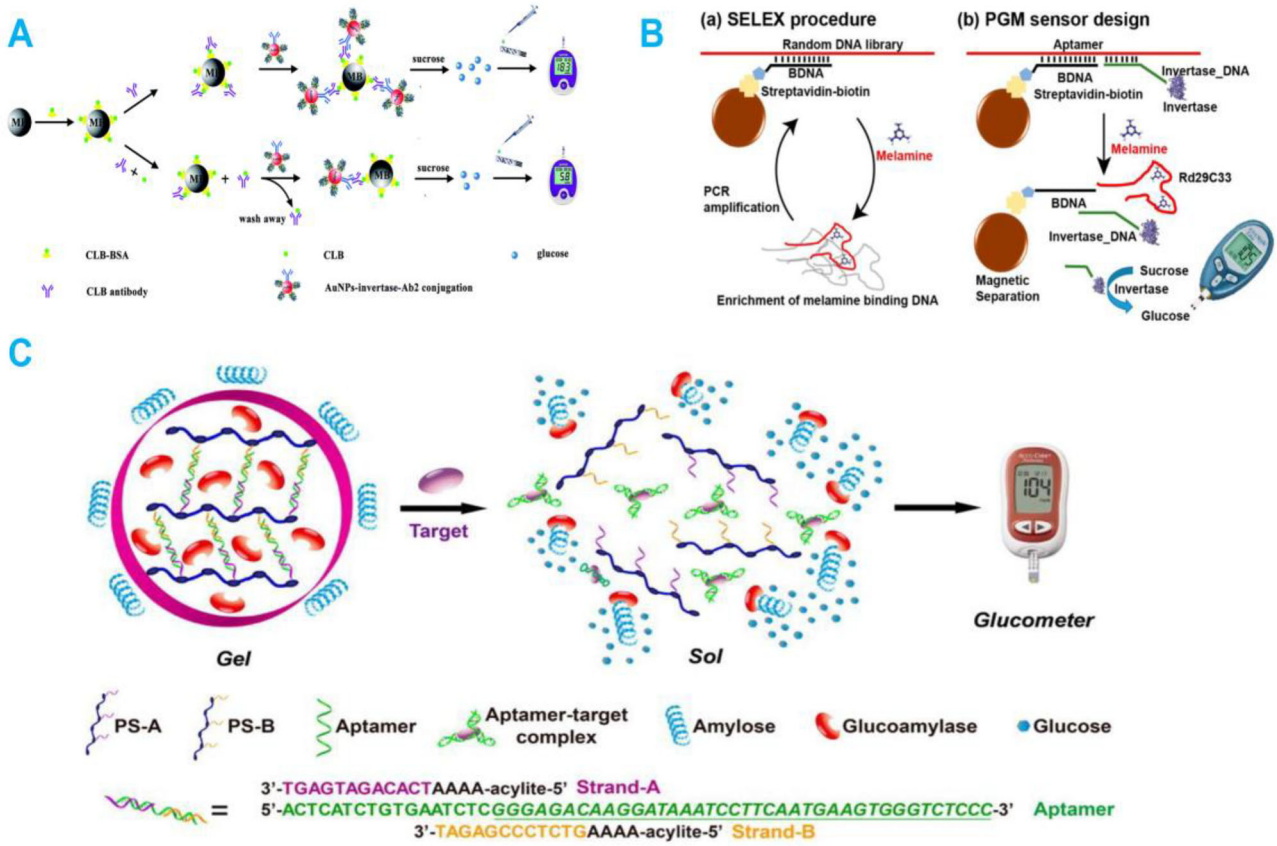

Figure 3. Illegal additives detection. (A) Principle of competitive ELISA combined PGM for CLB detection. Reprinted with permission from Ref. (Li et al. 2017) Copyright 2017 Royal Society of Chemistry. (B) SELEX based selection of a melamine aptamer and its use for a PGM sensor construction. Reprinted with permission from Ref. (Gu et al. 2015) Copyright 2015 American Chemical Society. (C) Target-Responsive "Sweet" Hydrogel with Glucometer Readout for Portable and Quantitative Detection of Non-Glucose Targets. Reprinted with permission from Ref. (Yan et al. 2013) Copyright 2013 American Chemical Society.

