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REVIEW

Detection of foodborne pathogens using bioconjugated nanomaterials

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Abstract This review focuses on applying nanotechnology to foodborne pathogen detection. Because of low infectious doses for most foodborne pathogens, the rapid and sensitive detection methods are essential to ensure the food safety. The advances in the development of nanomaterials have stimulated worldwide research interests in their applications for bioanalysis. The conjugation of biomolecules with nanomaterials is the foundation of nano-biorecognition. A variety of strategies including antibody–antigen, adhesin–receptor, antibiotic, and complementary DNA sequence recognitions have been explored for specific recognition between target bacterial cells and bio-functionalized nanomaterials. The incorporation of these bio-functionalized nanomaterials into current pathogen detection methods has led to rapid and nearly real-time pathogen detection (as short as a few minutes), improved sensitivity (single bacterial cell), and simultaneous detection of multiple micro-organisms from either nutrient broth, liquid or solid food products, or biofilms. The unique properties of nanomaterials in physical strength, chemical reactivity, electrical conductance, magnetism and optical effects make them promising in the development of practical biosensors with emphasis on device portability and simplicity in sample preparation, and the improvement of current pathogen detection methods.

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1 Introduction

1.1 Nanotechnology

Nanotechnology is defined by the National Nanotechnology Initiative of NSF as "the understanding and control of matter at dimensions of roughly 1–100 nm, where unique phenomena enable novel applications". As a result of their small size, nanomaterials display unique properties including physical strength, chemical reactivity, electrical conductance, magnetism and optical effects (Tan et al. 2004; Horák et al. 2007). The advances in the synthesis and characterization of nanoscale materials, e.g., nanowires, nanofibers, nanoparticles, nanobelts or nanoribbons, and nanotubes, have stimulated worldwide research interests in applying nanotechnology for discovering new applications, processes, phenomena, and science.

Nanobiotechnology is the convergence of biology, genomics and nanotechnology. When combined with molecular biological tools, nanomaterials offer more diverse capabilities in bioanalysis and biotechnological applications. Thus far, nanomaterials have found applications in bioimaging, biosensing, drug delivery, and design of multifunctional nanodevices (Chan 2006).

The food industry is turning to nanotechnology for innovations that could bring safer, healthier, and tastier products to consumers. Two major prospects for nanotechnology applications in food safety include: (1) development of sensitive biosensors for detecting the pathogens and toxins in

food products and food processing environments and (2) protection of food through immobilization of antimicrobials on nanomaterials for enhanced stability and activity (Doyle 2006; ENG 2006; IFST 2006). In this review, we focus our discussion on the application of nanotechnology for foodborne pathogen detection.

1.2 Foodborne pathogens and foodborne illnesses

Despite the fact that America's food supply is one of the safest in the world, the Center for Disease Control and Prevention (CDC), USA, reported that foodborne disease is a substantial health burden in the United States (Scallan 2007). The estimated foodborne illnesses in 1999 by CDC were 76 million cases annually in the United States (Mead et al. 1999). Based on the surveillance data for 2006, the Foodborne Diseases Active Surveillance Network (FoodNet) reported a total of 17,252 laboratory-confirmed cases of infections (CDC 2007). The overall incidence per 100,000 population was 14.81 for *Salmonella*, 12.71 for *Campylobacter*, 6.09 for *Shigella*, 1.91 for *Cryptosporidium*, 1.31 for shiga toxin-producing *Escherichia coli* O157 (STEC O157), 0.46 for STEC non-O157, 0.35 for *Yersinia*, 0.34 for *Vibrio*, 0.31 for *Listeria*, and 0.09 for *Cyclospora* (CDC 2007). Among various foodborne pathogens, *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 have been generally found to be responsible for the majority of foodborne disease outbreaks (Lazcka et al. 2007; Scallan 2007).

In considering the low infectious doses for most foodborne pathogens, the presence of foodborne pathogens needs to be monitored at each step of food production, processing, distribution and storage. Therefore, the availability of rapid and sensitive detection methods is essential to ensure the safety of our food supply. The traditional methods for food pathogen detection, based on the growth of micro-organisms, have to be performed in microbiological laboratories, and often require the complicated sample handling. Due to the perishable nature of most food products, there is an increased demand for the availability of detection methods which are rapid, specific, sensitive and field-applicable. In addition to conventional culture-based methods, a variety of rapid methods has been investigated for pathogen detection, such as typical or derived immunological assays, nucleic acid-based tests, and so on.

