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Review

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Analytical Applications of Nanomaterials in Monitoring Biological and Chemical Contaminants in Food

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The detection of food pathogens is an important aspect of food safety. A range of detection systems and new analytical materials have been developed to achieve fast, sensitive, and accurate monitoring of target pathogens. In this review, we summarize the characteristics of selected nanomaterials and their applications in food, and place focus on the monitoring of biological and chemical contaminants in food. The unique optical and electrical properties of nanomaterials, such as gold nanoparticles, nanorods, quantum dots, carbon nanotubes, graphenes, nanopores, and polydiacetylene nanovesicles, are closely associated with their dimensions, which are comparable in scale to those of targeted biomolecules. Furthermore, their optical and electrical properties are highly dependent on local environments, which make them promising materials for sensor development. The specificity and selectivity of analytical nanomaterials for target contaminants can be achieved by combining them with various biological entities, such as antibodies, oligonucleotides, aptamers, membrane proteins, and biological ligands. Examples of nanomaterial-based analytical systems are presented together with their limitations and associated developmental issues.

Keywords: Analytical nanomaterials, biosensor, food safety, pathogenic bacteria, food contaminants

Introduction

Over past decades, a number of new technologies have revolutionized the food industry, and the development and application of nanotechnology in food is one of the more notable of these technologies. Nanomaterials exhibit novel features that offer great opportunities for the development of innovative food products while ensuring food safety. Nanoscience is defined as the study of phenomena taking place at nanometer scale, and the utilization of novel properties by controlling the shapes and sizes of materials at the nanometer level. The preparation of food nanomaterials can be achieved using top-down or bottom-up approaches. The top-down approach involves physical processes, such as grinding or milling. On the other hand, the bottom-up approach utilizes the primary forces of self-assembly and self-organization exhibited by biological systems. The molecular arrangements or aggregations of starches, proteins, and fats at the nanometer scale cause changes in the physical properties of materials. Most nanomaterials developed by the food industry are restricted to nutraceutical or functional compounds with enhanced stabilities and bioavailabilities. However, the full commercial potential of nano foods may not be realized until consumer concerns about new food technologies have been assuaged. To achieve this, comprehensive guidelines are required regarding the definition and safety assessments of nano foods. In fact, the lack of such guidelines explains why the application of nanotechnology in the food industry has been largely restricted to food packaging and diagnostic systems for monitoring the presence of hazardous agents. In the food packaging sector, nanomaterials such as nanosilicates and metallic nanoparticles have been utilized in beverage containers to enhance barrier properties and protect contents

from UV. Nanosilicates intercalated or exfoliated throughout a polymer matrix significantly improve the barrier functions, because gases such as oxygen and carbon dioxide must pass through or around layers containing dispersed nanosilicates. Nanotechnologies being developed in the electronics, energy, and medical sectors are also being adapted to create sensors capable of detecting trace amounts of hazardous contaminants in food. In this review, we focus on nanomaterials that have been utilized for the development of such sensors.

The most challenging aspect of pathogen detection is the need to address zero tolerance; that is, the requirement that no viable pathogens be allowed in certain foods. Thus, to achieve zero-tolerance level detection, a method must be sensitive enough to detect a single pathogen in a sample. Current methods require several days to meet the standard because pathogen culture is required to increase pathogen numbers to a detectable level. New-generation detection systems currently under development utilize the spectroscopic, immunological, and genetic signatures of food contaminants. Most nanomaterials have a physicochemical property that differs greatly from their bulk counterparts. The surface plasmonic property of metallic nanoparticles and strong fluorescence with a sharp emission bandgap derived from the quantum confinement effect are examples of new phenomena that appear when the materials are in nanometer scale. The dimensions of carbon nanotubes, graphene, and nanopores are comparable to those of typical biomolecules, and as mentioned above, their electrical properties can be influenced by the physical status of target molecules. Furthermore, the optical and electrical characteristics of such nanomaterials are highly dependent on local environments. The introduction of an antibody or a specific ligand to those nanomaterials confers great selectivity, and signal readouts can be enhanced by highly sensitive optical or electronic readers. These new technologies offer faster and simpler means of detecting pathogens at much lower detection limits than conventional methods. However, there are a few technological hurdles that need to be overcome to fully realize nanomaterial-based analytical technologies. The challenges associated with sample preparation are discussed at the end of this review.

Inorganic Nanomaterials

Gold Nanoparticles

Gold nanoparticles (AuNPs) have been widely used in biosensors because they can be synthesized easily with defined sizes by chemical reaction and functionalized relatively simply using selected surface probes [20, 58]. The colorimetric characteristics of AuNPs are among the most intensively investigated, and in particular, the shift of maximum absorption wavelength of AuNPs is dependent on its degree of dispersion in solution [14]. For example, well-dispersed AuNPs in an aqueous solution impart a red color with a maximum absorption wavelength of 530 nm, whereas AuNP aggregation causes a blue shift. A melamine sensor was recently developed using functionalized AuNPs surface stabilized by citric acid. The specific interaction between the surface citric acid and melamine in solution results in color transition from red to purple with a detection limit of 0.05 mg/ml in milk [27]. This color change induced by the presence of melamine can be quantified by spectroscopy [32], and the detection limit can be further reduced by utilizing 18-crown-6, which has high affinity for melamine, or by using cysteamine, which reduces electrostatic repulsion between AuNPs [26]. Various colorimetric AuNP aggregation-based sensing systems have been developed for the detection of pesticides [31], aflatoxin B1 [13], *Salmonella* [64], microcystin-LR [63], and copper ions [69].