lean/fat ratio (Huang et al. 2019; Y. Tang, Huang, et al. 2016; Yan et al. 2015). Long term consumption of meat products containing CLB can cause serious physiological side effects, such as muscle pain, dizziness, heart rate acceleration, tension and even death. Melamine (1,3,5-triazine-2,4,6-triamine) is a kind of chemical intermediate based on triazine, which was often used to manufacture amino resin, plastics and flame retardant (Chen et al. 2010; Yin et al. 2010). Melamine itself has low toxicity, however, it can combine with analogues such as cyanuric acid to form crystals and cause significant renal toxicity and carcinogenic effects (Chan and Lai 2009; Sun et al. 2012). To pursuit illegal profits, criminals invented a new way to mix milk, infant formula, frozen yoghurt, pet food, biscuits, candy, and coffee drinks with melamine, faking the detection of protein with the high nitrogen content of melamine. According to reports, this adulteration once led to kidney complications and hospital admissions of hundreds of children. Cocaine is a central nervous system stimulant that can increase the level of dopamine and effectively inhibit the reuptake of neurotransmitter in the synapse. In Europe and the United States, it is the second most used illegal substance. Abuse of cocaine addiction can cause anxiety, paranoia, mood disorders, organ damage, and violent behavior characteristics (Adegoke et al. 2020; Aigner and Balster 1978; Shen et al. 2015). Regarding these cases, the development of fast, convenient, on-site quantitative methods to detect illegal additives in food is very important for food safety, quality management, and protection of the legitimate rights and interests of consumers.

Up to now, extensive efforts have been devoted to examine illegal additives. Li et al. once reported a competitive ELISA combined PGM method to detect CLB (Figure 3A) (Li et al. 2017). In this strategy, two nanocomposites of clenbuterol-bovine serum albumin (CLB-BSA) functionalized magnetic beads and gold nanoparticle-invertase-secondary antibody conjugates were synthesized. The presence of CLB was able to compete with CLB-BSA to bind with its antibody, prohibiting further the assembly of CLB-BSA functionalized magnetic bead with gold nanoparticle-invertase-secondary antibody conjugate. As a result, collected invertase was reduced associated with a comprised ability to transform sucrose into glucose. The signal-off model rendered a decreased glucose value by a PGM this was proportional to the CLB concentration and satisfied the testing of CLB from pork. Notably, when using nanometer carrier (such as colloidal gold) to co-couple invertase and antibody, the ratio between invertase and antibody, the pH value of the coupling buffer should be optimized and discussed. To detect melamine, Gu et al. firstly in vitro screened an aptamer of R29C33 against melamine with the use of the structure-switching selection method. After being cloned, sequenced, and demonstrated with high affinity ($K_d = 0.51 \pm 0.03\,\mu M$), the R29C33 aptamer was designed to build a sensor with a

PGM. In their work, the structural change of the R29C33 aptamer triggered liberation of the invertase-conjugated DNA from a magnetic bead-coupled ternary DNA complex, which can promote a catalytic reaction to produce signal that indicated the amount of melamine present and can be measured by a PGM. Its sensing performance met well with the threshold of 2.5 ppm for non-infant-formula products and 1 ppm for melamine in infant milk products defined by the US FDA (Figure 3B) (Gu et al. 2015). To tackle the illegal use of cocaine, Yang's lab presented an innovative design that linking a glucoamylase-trapped aptamer cross-linked hydrogel with a PGM (Figure 3C) (Yan et al. 2013). This hydrogel was formed by aptamer-assisted cross-linking of polymer strands A and B. In the absence of cocaine, glucoamylase was packaged in this hydrogel. In contrast, when cocaine was introduced, recognition between cocaine and its aptamer led to the breakdown of the hydrogel and release of the glucoamylase. The subsequent hydrolysis of amylose by glucoamylase thus allowed an efficient conversion of the cocaine detection event into a cascaded glucose production reaction for subsequent PGM readout. After careful system optimizations, a low detection limit of cocaine concentration at 3.8 μM (1.2 μg/mL) can be measured that was excitingly to be found even comparable than commercially cocaine test kits. Despite the desirable sensitivity and selectivity, in this method, there is no need to implement troublesome chemical modification to the enzyme so that the enzyme activity was maximally retained. However, even the best PGM can give readings that vary from a reference by 10% when measuring lower glucose. This is a critical side that cannot be ignored when for practical uses. In view of the successful examples for illegal additives profiling, it is hopeful to continually adopt PGMs to detect other illegal additives with low cost, rapidity, simplicity, and portability.