1.3 Traditional methods for foodborne pathogen detection

1.3.1 Culture-based methods

The culturing methods are based on bacterial isolation on selective media followed by biochemical confirmation. These methods remain the primary official methods for the detection of foodborne pathogens in food samples. For example, culture-based methods are required by the US Department of Agriculture–Food Safety and Inspection Service (USDA–FSIS) for detection of *L. monocytogenes* in meat and poultry products (Wallace et al. 2003; Silbernagel et al. 2005). Unfortunately, these culturing methods depending on different selective media are generally time-consuming and labor intensive. In the case of *Listeria* detection, 4–9 days are usually required for a presumptive result. This is an obvious inconvenience for food industrial applications. In addition, bacterial population could be underestimated due to the presence of injured or stressed cells, which may become unculturable on selective media.

1.3.2 Immunological assays

Immunoassays are based on the specific binding of antibodies to corresponding antigens including proteins, lipopolysaccharides or other molecules on the cell surface. Enzyme linked immunosorbent assays (ELISAs), including direct ELISAs, sandwich ELISAs and competitive ELISAs, are the most common formats used for immuno-detection of pathogens (Lazcka et al. 2007). Many of these ELISA methods are available as commercial kits and have been approved by regulatory agencies. Most assays produce comparable results to the FDA and USDA–FSIS culture-based methods for pathogen detection. The detection limits for pathogens are normally in the range of 10^3 and 10^5 CFU/ml, and the enrichment step is required to provide sufficient numbers of bacterial cells for ELISA (de Boer and Beumer 1999). The colorimetric reaction as the final step of signal amplification for ELISA could be eliminated by conjugating fluorescent labels to the antibodies. The use of fluorescent labels increases the sensitivity of ELISA assay and, however, also increases the cost of the immunoassay (Churchill et al. 2006).

Immunomagnetic separation (IMS) employs magnetic beads coated with specific antibodies for the targeted pathogens, and has been considered as an effective technique for the detection of pathogens in various sample matrices, especially food samples (Pyle et al. 1999). IMS is based on the principle that bacterial cells bound to magnetic beads by specific antibodies can be separated from the background interference in a magnetic field. Several advantages with the use of magnetic beads have been discussed in the literature: (1) the target bacteria are concentrated from the sample, (2) inhibitory agents are removed, and (3) the number of background bacteria is reduced significantly. In addition, IMS has found many other applications, such as cell separation, cell modification, nucleic acid and protein isolation, etc. (Sinclair 1998).

1.3.3 Molecular-based methods

As the principal tool of most molecular-based studies, polymerase chain reaction (PCR) is a technique to amplify small amounts or even a single copy of target DNA using a thermostable DNA polymerase and two primers (Monis and Giglio 2006). The presence of foodborne pathogens, either alive or dead, can be detected by simply determining if a specific bacterial gene of interest is present. The PCR is much less time-consuming than culture-based methods, and the technique is conducive to automation and high throughput.

Most commonly used PCR methods for bacterial detection include regular PCR, multiplex PCR, real-time PCR, and reverse transcriptase-PCR (RT-PCR) (Deisingh and Thompson 2004). Regular PCR methods are able to detect the presence of single pathogen, whereas a multiplex PCR allows the simultaneous detection of several microorganisms of interest or multiple genes of single microorganism (Kim et al. 2007b). Real-time PCR allows monitoring the gene amplification in real time by detecting fluorescence from fluorescent dyes upon their bindings to the targeted amplicons without the need to run agarose gel as for regular PCR amplicon detection (Kubista et al. 2006). Real-time PCR can be quantitative in that the resulting fluorescence of incorporated fluorescent marker is proportional to the number of pathogens present in original sample (Kubista et al. 2006). The RT-PCR is designed to detect only viable bacterial cells by amplifying messenger RNA (mRNA), which is a labile molecule and readily degraded after cell death. Based on the designed oligonucleotide probes complementary to the target nucleic acid sequence, fluorescent in situ hybridization (FISH) allows detecting and quantifying microbial community of interest. In contrast to PCR assays, comparatively large amounts of target DNA or RNA are required to perform hybridization. Furthermore, DNA microarray, based on DNA or RNA hybridization, can carry out multiple detections simultaneously, displaying its capacity of massive screening capacity (Call 2005).

1.4 Bacterial recognition by bio-functionalized nanomaterials

With the advance of nanoscience and nanotechnology, nanomaterials have been integrated into biological systems for various applications. These nanomaterials include polymeric nanoparticles (Yang et al. 2007a), liposomes (Ho and Hsu 2003; Chen et al. 2005; Chen and Durst 2006), vesicles, inorganic semiconducting and metallic, and magnetic nanoparticles carrying with specific properties such as versatile chemistry, unique optical properties, or strong ferromagnetic responses (Lin et al. 2002; Gu et al. 2003; Naja et al. 2007; Yang et al. 2007b). Nanomaterial-based genome and proteome detections have been documented in literature (Rosi and Mirkin 2003; Zhang et al. 2007). All these sensitive and selective methods for DNA or protein detection can be taken advantageous for the recognition of those related to pathogens by choosing suitable biomolecules. Therefore, the incorporation of these functionalized nanomaterials into current pathogen detection methods is likely to lead to the development of new generation methods with emphasis on device portability and simplicity in sample preparation.