A number of AuNP-based sensing systems that use other than aggregation-based detection platforms have been reported. For example, the kinetics of AuNP synthesis and the catalytic properties of AuNPs have been employed to develop colorimetric sensors [4, 44]. AuNPs are typically synthesized by reducing Au^{3+} in aqueous solution in the presence of reducing agents, and the rate of this reaction is retarded in the presence of substances that interfere with the actions of reducing agents. For example, the interference of 3,5-dihydroxybenzoic acid (a reducing agent) by melamine significantly inhibits AuNP synthesis, and this provided the basis for a melamine sensor [4]. This sensor is similar to aggregation-based sensors as it allows results to be determined by eye, but the detection mechanism involved is quite different.

The color transitions of AuNP-based supramolecules arise from interactions with target analytes and represent promising sensor platforms. The attachments (or detachments) of specific proteins to (or from) AuNP in the presence of competing interactions with target analytes can be monitored using color transitions of test solutions. For example, a facile method for detecting *Bacillus subtilis* was developed using AuNP complexed with lysozyme, which normally imparts a purple color [51]. Furthermore, lysozyme has selective affinity for peptidoglycans of gram-positive bacterial cell walls. When a gram-positive bacterium, such as *Bacillus subtilis*, is present in a test solution, the interaction

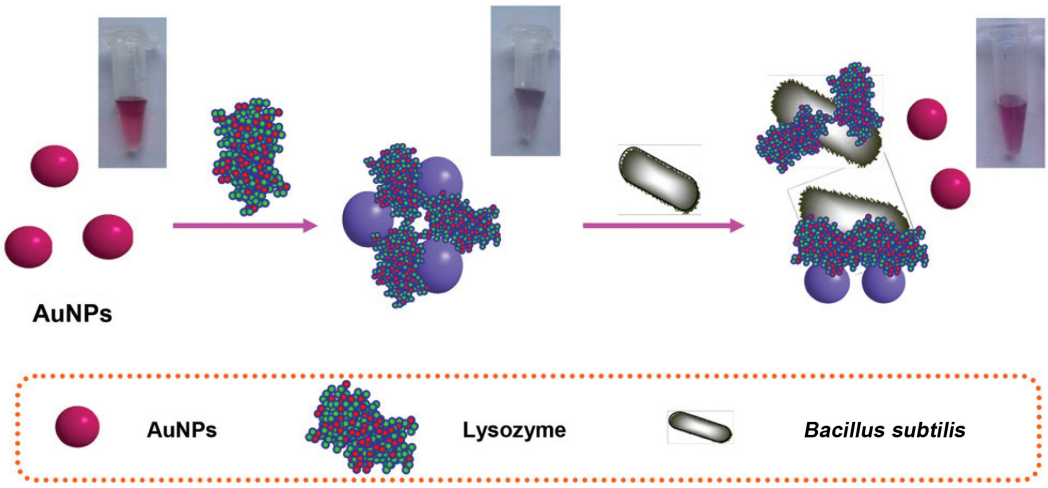


Fig. 1. Detection of *Bacillus subtilis* using gold nanoparticle/lysozyme supramolecules.
Reproduced with permission from The Royal Society of Chemistry [51].

between lysozyme and the cell wall causes the release of lysozyme from AuNP-based supramolecules and cause a purple to red color transition (Fig. 1). The supramolecule consisting of β-galactosidase and AuNPs also has specific affinity for target bacteria and provides another example of a colorimetric sensor. In this case, when AuNPs bind to the target bacterial surface, β-galactosidase is detached from the AuNP-based supramolecule, and the release of galactosidase causes a yellow to red color change in the presence of the chromogen, chlorophenol red β-D-galactopyranoside [41]. The detection sensitivity of these types of supramolecules can be further enhanced by tuning the physical properties of AuNP and by employing elaborately designed recognition molecules such as antibodies and aptamers.

The lateral flow assay test provides another example of an AuNP-based sensor platform. For this test, antibodies are immobilized on the surfaces of AuNPs and the detection line of strip membrane. The antibody-functionalized AuNPs undergo sandwich binding to the detection line in the presence of the target antigen to produce a visible red line on the strip. Various types of lateral flow assay platforms have been developed to detect aflatoxin B1 [34], staphylococcal enterotoxin B (SEB) [54], and genetically modified organisms (GMOs) [23].