Detection of pathogenic bacteria

Foodborne illnesses caused by pathogenic bacteria represent a widespread and growing problem to public health since there are varied species of pathogenic bacteria can contaminate daily food. *Escherichia coli* (*E. coli*), *Salmonella, Cronobacter sakazakii* (*C. sakazakii*), *Vibrio parahaemolyticus, Campylobacter jejuni, Listeria monocytogenes*, and *Staphylococcus aureus* were the typical pathogens (Alamer et al. 2018; Ogihara et al. 2000; Velusamy et al. 2010; J. Wang, Xie, et al. 2015; Yao et al. 2017). Among them, *E. coli O157:H7* can easily contaminate beef, milk, poultry products, fruit wine, sandwiches, vegetables and drinking water (Liu and Li 2001). It can cause gastrointestinal tract infection or urinary tract infection, and even death. Infection of *Salmonella* can be from contaminated poultry, eggs, and dairy products, causing clinical symptoms of fever, nausea, vomiting, diarrhea, and abdominal colic (Besser and John 2018). *C. sakazakii* is also a pathogen has been found in commercial milk (Ripollés et al. 2017). Infants who are contaminated with *C. sakazakii* will lead to enterocolitis, meningitis and septicemia necrosis, with a mortality rate of 40%–80% (Hu, Yu, and Xiao 2017; Ye, Zhao, and Dou 2017). According to the statistics of the World Health Organization (WHO), about 70% of food poisoning events in the world were caused by pathogenic bacteria (Teng et al. 2017). The Centers for Disease Control and Prevention (CDC) of United States estimated that 47.8 million people were affected by intestinal pathogens alone each year (Cohen 2000). Accordingly, it is worth noting that there is an urgent need for pathogenic bacteria detection to meet the demands in reducing the role that these pathogens play in human foodborne illnesses.

Recently, an invertase and IgG co-modified MNPs (IMIc) were synthesized to enrich the pathogenic bacteria of *E. coli O157:H7* from physiological saline solution or milk by Dou's group. The IMIc also acted as the label probe on an immuno-chromatographic test strip. The absorbent pad to fabricate the strip was pretreated with sucrose solution and desiccated. Addition of IMIc enriched *E. coli O157:H7* on the strip can be trapped in the test line immobilized with monoclonal anti-*E. coli O157:H7* antibody. As the invertase was coupled at the MNPs surface, the catalysis of sucrose on the absorbent pad into glucose was realized. Final treatment of the absorbent pad of each test strip into a disposable syringe to squeeze out the glucose solution made the concentration of glucose can be determined by a PGM (Figure 4A) (Huang, Zhao, and Dou 2018). This method, which combined PGM with LFS, has the advantages of easy to carry, low cost, and wide application range. It can be used for the quantitative detection of pathogenic bacteria in food and also is a potential quantitative detection method in the POCT. Likewise, the same group has carried out the detection of *C. sakazakii* by a sandwich immunoassay linked PGM (Figure 4B) (Ye, Zhao, and Dou 2017). They constructed two nanoconjugates of antibody and glucose oxidase coated silica nanoparticles (SiNP-GOX-IgG) and antibody functionalized silica-coated magnetic nanoparticles (MNP-IgG). In detecting a *C. sakazakii* contaminated powdered infant formula, a sandwich immunocomplex of SiNPs-GOX-IgG/*C. sakazakii*/MNPs-IgG was formed. The change in the glucose content resulted from the hydrolysis by glucose oxidase can be monitored by a commercial PGM. Lin's group prepared a concanavalin A (Con A)-invertase-$CaHPO_4$ nanoflowers by a one-pot bioinspired synthesis and used it to construct a sandwich assay for a PGM assisted *E. coli O157:H7* screen (Figure 4C) (Ye et al. 2016). The Con A has a high binding affinity to *E. coli* surface O-antigen and the $CaHPO_4$ provided biocompatible sites for the modification of Con A and invertase, and improve the activity of invertase, making the nanoflowers not only can be used for *E. coli O157:H7* recognition, but can also loaded with sufficient invertase with enhanced activity. The conferred high bio-recognition of the analyte and signal amplification of the nanoflowers were favorable for point of care testing of *E. coli O157:H7* with the detection sensitivity of 10^1 CFU/mL and the linear range of 10^1 to 10^5 CFU/mL. The advantage of protein-inorganic-hybrid nanoflowers is that the protein-inorganic nanoflowers can endow the recognition ability or biological activity of other molecules by only changing the type of protein. By taking advantage of the inherent property of *E. coli* to produce the enzyme