The conjugation of biomolecules with nanomaterials is the foundation of nano-biorecognition. Each nanoparticle with diameter of around 100 nm could efficiently conjugate about 150–200 molecules of antibody and result in more than 300 active binding sites (two binding sites for each antibody) (Soukka et al. 2003). Coating biomolecules on nanoparticles allows multiple contacts between nanomaterials and target cells, and therefore, the functionalized nanomaterials display higher binding affinity than free biomolecules. Soukka et al. (2001) demonstrated that the binding affinity constant for antibody–nanoparticle bioconjugates was eightfold higher than the intrinsic affinity of the free antibody. The affinity of magnetic nanoparticles coated with mannose was 200-fold higher than that of the monomeric mannose (El-Boubbou et al. 2007).

A variety of strategies have been developed toward the surface modification of nanomaterials. These strategies are usually categorized into two modes, either direct or indirect. In the direct methods, biological molecules can be connected to nanoparticles through physical adsorption, or covalent coupling. Both hydrophobic and electrostatic interactions are the most likely mechanisms involved in adsorption of proteins. The simple adsorption of biomolecules, ranging from low-molecular-weight organic substances (e.g. vitamin C) to large protein/enzyme molecules, on gold nanoparticle and some semiconductor dots was reviewed by Katz and Willner (2004).

In covalent coupling, the surface of nanomaterials is modified to contain functional groups of either sulfide, amine, or carboxyl (Tan et al. 2004). The biomolecules are conjugated to nanomaterials through covalent binding. For example, one of the well-established methods was to introduce carboxylic functional groups to nanomaterials and then connect biomolecules via carbodiimide coupling (Tan et al. 2004; Zhao et al. 2004). This diimide-activated amidation includes two steps: (1) carboxylated nanomaterials are activated by *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) to form a stable active ester and (2) amide bonds are formed between the nanomaterials and the proteins through the reaction between the active ester and the amine groups of protein.

This approach provides a universal and efficient method for attaching biomolecules, such as sugar moieties, oligonucleotides, peptide nucleic acids (PNA) and proteins to the carboxylic groups of nanomaterials (Tan et al. 2004; Zhao et al. 2004; Horák et al. 2007).

In the indirect method, biomolecules are conjugated to nanomaterials through bridge molecules having high affinity to each other. The biotin and avidin interaction is often the method of choice. For this approach, the avidin-coated nanomaterials can then be conjugated with biotinylated molecules based on the strong avidin–biotin affinity. The capability of silica to bind with avidin allows the use of nanoparticles (NPs) to assays requiring commonly used and widely available biotinylated compounds. The biomolecules can also be conjugated to nanomaterials through protein A, protein G or aptamer (Chen et al. 2005; Long and Keating 2006; Naja et al. 2007).

Based on the nature of biomolecules conjugated to nanomaterials, there are antibobody–antigen, adhesin–receptor, antibiotic, and complementary DNA sequence recognitions.

1.5 Antibody–antigen recognition

Antibody–antigen recognition is the most widely used strategy for nanomaterial biofunctionalization. Antibody specifically recognizes the corresponding antigen through a highly variable N-terminal region. The ideal antibody conjugation is that the antibody binding sites orient away from the nanomaterial surface. Wang et al. (2007) compared three different antibody conjugating strategies: (A) antibody was directly immobilized onto carboxylated nanoparticles (COOH-NP): NP-antibody, (B) antibody was immobilized onto COOH-NP through streptavidin and biotin: NP–streptavidin–biotin–antibody, (C) antibody was immobilized onto NH_2-modified NPs through PEG, streptavidin and biotin: NP–PEG–biotin- or streptavidin–biotin–antibody. Their results revealed that, for strategy A, some of the antibodies lost their ability to bind to a target bacterial cell due to the attachment of antibody onto the NPs through binding sites. Strategy C exhibited the best colloidal stability and binding performance because the addition of hydrophilic PEG linkers allowed the conjugated antibody to extend out from the NP surface. In addition, strategy C reduced the binding steric hindrance and thus, improved the binding efficiency of biofunctionalized nanoparticles.