Nanorods

Nanorods are typically synthesized from metals or semiconducting materials and have aspect ratios of 3–5. Their optical, electromagnetic, and photoelectric properties have led to their applications in displays, theragnosis, energy harvesting, light emitting devices, biosensing, and others. The recent upsurge in research activities associated with the use of nanorods for biosensing is the result of the unique nature of these materials. Increased popularity of surface-enhanced Raman scattering (SERS) biosensors has resulted in the developments of a number of clever metal nanorod sensing strategies that enhance Raman scattering signals derived from specific target molecules [29, 57]. Generally, metal nanorods are synthesized by seed-mediated sequential growth using surfactants or crystal growth in confined region, such as, on anodized aluminum oxide (AAO) membranes [6, 19]. Absorption spectra of nanorods are dependent on the aspect ratio, and this relation is utilized in various optical biosensors [47]. The effective excitation wavelengths and maximal scattering intensities of the SERS spectrum are highly dependent on the gap distance between nanorods [33]. Recently, Peng *et al*. [49] reported that 2D gold nanorod arrays with a 0.8 nm gap enabled the detection of food contaminants, such as melamine, BBP, and DEHP, at levels as low as 0.9 fM by SERS [49]. In a related approach, researchers utilized vertically synthesized silver nanorods of specific angle (86°) to enhance scattering signals and used them to detect pathogenic bacteria [7]. The results of this study indicated that the developed SERS substrate could identify bacterial strains and differentiate live and dead cells without additional chemical treatments.

Metal nanorods have also been utilized as labeling materials for the fluorescent or visible detection of hazardous molecules. For example, Fu *et al*. [16] described the use of a gold/silicon hetero nanorod (Au/Si hetero nanorod) detection system for *Salmonella* using fluorescent

analysis. This group functionalized the surface of hetero nanorods with anti-*Salmonella* antibody and fluorescent dye to the gold and silicon sides of the nanorod, respectively. Using these bifunctional nanorods, they showed that the intensity of the fluorescence signal was proportional to *Salmonella* concentration. Gold nanorods can also be used in a colorimetric detection method like gold nanoparticles. The color transition associated with the degree of dispersion has been used to monitor the presence of gentamicin using gold nanorods [39]. When plasmonic nanorods approach each other in bulk solution, the red-shift of surface plasmon resonance caused band coupling results in a distinct color change. They utilized two types of gold nanorods with surfaces functionalized with an anti-gentamicin antibody or a gentamicin analog. In the absence of gentamicin, these anti-gentamicin antibody and gentamicin analog nanorods aggregated via specific antigen-antibody interactions. On the other hand, aggregation of nanorods by antigen-antibody interaction was inhibited when free gentamicin was present because of competition between free gentamicin and the immobilized gentamicin analog for the surface-bound antibody. Differences in the degrees of dispersion in the presence or absence of target molecules (gentamicin in this case) are reflected by distinctive color changes. This system was found to have a detection limit of 0.05 ng/ml for gentamicin when coupled with visible spectroscopy.