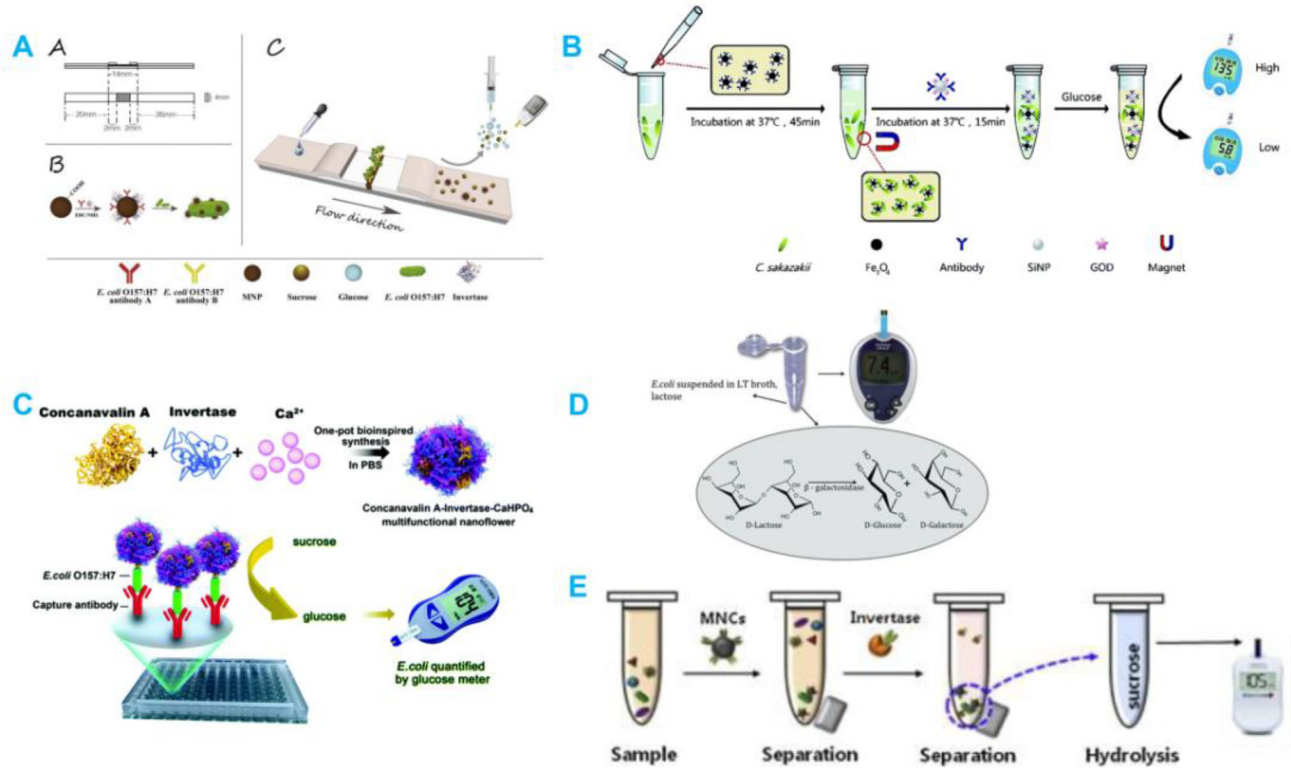

Figure 4. Pathogenic bacteria detection. (A) Scheme of IMIc for *E. coli O157:H7* capture and its flow on an immunochromatographic strip using a PGM as readout. Reprinted with permission from Ref. (Huang, Zhao, and Dou 2018) Copyright 2018 Elsevier. (B) procedure of detection of *C. sakazakii* using two nanocomposites of SiNPs-GOX-IgG and MNPs-IgG on a PGM. Reprinted with permission from Ref. (Ye, Zhao, and Dou 2017) Copyright 2017 Royal Society of Chemistry. (C) Illustration of the one-pot bioinspired synthesis of Con A-invertase-$CaHPO_4$ nanoflowers for sandwich recognition-based point-of-care testing of *E. coli O157:H7* in milk. Reprinted with permission from Ref. (Ye et al. 2016) Copyright 2016 Royal Society of Chemistry. (D) Principle of *β*-galactosidase mediated cleaving of the galactose-glucoside linkage for *E. coli* detection with a PGM. Reprinted with permission from Ref. (Chavali et al. 2014) Copyright 2014 Royal Society of Chemistry. (E) Principle of capturing of *Salmonella* with MNCs to combine with polyclonal antibody-functionalized invertase for PGM based *Salmonella* sensing. Reprinted with permission from Ref. (Joo et al. 2013) Copyright 2013 Elsevier.

β-galactosidase, Mitra et al. also implemented the *E.coli* detection by a PGM (Figure 4D) (Chavali et al. 2014). The principle was dependent on the *β*-galactosidase mediated cleaving of the galactose-glucoside linkage (*β*-1,4 linkage) of lactose, a usual disaccharide substrate, to generate glucose. Along this line, if the food was contaminated by *E. coli*, it can be directly signaled by a PGM without any complex operations. Such a system has the potential to be explored as a *E. coli* test kit. To answer to the *Salmonella* contamination and infection, Joo and his coworker proposed a facile method for the detection of *Salmonella* in milk using a PGM (Figure 4E) (Joo et al. 2013). The *Salmonella* was firstly accumulated from the milk substrate by a monoclonal antibody-functionalized magnetic nanoparticle clusters (MNCs). Following