For bacteria, there are many surface antigens available for specific recognition by using antibody-conjugated nanomaterials (Fig. 1). Antibody functionalized liposome, fluorescent dye-doped silica nanoparticles, polymeric nanoparticle, gold nanowire, quantum dot and carbon nanotubes have been used for specific recognition of

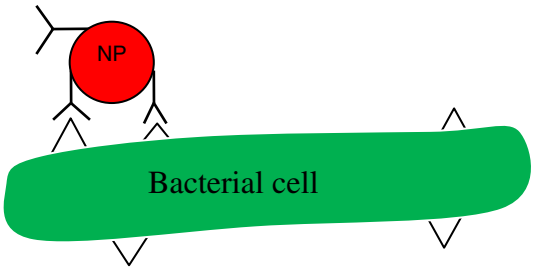

Fig. 1 Illustration of bacterial recognition by nanomaterials conjugated with specific antibody

pathogenic *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. (Ho and Hsu 2003; Zhao et al. 2004; Elkin et al. 2005; Kim et al. 2007a; Yang et al. 2007a). Using different antibodies specific for various bacterial pathogens, simultaneous detection of a wide variety of bacterial pathogens in food samples could be achieved. When polyclonal antibodies against three pathogens were applied, universal G-liposomal nanovesicles-based immunoassay simultaneously detected *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* at the concentrations of 3.1×10^3, 7.8×10^4, and 7.9×10^5 CFU/ml, respectively (Chen and Durst 2006).

In addition to above-mentioned nanomaterials, nanomaterials encapsulated with Fe_2O_3 magnetic have drawn a great interest in pathogen detection (Campion and Kambhampati 1998; Chen et al. 2005; Gu et al. 2004; Mao and Koser 2006; Yang et al. 2007b). As reported by Yang et al. (2007b), the detection limit of *L. monocytogenes* in milk samples was ca. 10^2 CFU/0.5 ml, about 1.4–26 more sensitive than Dynabead®-based immunomagnetic separation. In that study, the polymer-inorganic heterodimeric nanoparticles tethered with carboxylic acid were conjugated with rabbit anti-*L. monocytognenes* via EDC coupling. Similar heteronanoparticles were developed with magnetic cores for separation and purification, and with luminescent surface (quantum dots) (Gu et al. 2004) or plasma surface (silver or gold nanoparticle) (Campion and Kambhampati 1998; Dinsmore et al. 2002) for optical detections. By combining immunomagnetic separation with advanced detection systems such as time-resolved fluorometry and electrochemiluminescence Yu and others (1996, 2003) can detect ca. 10^2 to 10^3 CFU *E. coli* O157:H7/ml. The immunomagnetic nanoparticles can also be detected based on their Brownian relaxation time constant which is usually the reciprocal of the optimum pumping frequency in microfluidic devices (Mao and Koser 2006). Furthermore, the bacterial cells touched with immunomagnetic nanoparticles can be imaged with transmission electron microscopy, which has been an important tool to analyze other immuno-nanoparticles or nanoparticles conjugated with other cell-surface-specific ligands on the surface of *E. coli* bacteria (Lin et al. 2002).

1.6 Adhesin (lectin)–receptor (carbohydrate) recognition

Many species of bacteria express surface lectins that adhere to complementary receptors present on the host cell surfaces (Sharon 2006). A variety of carbohydrates have been recognized as receptors for attachment of pathogenic micro-organisms to epithelial cells. For example, galactose, glucose, fructose, fucose, mannose and sucrose are corresponding carbohydrate receptors on epithelial cells for the lectins of *E. coli* (Sharon 2006). Functionalization of nanomaterials with carbohydrates involved in adhesion interaction between bacteria and host cells may serve as a platform for specific recognition of target bacteria (Fig. 2). El-Boubbou et al. (2007) functionalized silica-coated magnetic nanoparticles with D-mannose. These mannose-coated magnetic nanoparticles allow differentiation of three *E. coli* strains with different mannose binding affinity.

As the emerging new materials, carbon nanotubes have broad physical and chemical properties. The unique one-dimensional flexible tubular structures with hydrophobic core as well as defect sizes provide the versatile functionalities. Several saccharides as cell-surface ligands were functionalized onto carbon nanotubes via amide formation in their defect sites, and the resulting carbohydrate-conjugated nanomaterials displayed strong adhesion-specific interaction with *E. coli* (Gu et al. 2005; Qu et al. 2005). The galactose functionalized single-walled nanotubes (SWNT) were found to agglutinate *E. coli* O157:H7, whereas the mannose functionalized SWNT-aggregated *E. coli* O178 as analyzed with optical microscopy and electron microscopy, even visible with naked eyes (Gu et al. 2005). These mannosyl SWNTs also exhibited strong binding to *Bacillus anthracis* spores in the presence of dications Ca^{2+} (Wang et al. 2006). Chen et al. (2006) also developed α-*N*-acetylgalactosamine (α-GalNAc)-coated carbon nanotubes via self-assembly to efficiently interact with Chinese hamster ovary (CHO) living cells. In addition to these carbon nanomaterials, the prospective conjugated polymers functionalized with saccharides such as mannosylated polyphenyleneethylene, were able to detect the presence of a pathogen in 10–15 min via bright fluorescence emission after multivalent interactions (Disney et al. 2004).