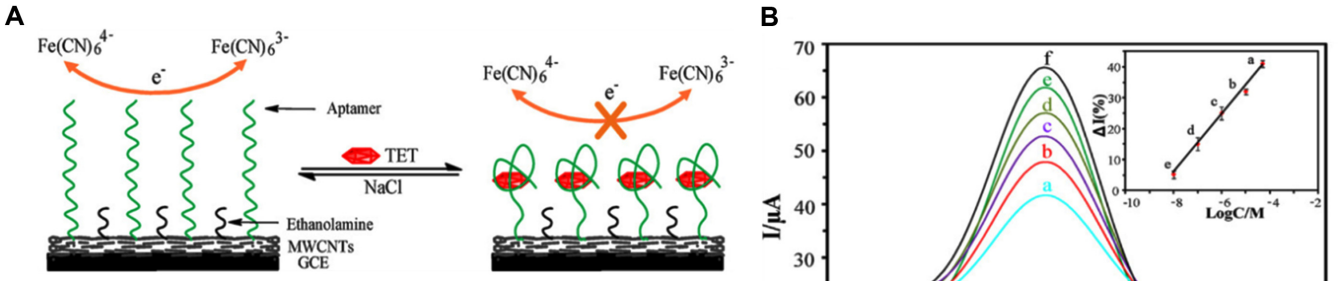
Quantum Dots

Quantum dots (QDs) are nanoscale semiconductor materials, such as cadmium selenide (CdSe), and their sizes define specific photoluminescence wavelengths [10, 43]. The unique optical properties of QDs make them attractive fluorophores in a variety of biological situations. The advantages of QDs over traditional organic dyes are their brightness and stability [67]. A number of researchers have utilized QDs as labeling materials for optical imaging and for biosensors [40]. Here, we mention a few examples of the use of QD-based biosensors in the context of food safety. Kim and Son [24] successfully demonstrated the detection of pathogenic *E. coli* O157:H7 using single-stranded DNA-conjugated QDs and magnetic microparticles. The QDs and magnetic microparticles were complexed by sequence-specific hybridization to probe DNAs containing the target *eaeA* gene (a specific marker for *E. coli* O157:H7). After magnetic separation of the QDs/magnetic microparticles, QD fluorescence intensity was used to quantify target bacteria. Using a similar approach, aptamer-conjugated QDs and magnetic particles were employed to detect *Campylobacter* in various food samples at a detection limit of 10−250 CFU [3]. Using the sandwich-binding technique, they separated target bacteria from food samples and measured fluorescence signals from complexed QDs on bacteria/magnetic beads. Recently, Dong *et al*. [11] used polyamine-functionalized carbon QDs to detect Cu^{2+}, based on the selective interaction between polyamine on the surfaces of QDs and Cu^{2+} in samples [11]. The photoluminescence intensity of the carbon QDs was found to be dramatically decreased by the inner filter effect of adsorbed Cu^{2+}. The detection limit of this system for Cu^{2+} ions was reported to be as low as 6 nM.

A herbicide sensor was developed based on the quenching of QD photoluminescence by an enzymatic chain reaction [71]. In this detection system, the inhibitory effect of organophosphorus herbicides on the activity of acetylcholinesterase (AChE) was utilized. Hydrogen peroxide is generated from acetylcholine by the continuous reaction between AChE and choline oxidase. This hydrogen peroxide acts as a photoluminescence quencher for silicon QDs, and thus, it dramatically decreases light emission from QDs. However, the catalytic activity of AChE is inhibited by herbicides, which results in no hydrogen peroxide generated as well as no photoluminescence quenching. Degrees of quenching in this system are inversely proportional to herbicide concentration. Together with the advantages associated with size, the superior optical and fluorescence properties of QDs have enabled the development of more sensitive and reliable sensing systems.

Magnetic Nanoparticles

The rapid development of magnetic nanoparticles with defined sizes and functionalities has resulted in a range of novel applications in imaging, medicine, and biosensors [15, 42, 61]. In particular, iron oxide (Fe_3O_4) nanoparticles are the most widely used magnetic nanoparticles. These nanoparticles are mainly used to separate and concentrate target molecules or cells from complicated biologic matrices [28]. In order to selectively separate a targeted substance, the magnetic nanoparticles must be functionalized with probe molecules, such as an antibody or aptamer with selective binding ability for the target. Magnetic nanoparticles are among the most promising materials for the separation and concentration of target pathogens from food samples. However, the physical and chemical characteristics of magnetic nanoparticles or of materials conjugated to magnetic nanoparticles could also be utilized to detect the presence of target bacteria. Recently, Luo *et al*. [39] reported a new approach to *E. coli* O157:H7 detection using



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Sample	Standard value of TET (M)	Found (M)	Recovery (%)	Standard deviation
1	5×10^{-8}	4.6×10^{-8}	92	0.092
2	5×10^{-7}	4.4×10^{-7}	88	0.084
3	5×10^{-6}	4.8×10^{-6}	96	0.105
4	2.5×10^{-5}	2.2×10^{-5}	88	0.056

Fig. 2. Application of aptamer-functionalized carbon nanotubes for monitoring the presence of tetracycline in milk.
(**A**) Schematic diagram illustrating the electrochemical detection of tetracycline using an aptamer-functionalized CNT network. (**B**) Representative DPV analysis result for the detection of tetracycline by the device. (**C**) Recovery of tetracycline from milk using the developed device. Reproduced with permission from Elsevier [77].

polyaniline-coated magnetic nanoparticles. Target bacteria together with captured magnetic nanoparticles were concentrated on the specified area of a lateral flow strip sensor by immobilized antibody. The polyaniline coated on the surfaces of the magnetic nanoparticles becomes electrically conductive upon exposure to an acidic environment. *E. coli* O157:H7 was detected at down to 67 CFU/ml by utilizing the correlation between electrical current across electrodes and target bacteria concentration. The characteristic infrared spectra of magnetic nanoparticle have also been used for pathogen detection [52]. The locations and amplitudes of characteristic bands are sensitively affected by the environment surrounding the magnetic nanoparticles. In this study, antibody-conjugated magnetic nanoparticles were used to concentrate the target pathogen, and changes in the infrared spectra caused by the interaction between the magnetic nanoparticles and target pathogen were utilized to quantify the target bacteria. This system showed high specificity for the target bacteria, but its sensitivity was low (detection limit 10^4–10^5 CFU/ml).

Carbon-Based Nanomaterials

Carbon Nanotubes

Carbon nanotubes (CNTs) are an allotrope of carbon with a cylindrical nanostructure and a length-to-diameter ratio typically over millions to one. Their extraordinary thermal conductivities and mechanical and electrical properties mean CNTs have applications ranging from structural material additives to analytical devices [50, 59]. To produce CNT-based biosensors, functionalization of the CNT surfaces with specific probes by chemical or physical conjugation is required [37]. The electrical properties of CNTs are highly sensitive to changes in the local environment owing to the binding of target analyte to CNTs. CNT-based biosensors can be classified as electrochemical or field-effect transistor sensors. In this review, we focus on electrochemical sensor systems.