addition of polyclonal antibody-functionalized invertase to attach the MNC-*Salmonella* complexes bridged the *Salmonella* concentration with the absorbed invertase. With the production of glucose from sucrose due to enzymatic activity of invertase and experimental optimizations, the detection sensitivity to 10 CFU/mL *Salmonella* can be obtained. These studies will be the key research direction for food-borne pathogens detection by PGM and push the frontier of current research forward in areas of pathogenic bacteria, making the PGM-based strategy a hopeful method for use in the food safety control.

Detection of pesticides and veterinary drug residues

Use of pesticides and veterinary drugs is aimed to prevent the devastating loss from largescale pests and diseases but inevitably brought the risk of drug residues that is harmful to human health when overuse and abuse of them (Fang et al. 2014, 2018; Guo et al. 2013; Zhou et al. 2018). Although laws and regulations have been enacted to restrict their usage, there were still increasing food safety incidents reported with drug residues (Winter 2012). An amazing case was that from August 2016 to October 2016, 172 kinds of pesticide residues were measured on fresh picked grapes in Turkey, among which about 60% of fresh grapes were detected with one or more kinds of pesticide residues (Golge and Kabak 2018). Intake pesticides and veterinary drugs from contaminated food might induce serious health problems. For example, organophosphorus pesticides (OP) play an important role in preventing crop losses by insects and account for 38% of the world's total pesticides. The hazard of OP to the human body is mainly acute toxicity. Large doses or repeated exposure to OP will occur sweating, tremors, mental confusion, language disorders, and even death (Pundir, Malik, and Preety 2019; Singh 2009).

Chloramphenicol (CAP) is a widely used broad-spectrum antibiotic in the treatment of typhoid, dysentery, *E. coli*, Influenza, Brucella, Pneumococcus, and other infections. Accumulation of CAP even at trace amount in the body