1.7 Antibiotic recognition

Vancomycin, a glycopeptide antibiotic, was applied to recognize Gram-positive bacteria by its binding to terminal peptide (D-Ala–D-Ala) on the cell walls of Gram-positive bacteria via hydrogen bonds (Fig. 3). Magnetic nanoparticles functionalized with vancomycin for protein separation and pathogen detection were reviewed elsewhere (Gu et al. 2006). Lin et al. (2005) employed vancomycin-modified magnetic nanoparticles for selective isolation of Gram-positive pathogens from pure sample solutions. The isolated cells were further characterized by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), a straightforward means to differentiate micro-organism species based on mass spectral fingerprinting. The lowest detection limit for both *Staphylococcus saprophyticus* and *Staphylococcus aureus* in a urine sample was ca. 7×10^4 CFU/ml. Recently, vancomycin functionalized magnetic nanoparticles have demonstrated their capacity in detecting both Gram-postive and Gram-negative bacteria at ultra-low concentrations, i.e., *S. aureus* at 8 CFU/ml, *S. epidermidis* at 10 CFU/ml, coagulase negative staphylococci (CNS) at 4 CFU/ml, *E. faecalis* (ATCC 29212) at 26 CFU/ml, and *E. coli* at 15 CFU/ml, respectively (Gu et al. 2003; 2004; 2006).

1.8 Complementary DNA sequence recognition

Nucleic acid sequences are unique for every living organism. The property of interacting with complementary DNA

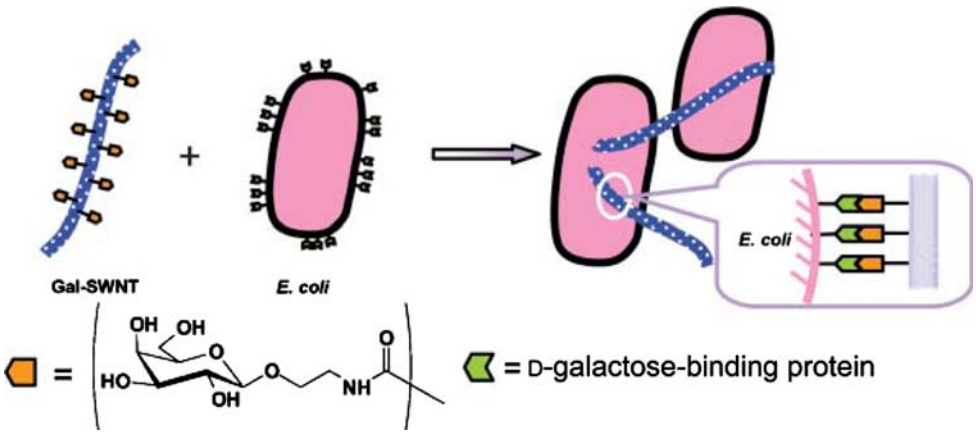

Fig. 2 Illustration of bacterial recognition through the binding of galactose functionalized single-walled carbon nanotubes to an *E. coli* cell. Reprinted from Gu et al. (2005) with permission

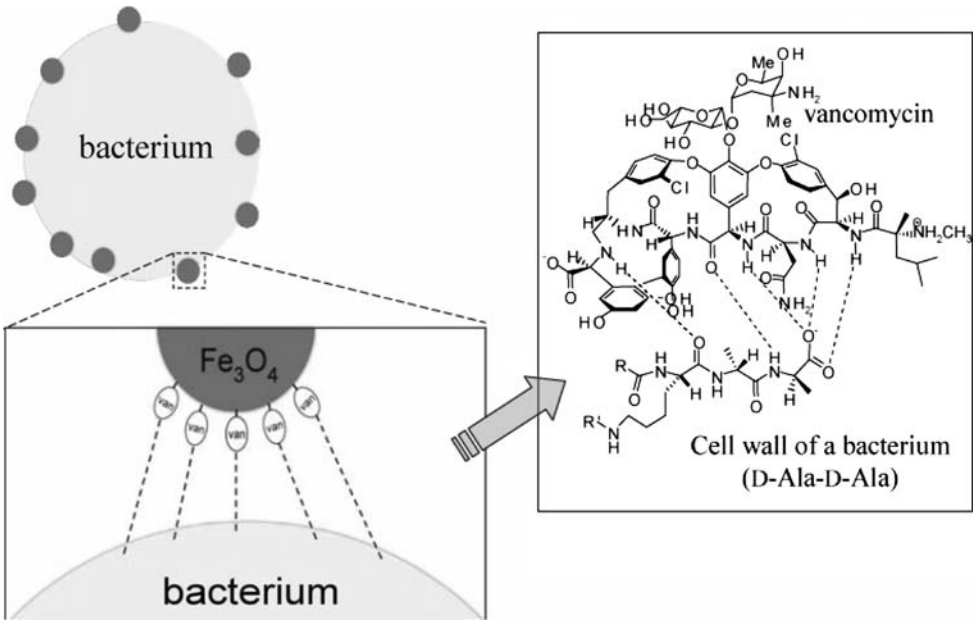

Fig. 3 Illustration of bacterial recognition through the binding of vancomycin immobilized magnetic nanoparticles to the terminal of D-Ala–D-Ala of the peptides on the cell wall of a Gram-positive bacterium. Reprinted from Lin et al. (2005) with permission

sequences has been exploited for bacterial recognition. Oligonucleotides-functionalized nanomaterials are used for selective separation of target DNA and RNA from a mixture. Amagliani et al. (2006) immobilized oligonucleotide probes to magnetic nanoparticles for selective DNA purification. Followed by PCR, *L. monocytogenes* cells were detected from milk samples at a 10 CFU/ml contamination rate. However, a dose-dependent inhibitory effect of the nanoparticles on PCR was observed.