The utilization of CNTs as sensing elements has provided a simple and effective means of monitoring electrochemical changes in CNT electrodes associated with binding of a specific target analyte. For example, an aptamer-functionalized CNT network deposited on an electrode was used to monitor the presence of tetracycline in milk [77] (Fig. 2). The structural transformation of aptamers in the presence of tetracycline significantly lowered the oxidation-reduction rate of the ferricyanide probe on the surfaces of the aptamer-functionalized CNT electrodes. Changes in electrochemical reactions were analyzed using redox couples by differential pulse voltammetry. The inhibition rate of the electrochemical reaction was found to be proportional to the tetracycline concentration. The detection limit of the aptamer-functionalized CNT electrode was 5 nM for a target in water and 50 nM for that in a milk sample.

In addition, enzymes have been incorporated into CNT-based sensors to amplify electrical signals, and thus increase sensitivity. Horseradish peroxidase (HRP)-tagged

antibody is widely used in ELISA because it can induce color development when bound to the target in sandwich form. A CNT-based sensor surface functionalized with HRP-tagged antibody was developed to detect *Shigella flexneri* in food. This sensor utilized the phenomenon that the activity of the surface-bound HRP is considerable reduced by binding to target bacteria. The detection limit of this enzyme-linked CNT sensor was 3.1×10^3 CFU/ml [75]. The results obtained using this sensor were comparable to that of conventional ELISA with a correlation >95% despite its use of only one antibody as compared with the two or more antibodies typically used for ELISA. In another study, short-length CNTs were used in combination with a HRP-conjugated antibody to detect staphylococcal enterotoxin B [60]. The introduction of HRP and an antibody significantly enhanced the sensor sensitivity (detection limit 10 pg/ml). In addition, the performance of the developed system for SEB in a food matrix was comparable to that of the ELISA method. This system is relatively simple and rapid as compared with ELISA and offers a promising alternative to conventional technologies.

Specific probes, such as antibody, aptamer, oligonucleotide, and specific receptors, importantly provide sensor specificity, but the intrinsic affinity of CNTs for certain contaminants or hazardous molecules can also be utilized to make CNT-based electrochemical sensors. The high affinities of specific colorants for CNT and graphene (derived from structural complementation) was utilized to develop a sensor for colorants with a detection limit of 15–50 μg/l [74, 76], and the intrinsic affinity of CNT for malachite green was used to develop a sensor to detect the presence of malachite green, which has sometimes been used illegally by fish farmers [70].

CNT has also been employed as a physical mediator in electrochemiluminescent (ECL) analysis to supply electrons. The target analyte brings about the binding of secondary antibody to the primary antibody on the CNT-functionalized electrode. The secondary antibody is tagged with a molecule that decomposes through a series of electrochemical redox reactions and generates luminescence signals. In this platform, CNTs deliver electrons from the electrode to luminescence molecules tagged on a secondary antibody in the presence of the target analyte. The intensity of electrochemiluminescence is proportional to the concentration of target analyte, which suggests that quantitative detection is possible. A CNT-based ECL device was utilized to detect palytoxin in fish with a detection limit of 2.2 μg/kg [73].

The structural and electrical properties of CNTs are attractive for biosensors. A large surface area, excellent electrical conductivity, and the sensitivity of electrical responses associated with their molecular-scale dimensions have been actively exploited to develop more advanced forms of analytical devices for food safety.

Graphene

Graphene is another allotrope of carbon and consists of a two-dimensional honey-comb lattice composed of sp^2-hybridized carbon atoms [45]. A single graphene sheet is about 0.3 nm thick and nearly transparent. Graphene effectively conducts heat and electricity, and its electrical properties are highly sensitive to the local environment and adsorbed surface molecules [18, 56]. Accordingly, graphene has been widely used to develop biosensors. Wan *et al*. devised a sulfate-reducing bacteria (SRB) sensor using reduced graphene sheet nanofilm with chitosan deposited on its surface [62]. This surface was further functionalized with anti-SRB antibody and used to detect target bacteria by impedance spectroscopy to a detection limit of 18 CFU/ml. In another study, a graphene electrode functionalized with tyrosinase was used to monitor the presence of organophosphorus herbicides [36]. The electrochemical response in this case originated from the reduction of quinone generated by the enzymatic oxidation of catechol by tyrosinase immobilized on the graphene surface. Organophosphorus herbicides inhibit tyrosinase activity and the degree of reduction is dependent on the herbicide concentration. The results obtained showed that the developed system could detect the organophosphorus herbicides at a lower detection limit of 0.2–3 ppb.

Graphene-based optical biosensors have been developed using a variety of optical techniques, such as electrochemical luminescence, enzymatic luminescence, and fluorescence resonance energy transfer. Recently, Xie *et al*. [68] reported a graphene-SERS system for the detection of a certain colorant, with restricted use in food. Silver nanoparticles, formed on the surface of a graphene sheet by reduction, enabled the detection of various colorants by SERS analysis. Experimental results showed that the silver nanoparticle-decorated graphene sheets could identify different colorants by generating different SERS peaks due to the strengths of interactions between graphene and the adsorbed colorants.