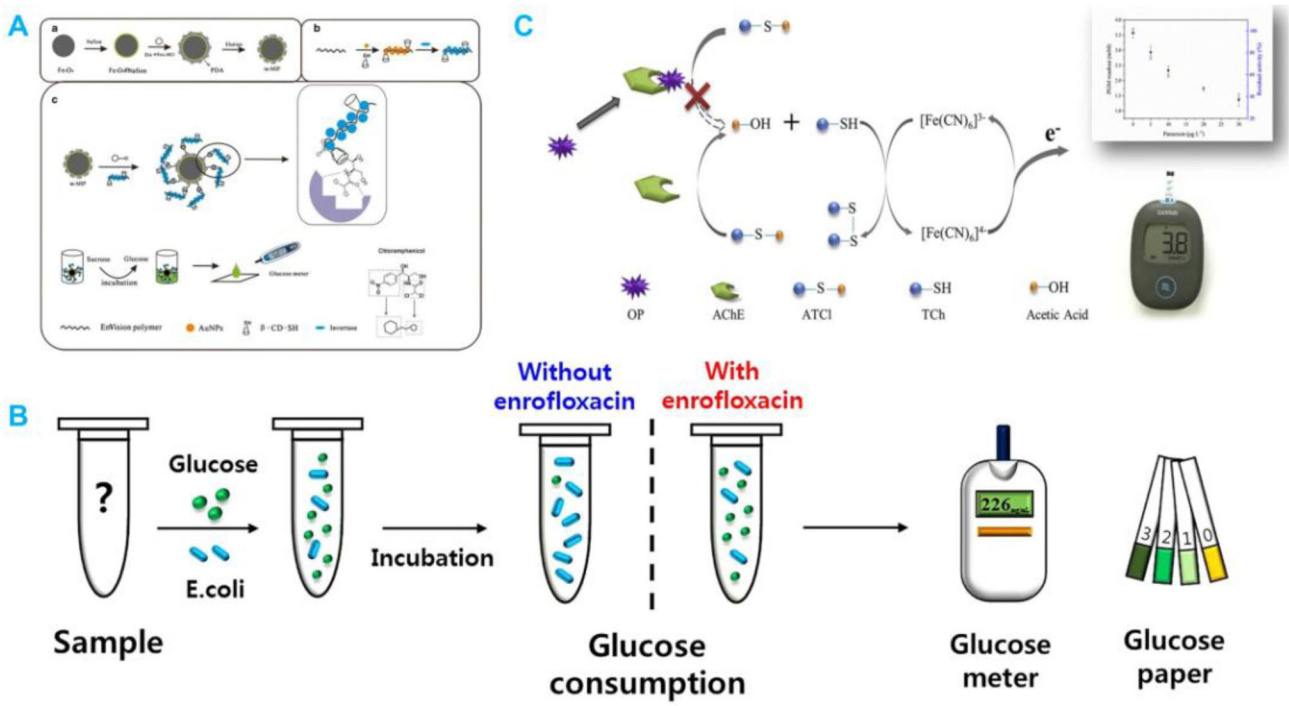

Figure 5. Pesticides and veterinary drug residues detection. (A) Schematical illustration of m-MIP and EV-Au-β-CD/INT cooperated sandwich assay on CAP. Reprinted with permission from Ref. (Chen, Gan, et al. 2015) Copyright 2015 Springer. (B) Construction of an enrofloxacin assay on a PGM based on the utilization of the antibacterial activity of enrofloxacin. Reprinted with permission from Ref. (Kwon et al. 2018) Copyright 2018 Elsevier. (C) OP sensing with the use of a new mediator of $[Fe(CN)_6]^{3-}$ to produce electrochemical response on a PGM. Reprinted with permission from Ref. (Tang et al. 2019) Copyright 2019 Elsevier.

may cause allergic reaction, reduce human immunity, and other toxic effects (Stolker and Brinkman 2005; Zhang, Zuo, and Ye 2008). Enrofloxacin is the third generation of fluoroquinolones, which was usually used as a kind of animal medicine in the treatment of gram-negative bacterial infection (Paudel et al. 2019). Excessive exposure to enrofloxacin can cause symptoms such as nausea, vomiting, diarrhea, headache, and insomnia. However, abuse of enrofloxacin caused residue of enrofloxacin was found in most meat products, including beef, chicken, and so on (Chen and Schneider 2003; Junping et al. 2009; Yu et al. 2014). Therefore, detection of drug residues is also very important that cannot be ignored to ensure food safety.