2 Nanomaterials in biosensors used for foodborne pathogen detection

The development of practical biosensors using nanomaterials is promising in eliminating the need for expensive or complicated instruments and allowing the rapid detection of foodborne pathogens on a portable or hand-held device. The detection of pathogens can be improved in conventional pathogenic biosensors by using immunonanoparticles. For example, the sensitivity of the impedimetric biosensor for *S.* Enteritidis cells was improved from 10^6 to 10^4 CFU/ml at 100 Hz of input frequency by incorporating anti-*Salmonella* antibody-conjugated nanoparticles (Kim et al. 2007a). By further coupling immunonanoparticles with enzymatic catalysis, the detection of electrochemical immunosensor could be rapid, efficient and accurate. The screen-printed electrode coated with agarose/nano-Au membrane and horseradish peroxidase (HRP) labeled with anti-*Vibrio parahaemolyticus* antibody (HRP-anti-VP) detected VP with the detection limit of 7.4×10^4 CFU/ml. The mechanistic principle is that VP in the vicinity of the active center of HRP partially inhibited the catalytical oxidation of thionine by H_2O_2 (Zhao et al. 2007). By combining immunomagnetic separation with enzymatic *p*-nitrophenyl phosphate hydrolysis by alkaline phosphatase (EC 3.1.3.1), *E. coli* O157:H7 was detected in a range of 3.2×10^2 to 3.2×10^4 CFU/ml by measuring the absorption of *p*-nitrophenol product at 400 nm from the catalysis of the "sandwich" structure complexes (antibodies-coated micromagnetic beads–*E. coli* O157:H7–antibodies-conjugated enzymes) (Lin et al. 2002).

Varshney et al. (2005) used magnetic nanoparticles conjugated with anti-*E. coli* (specific for O and K antigens) for separating *E. coli* O157:H7 from ground beef. Their study demonstrated that due to the efficient diffusion and rapid binding kinetics of nanoparticles, no mechanical mixing was needed for nanoparticle-based immunomagnetic separation. For this reason, nanoparticles may have distinct advantages in their application in microfluidic devices by offering a more efficient mass transfer.

Due to the small size of nanomaterials, a target bacterial cell binding event can have a significant effect on their optical, physical and chemical properties, thereby providing a mode of signal transduction or amplification which makes the detection of pathogenic bacteria in real time. Basu et al. (2004) used anti-*E. coli*-bound gold nanowire arrays (GNWA) prepared on anodized porous alumina template for capturing *E. coli* O157:H7. The formation of bacteria–antibody complex changes the surface properties of the sensor, such as capacitance of the biomembrane. Such change was measured by electrochemical impedance spectroscopy (EIS) and then the amount of bound *E. coli* was determined. Their preliminary results indicated that

the GNWA biosensor could detect ten *E. coli* cells with the sensor area of 0.173 cm^2.

In a study reported by Zhou et al. (2006), the attachment of SWNT both enhanced and reversed bacterial dielectrophoresis (DEP) mobility. Consequently, the SWNT–bacteria aggregates assemble rapidly (<5 min) into conducting bridges between two electrodes by positive-alternating current DEP. This strategy showed a detection threshold of 10^4 CFU/ml of *E. coli*. Therefore, the functionalized SWNT may serve as absorbers and transporters of pathogens in biosensors.

3 Advantages of using nanomaterials for foodborne pathogen detection

3.1 Rapid and real-time detection

Traditional culture-based methods rely on the multiplication of bacterial cells, and could take at least 24 h of incubation in a laboratory setting. Although DNA- and protein-based detection methods are quicker, these methods still require at least several hours to perform. In order to detect a few bacterial cells in a food sample, a culture enrichment step ranging from a few hours to overnight is typically required. In food industry, such a long waiting for the results can be expensive and inconvenient.