Nanopore-Based Detection Systems

Protein Nanopores

A protein nanopore is a nanometer-sized hole created by a pore-forming protein. When a nanopore is present in an

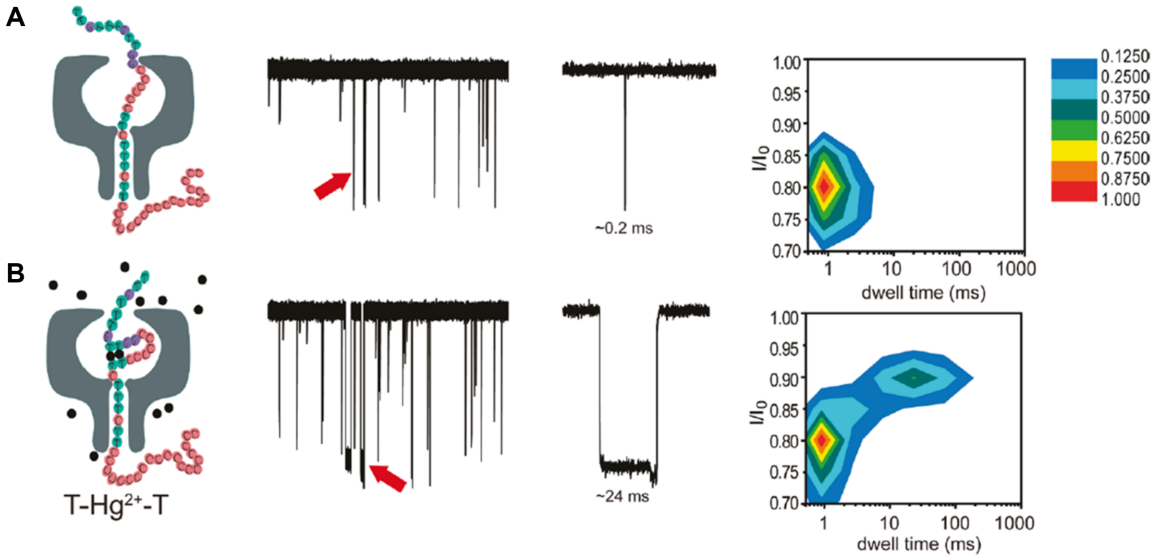

Fig. 3. Structure and translocation result of (**A**) ssDNA aptamer and (**B**) ssDNA aptamer-Hg^{2+} complexed hairpin.
Reproduced with permission from the American Chemical Society [66].

electrically insulating membrane, such as a lipid bilayer, the hole can be used as a single molecule detector. For example, α-hemolysin (α-HL) secreted by *Staphylococcus aureus* forms a nanopore in lipid membranes, and is the most widely used nanopore platform for biosensor development [8]. When an electrical bias is applied across the insulating membrane containing a single nanopore, ions in electrolyte solution in both *cis* and *trans* chambers travel in directions dictated by their charges through the nanopore, and these movements are reflected by electrical currents. When a negatively charged biomolecule, such as DNA, is introduced to the chamber, it is electrophoretically driven through the nanopore to the positively charged side. The open current of the nanopore is interrupted when DNA moves through it, and the amplitude and duration of this interruption are determined by the thickness and length of the DNA molecules [1, 55]. In other words, the folded structure of a DNA or protein molecule can be readily determined from the pattern of current blockade. Because nanopore diameters are comparable to those of small molecules, nanopores could be regarded as high-resolution microscopes that provide structural information.

Wen *et al*. [66] reported a sensing system for Hg^{2+} using a protein nanopore, α-HL, and a DNA aptamer specific for Hg^{2+}. The negatively charged DNA aptamer passes through the nanopore from anode to cathode. Upon binding Hg^{2+}, the DNA aptamer forms a hairpin structure that takes longer to pass through the nanopore than bare DNA aptamer. The authors showed that the developed system could be used to monitor Hg^{2+} with a detection limit of 7 nM by analyzing translocation events and dwell times (Fig. 3). Wang *et al*. [65] also devised a nanopore sensor for the detection of botulinum neurotoxin. The toxin effectively digests the target substrate, synaptobrevin, generating two fragmented products, the smaller of which could pass through the protein nanopore. Because intact synaptobrevin is too big to be translocated through the protein nanopore, no translocation signal is observed in the absence of the target toxin. The technique was able to detect the presence of botulinum neurotoxin type B within minutes at subnanomolar concentrations by observing the current blockade signal generated by the translocation of the synaptobrevin fragment. Accordingly, this technique enables the direct detection of hazardous molecules in food by utilizing structural signatures or by employing secondary components that are structurally altered by reaction with the targeted analyte.