Faced with these issues, Li and his colleague developed a sandwich assay to recognize CAP (Figure 5A) (Chen, Gan, et al. 2015). The recognition units were a magnetic molecularly imprinted probe (m-MIP) nanoparticle and a β-cyclodextrin/invertase-functionalized signal tag (EV-Au-β-CD/INT). Formation of the complex of m-MIP/CAP/EV-Au-β-CD/INT can be magnetically separated form complex substrates and then applied to cause the hydrolysis of sucrose to glucose. The uniqueness is that this is a positive trial to build an antibody-free sandwich assay with desirable dynamic range (0.5–50 ng/mL) and detection limit (0.16 ng/mL) for on-site screening of CAP in animal-derived food. This non-antibody sandwich detection method greatly reduces the cost, and the disadvantages of enzyme inactivation caused by chemical crosslinking does not need to take account. By virtue of a PGM, Kwon et al. demonstrated a simple method for easy detection of enrofloxacin in water and milk (Figure 5B) (Kwon et al. 2018). Their assay design facilely made use of the antibacterial activity of enrofloxacin to obstruct the metabolism process of glucose consuming by *E. coli*. This means that without the interference of enrofloxacin, the glucose consumption by *E. coli* can be smoothly proceeded, resulting in a low glucose value. If the enrofloxacin was presented, the glucose cannot be metabolized and was kept at a high level for a high glucose value output. Based on this difference, as low as 5 ng/mL enrofloxacin was examined form both the water and milk matrices. More than that, they simultaneously realized the qualitatively monitoring of the glucose change by the naked eye using glucose test strips.

In addition to CAP and enrofloxacin, the OP was also detected by a PGM in Tang and his coworker research (Figure 5C) (Tang et al. 2019). They exploited the inhibition ability of organophosphorus compounds on the catalysis activity of acetylcholinesterase (AChE) to hydrolyze acetylthiocholine chloride into thiocholine. Such reduced yielding of thiocholine in turn decreased the reduce of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ and restrained the electrons transfer that can be quantified using a PGM. Unlike most methods with a mediator of invertase, the discovery of the mediator, $[Fe(CN)_6]^{3-}$, based on the principle of measuring glucose use a glucose meter, the electrons transfer process is directly affected, which is a more meaningful protocol. In this respect, the application of PGM show a broader application prospect in detecting common drug residues.

Outlook and prospective

Across the globe, the increased concerns on food safety and quality matters show the fundamental importance of food

Table 1. Overview of the process of PGMs on the food hazards detection.

Food hazards	Mediator 1	Mediator 2	Test sample	Detection limit	Detection range	Ref.
OTA	Antibody	Invertase	Buffer (PBST)	6.8 ng/mL	N	Xiang and Lu (2012a)
AFB1	Antibody	Liposome	Peanut/Serum	0.6 pg/mL	0.001 to 10 ng/mL	J. Tang, Huang, et al. (2016)
AFB1	Antibody	Mesoporous silica	Peanut	5 ng/kg	0.01 to 15 μg/kg	Tang et al. (2014)
CLB	Nanoparticle	Invertase	Pork	0.1 ng/mL	0.1 to 100 ng/mL	Li et al. (2017)
Cocaine	Aptamer	Invertase	Serum/Blood	3.4×10^{-6} M	0 to 500 μM	Xiang and Lu (2011)
Cocaine	Aptamer	Invertase	Cocaine sample solution	7.7×10^{-6} M	0 to 300 μM	Zhang et al. (2016)
Cocaine	Aptamer	Glucoamylase	Urine	3.8×10^{-6} M	0 to 750 μM	Yan et al. (2013)
Cocaine	Aptamer	Mesoporous titania	Cocaine standards/ New-born cattle serum	5.2×10^{-9} M	10 to 600 nM	Li and Tang (2017)
Melamine	Aptamer	Invertase	Milk	0.53 μM	N	Gu et al. (2015)
Salmonella	Antibody	Invertase	Milk	10 CFU/mL	N	Joo et al. (2013)
C. sakazakii	SiNPs-Antibody	Glucose oxidase	Milk	4.2×10^{1} CFU/mL	9.0×10^{2} to 9.0×10^{7} CFU/mL	Ye, Zhao, and Dou (2017)
E. coli	Aptamer	Invertase	PBS	10^{6} CFU/mL	1.2×10^{5} to 1.2×10^{8} CFU/mL	Wan et al. (2016)
E. coli	Electrostatic interactions	Glucoamylase	Lake water	20 bacteria/mL	20 to 1×10^{5} bacteria/mL	Z. Wang, Xie, et al. (2015)
E. coli	Paracetamol	N	PBS or Citrate buffer	100 CFU/mL	10^{1} to 10^{8} CFU/mL	Das et al. (2018)
E. coli	N	N	Potable water	2 CFU 100/μL	N	Chavali et al. (2014)
Enrofloxacin	N	*E. coli*	DI water/Milk	5 ng/mL	N	Kwon et al. (2018)
CAP	Molecularly imprinted polymer	Invertase	Fish/Pork	0.16 ng/mL	0.5 to 50 ng/mL	Chen, Gan, et al. (2015)
OP	$[Fe(CN)6]^{3-}$	AChE	Apple/Cucumber	5 mg/L	0 to 30 μg/L	Tang et al. (2019)
Dopamine	Aptamer	Invertase	Human serum	3×10^{-9} M	0.08 to 100 umol/L	Hun et al. (2015)