Usually, in a nanomaterial-based method, the target cells are captured, removed or concentrated from testing samples by biofunctional nanomaterials. The complex of bionanomaterial-bacterial cells could be then detected or confirmed within 3 h by means without bacterial culture and enrichment (Qu et al. 2003; He and Liu 2004; Zhao et al. 2004; Edgar et al. 2006; El-Boubbou et al. 2007; Kim et al. 2007a; Wang et al. 2007). However, sometimes the procedures involved the use of impractical and expensive instruments in a laboratory setting, such as scanning electron microscopy (SEM) (Gu et al. 2006), fluorescent microscopy (Gao et al. 2006), confocal scanning laser microscopy (Yang et al. 2007a), to speed up the detection time.

The development of practical biosensors using nanomaterials is promising in eliminating the need for expensive or complicated instruments and allowing the rapid detection of foodborne pathogens on a portable or hand-held device. As an example, Zhao et al. (2004) reported a nanoparticle-based test which can finish the detection of a single *E. coli* O157 cell in ground beef sample in less than 20 min as compared with up to 48 h for conventional tests. In their study, fluorescent silica nanoparticles conjugated with anti-*E. coli* O157 were added to ground beef inoculated with *E. coli* O157. Antibody-conjugated nanoparticles bound to target cells were detected by a flow cytometer, which gave a fluorescent spike when the target cell was flowed through. This bioassay was finished within 20 min from bacterial binding to detection without any cell amplification or enrichment.

Due to the small size of nanomaterials, a target bacterial cell binding event can have a significant effect on their optical, physical and chemical properties, thereby providing a mode of signal transduction or amplification which make the detection of pathogenic bacteria in real time. Various functionalized nanomaterials have been studied for their incorporation into biosensors as absorbers and transporters of pathogens. These nanomaterial-based biosensors allow the detection process to be finished within 10 min. In Kim et al. (2007a) study, a nanoparticle-enhanced impedimetric biosensor was used to detect *S.* Enteritidis by measuring the impedance changes caused by the binding of target cells to the anti-*Salmonella* immobilized on interdigitated gold electrodes. The nanoparticle-based biosensor was able to detect 10^4 CFU *S.* Enteritidis/ml in phosphate-buffered saline (PBS) with a detection time of 3 min.

3.2 Improved detection sensitivity

Even the presence of low number foodborne pathogens in food can be dangerous given their severity of infections. For example, the United States has 'zero-tolerance' policy for the presence of *L. monocytogenes* in ready-to-eat (RTE) food (Donnelly 2001). The critical issue facing the implementation of any "zero-tolerance" policy relates to the lack of rapid and reliable procedures for the detection of low numbers of *Listeria* in foods. Application of nanomaterials will be helpful in detecting low levels of foodborne pathogens quickly and accurately.

Fluorescent dye-doped nanoparticles were developed as markers for sensitive bacterial detection due to their favorable properties such as high fluorescence quantum yields, photo-stability, and tunable fluorescence bands (Lian et al. 2004; Zhao et al. 2004). A single nanoparticle of diameter about 100 nm may contain hundreds and hundreds of fluorescent dye molecules. Thus dye-doped nanoparticles are much brighter than single fluorescent dye molecule (Lian et al. 2004). Zhao et al. (2004) developed a bioassay based on fluorescent nanoparticles conjugated with anti-*E. coli* O157 for detection of *E. coli* O157 in ground beef. Due to the size difference between fluorescent nanoparticles and *E. coli* O157 cell, thousands of anti-*E. coli* O157-conjugated fluorescent nanoparticles were bound to a single bacterial cell. Since anti-*E. coli* O157-conjugated fluorescent nanoparticles are 1,000 times brighter than that dye molecule-labeled antibody, a single *E. coli* O157 cell was detected by measuring the enhanced fluorescent signals from the bacterial surfaces.

Other fluorescent nanomaterials have also been reported for sensitive detection of foodborne pathogens. The protein G-tagged liposomal nanovesicles were successfully used in an immunomagnetic bead sandwich assay for the detection of *E. coli* O157:H7 with a detection limit of approximately 100 CFU/ml (Chen et al. 2005). Each liposomal nanovesicle, i.e., liposome, can be filled with several million fluorescent dye molecules. Because of providing greatly enhanced signals, liposomal nanovesicles have been successfully used as reporter particles in immunoassays. Luminescence colloidal semiconductor nanocrystals, known as quantum dots (QDs), were used for detecting *Cryptosporidium parvum* oocyst (Lee et al. 2004). As compared with organic fluorescent dye, QDs displayed the advantage of high photobleaching threshold. Combining biotin-tagged bacteriophage and streptavidin-coated QDs, *E. coli* (10 CFU/ml) could be detected within an hour (Edgar et al. 2006). Furthermore, by using multiple host-specific phages and QDs of different emission colors, it is possible to expand this QDs-phage-based method to the detection of multiple bacterial strains.