Nanoporous Membranes

Anodized aluminum oxide (AAO) is commonly used to prepare well-defined nanoporous membranes [9, 30]. Pore sizes can be readily controlled by adjusting the oxidizing conditions and the manufacturing process is compatible with mass production [17]. There are many advantages of nanoporous membranes, including high surface area and ion permeability, and a number of researchers have utilized AAO membranes for biosensor applications. A nanoporous membrane-based impedance sensor for

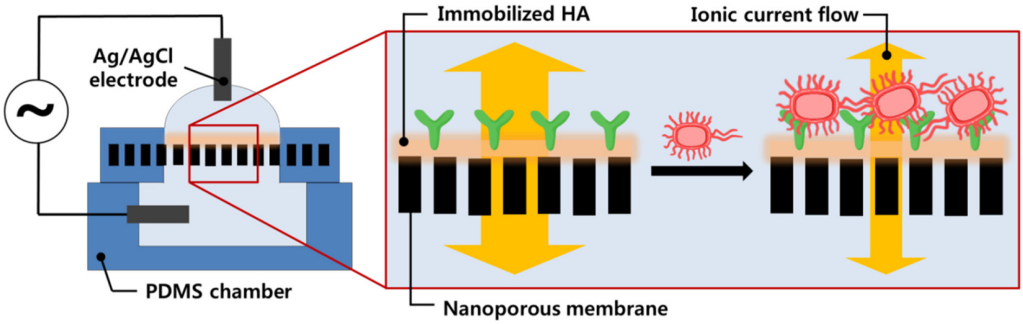

Fig. 4. Schematic diagram illustrating the detection of *E. coli* O157:H7 using a hyaluronic acid (HA) coated nanoporous membrane. Reproduced with permission from Elsevier [21].

pathogenic bacteria was devised by Yu *et al.* [72] using an antibody-functionalized AAO membrane between the anode and cathode electrode. The experimental data obtained showed that *E. coli* O157:H7 and *Staphylococcus aureus* could be detected at a limit of 10^2 CFU/ml by monitoring impedance changes at a specific AC frequency. Bacteria captured on the surface of the AAO membrane by specific antigen-antibody interaction reduced the current flow through the nanoporous membrane as indicated by impedance changes. To enhance the sensitivity of this system, Chan *et al.* [5] employed immuno-magnetic particles to enhance the sensitivity for the detection of *E. coli* O157:H7 to 10 CFU/ml. More recently, Joung *et al.* [21] demonstrated that by reducing nonspecific binding, enhancing the antibody capture efficiency by introducing hyaluronic acid (HA), and by using a passivation layer on the AAO membrane, the detection sensitivity of the nanoporous membrane sensor was significantly increased. In fact, the detection limit of this HA-functionalized nanoporous membrane sensor for *E. coli* O157:H7 in a milk sample was 83 CFU/ml. It is noteworthy that the simplicity and anti-fouling characteristics of the HA-functionalized AAO sensor enabled the testing of whole milk without any pretreatment, which is essential for field diagnosis (Fig. 4).

Polydiacetylene Nanovesicles

Polydiacetylene (PDA) is a self-assembled polymer with a closely packed and well-aligned conjugated backbone [2, 38], and is a promising sensor platform due to its unique optical properties; that is, the color of PDA switches from blue to red in response to external stresses including pH change, heat, and mechanical perturbation [46]. PDA monomers spontaneously form nano-sized vesicles by self-assembly in aqueous solution. To confer specificity for a target analyte, the surfaces of PDA vesicles are functionalized using a specific probe [53]. Upon binding to a target analyte, PDA vesicles undergo a color change from blue to red due to the physical stress induced by the interaction between the immobilized probe and analyte. The color transition of PDA vesicles indicates the presence of target analyte, and concentrations can be determined by quantifying the degree of color transition [25]. PDA-based sensors are attractive because detection is straightforward, but they have one critical drawback that limits their wide application. More specifically, the binding of small target analytes to the surfaces of PDA vesicles typically does not generate stresses sufficient to induce color transition. Therefore, the application of PDA vesicle sensors is limited to sample containing the analyte at high concentration. In an effort to enhance the sensitivity of PDA vesicle sensors, Lim *et al.* [35] have integrated PDA vesicles with silica microbeads, which were utilized to maximize physical stress when they aggregate in the presence of the PAT protein, a marker protein of genetically modified organisms (Fig. 5A). A complex of antibody-conjugated PDA vesicles and silica microbeads was used to detect phosphinothricin acetyltransferase (PAT) expressed in herbicide-resistant GMOs. The results revealed that the detection sensitivity (20 nM) of the developed system was 100-fold higher than that of PDA vesicles not conjugated to microbeads. In another approach taken to enhance the sensitivity of the PDA-based sensor for PAT protein, researchers utilized a hydrogel matrix to encapsulate antibody-functionalized PDA vesicles in a confined volume [22]. They found that the confinement effect of hydrogel beads containing activated PDA vesicles significantly enhanced sensitivity to the nanomolar level (Fig. 5B). In another study, researchers devised a novel system to identify food pathogen using a PDA vesicle indicator [12]. Park *et al.* [48] reported a direct and multiplex detection platform for pathogenic bacteria