"N" means not mentioned.

safety for food processing industry, food retailers and distributors, and competent authorities. For that reason, various PGMs based devices have been successively developed over the past decades to serve as precise and promising methods to substitute the traditional instruments (e.g., HPLC, SERS, ELISA) for ensuring food quality and food value for the human body. In Table 1, a summary of using PGMs for food hazards detection in recent years are listed. Based on its appealing features of portability, simplicity, rapidity, low cost, sensitivity, specificity, and reliability, the PGMs have been demonstrated with a powerful applicability in the detection of mycotoxins, pathogen bacteria, illegal additives, and pesticides and veterinary drug residues.

Despite the progress, we must admit that the application of PGMs is still in their infancy. There are several challenges left needed to be addressed as follows. First, although assay sensitivity can be guaranteed and application field can be broadened by PGMs, bridging the relationship between PGMs with mediator enzymes (e.g., invertase and oxidase), and then with interrogated target analytes often increases the costs and sacrifices its simplicity and rapidity. How to simply the signal conversion process or find more easier used mediators should be seriously considered. Nanocarriers, "Sweet" Hydrogel and other non-enzymatic systems may be the focus of future research. Second, multiple hazards coexist in contaminated food is very usual. Almost all current PGMs are only developed to focus on one interested target. Its application for co-analyzing multi-analytes is difficult and required to be solved. Third, the narrow range of a typical PGM from 1.1 to 33.3 mmol/L results in a limited detection of target hazards. For instance, if food hazards were at high concentrations, they cannot be tested with a PGM since they are beyond the maximum measuring range of a PGM. Fourth, the research of personal glucose sensor is still at the laboratory stage. To date, there is no mature detection device on the improving of PGMs can be commercially applied in practice. Many respects, such as the cost, shelf life and storage condition requirements for the PGM-based methods for food analysis should be considered before their commercial applications. For instance, the cost with the using of enzymes and antibodies is increased. To make it affordable for publics, the cost for per test should be cheap. The storage conditions should also be considered to make the PGM based device hold a longer shelf life for market use. In addition, many foods often contain endogenous sucrose and glucose, ruling out the availability for applying PGM based sensors in this kind food. Therefore, it is imperative to identify the suitable foods or to find reasonable way to eliminate the interference of endogenous sugar sources. These problems must be discussed and seriously faced before their commercial applications.

Conclusion

In this review, we give a brief overview of the development of PGMs for detection of non-glucose targets and attempt to draw a diverse figure of awareness into a unified vision on PGM-based sensing of food hazards. The discussed achievements indicate that the PGMs are useful tools for challenging with food safety issues, which remains an emerging area and undergoing substantial developments. At the same time, although many challenges lie ahead of us, such numerous pioneered literatures on the using of PGMs to test non-

glucose targets pave us the way to expand the related studies. And challenges also mean opportunities, it will encourage us to pay much endeavor on the continuing development of versatile PGM to face the growing food safety issue. Overall, we believe the portable PGM can enrich the food safety detection tools and play more functions on food safety control.

Author contributions

Xiuguang Xing: Writing-Original Draft. **Li Yao**: Writing-Original Draft. **Chao Yan**: Writing-Original Draft. **Zhenlin Xu**: Writing-Review and Editing. **Jianguo Xu**: Conceptualization, Writing-Review & Editing. **Guodong Liu**: Conceptualization, Supervision. **Bangben Yao**: Conceptualization, Writing-Review & Editing. **Wei Chen**: Conceptualization, Supervision, Writing-Review & Editing.

Disclosure statement

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