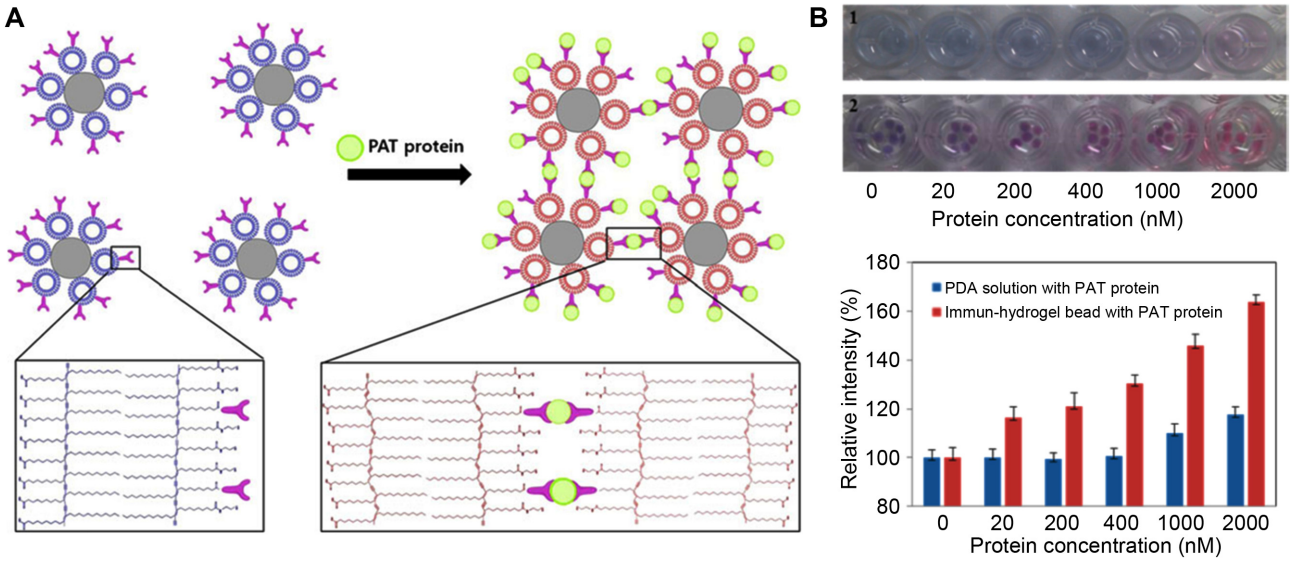

Fig. 5. Applications of polydiacetylene (PDA) vesicle-based detection systems.
(**A**) Schematic illustration of the microbead-assisted detection of PAT protein (a GMO marker protein). (**B**) Digital image and detection signal for the PAT protein obtained using hydrogel beads containing antibody-functionalized PDA vesicles. Reproduced with permission from Springer [35] and American Chemical Society [22].

based on PDA vesicles immobilized on a solid substrate. The results showed that they were able to quantify six species of pathogenic bacteria by measuring the fluorescence intensities of the PDA spots. In addition, the fluorescence intensities emitted by these immobilized PDA vesicles were 10 times higher than the non-immobilized control. When integrated with smart strategies that enhance their sensitivity and specificity, it is likely that PDA-based sensors will be of considerable diagnostic use.

Concluding Remarks and Future Perspectives

As mentioned above, a number of new nanomaterials and nanoparticle-based assay systems have been devised that greatly enhance our ability to detect pathogenic agents in foods. These systems are rapid, simple, and accurate, and have extremely low detection limits, and some provide results in real time. The biggest hurdle confronting these technologies is sample preparation. Detection systems employing nanomaterials rely mainly on indirect signatures derived from analyte-induced differences in electrical, optical, or mechanical characteristics. However, foods contain many components, such as polysaccharides, fats, proteins, and salts, that could interfere with specific target-associated reactions, and in particular, such interferences increase the background noise. Therefore, it is critical that major food components be removed or that target pathogens be selectively separated to ensure sensitivity and accuracy. However, sample preparation for downstream analysis is tedious and time consuming because the target pathogen is not easily separated from food matrices. Furthermore, it also requires concentrating target bacteria present to levels commensurate with the detection limit of the technique used. Solid-phase extraction is one of the most promising sample preparation methods under development, and can achieve high recoveries and enrichment levels. The adsorbents can be used in columns or directly dispersed in sample solutions to separate the target analyte. Magnetic materials such as iron oxide (Fe_3O_4) nanoparticles have made it easier to separate and concentrate target analytes, as together with the captured analytes they can easily be separated by applying an external magnetic field and re-dispersed to wash out contaminants. The surfaces of magnetic materials are usually coated with polymeric materials or silica to enhance nanoparticle stability and facilitate surface functionalization using a specific ligand or antibody. Finally, sample preparation should be physically and functionally compatible with the detection unit, such that the system can eventually be realized in a portable form and suitable for field diagnosis. The detection of pathogens in food is a complex issue that requires advances in food science, engineering, and the pure and material sciences, and is currently a topic for active development.

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