The large surface area to volume ratio of nanomaterials provides them higher capacity as substrates for biomolecule immobilization. He and Liu (2004) applied nano-membranes to a DNA biosensor for detecting *Pseudomonas aeruginosa*. The surface area of nano-sized membranes is approximately 1 or 2 orders of magnitude more than that of continuous thin films. Their results found that using nano-membrane, the amount of bound DNA was increased 3–5 times and the response sensitivity was improved about three times. Chang (2007) reviewed the advantages of using nanobead as microfluid platform for multi-target pathogen detection. The low capture efficiency and long diffusion time accounting for the slow response time and low sensitivity are the bottlenecks of microfluidics. Using nanomaterials is one of the future research trends to overcome those obstacles since the large local density of nanomaterial-bound biomolecules offers a higher capacity for capturing target bacterial cells present in testing samples, and, thus, improves the sensitivity and reduces diffusion time.

Taking advantage of the high surface-to-volume ratio and faster reaction kinetics, the functionalized magnetic nanomaterials display higher capture efficiencies in immunomagnetic separation than microbeads do. A minimal capture efficiency of 94% for *E. coli* O157:H7 ranging from 1.6×10^1 to 7.2×10^7 CFU/ml was reported by using magnetic nanoparticle-anti-*E. coli* O157 conjugates (Varshney et al. 2005). In the presence of *S.* Typhimurium DT104 cells as background flora, carbon magnetic nanotubes conjugated with anti-*E. coli* O157 were capable of capturing *E. coli* O157:H7 at a relatively low concentration of 40 CFU/0.1 ml without cross reaction between species (Lin et al. 2006). Yang et al. (2007b) developed a method combining nanoparticle-based immunomagnetic separation (IMS) with real-time PCR for a rapid and quantitative detection of *L. monocytogenes*. In their study, the capture efficiencies of anti-*L. monocytogenes*-magnetic-based IMS were 1.4–26 times higher than those of Dynabeads®-based IMS depending on the initial cell concentrations inoculated into milk samples. When combined with real-time PCR, *L. monocytogenes* DNA was detected in milk samples with *L. monocytogenes* $\geq 10^2$ CFU/0.5 ml.

As compared with food products, pathogen detection in biofilm can be a very challenging task. A microbial biofilm is described as the adherent micro-organisms within polymeric matrix in a single layer or a three-dimensional structure. Many foodborne pathogens can form biofilms with other micro-organisms in food and processing environments. Inside biofilms, microbial cells become more resistant than planktonic cells to routine sanitizing procedures and, thus, were more difficult to remove (Chmielewski and Frank 2003; Frank and Koffi 1990). Consequently, there is an increasing concern in the food industry with the growth and presence of foodborne pathogens, such as *L. monocytogenes,* in biofilms. A study conducted by Yang et al. (2007a) demonstrated that immuno-nanoparticle-based immunoassays were more sensitive than traditional immunoassays for detecting *L. monocytogenes* in mono- or two-species biofilms including *P. aeruginosa* ATCC 27853. Under the same confocal laser scanning microscopy conditions, nanoparticles coated with anti-*L. monocytogenes* generated higher intensity of fluorescent signals ranging from 0 to 4,000, than anti-*L. monocytogenes* alone did, which was in the range of 0–250. Individual *L. monocytogenes* cells at different depths (0–5 μm) of two-species biofilms were successfully detected due to the signal amplification system by using immuno-nanoparticles.

3.3 Simultaneous detection of multiple foodborne pathogens

The optical properties of nanomaterials, such as emissive, absorptive, and light-scattering properties, are directly related to their sizes, composition, and shapes. For example, both the absorption and emission energies of QDs shift to higher energies as the size of the nanoparticles decreases (Bruchez et al. 1998). These features make nanomaterials ideal for multiplexed detection. Yang and Li (2006) explored the use of semiconductor QDs as fluorescence labels in immunoassays for simultaneous detection of two species of foodborne pathogens, *E. coli* O157:H7 (1.95×10^3 CFU/ml) and *S.* Typhimurium (3.35×10^4 CFU/ml). The varying numbers and ratios of different quantum dots

Table 1 Summary of pathogen detection using nanomaterials

Micro-organisms	Sample matrix	Detection limit	Nanomaterial	Recognition	Detection methods	Analysis time	References
In buffer or broth							
Individual detection							
E. coli	–		Gold nanoparticles	Adhesin–receptor	TEM		Lin et al. (2002)
E. coli	–	10 cells/0.173 cm^2	Gold nanowire arrays	Antibody–antigen	Electrochemical impedance spectroscopy		Basu et al. (2004)
E. coli	PBS		Polymeric nanoparticles	Adhesin–receptor	TEM		Qu et al. (2005)
E. coli	–	10 CFU/ml	Quantum dot	Antibody–antigen	Fluorescence microscopy	<1 h	Edgar et al. (2006)
E. coli	Deionized water	10^4 CFU/ml	Carbon nanotubes		DEP		Zhou et al. (2006)
E. coli	–	10^4 cells/ml	Magnetic nanoparticles	Adhesin–receptor	Fluorescent microscopy	<5 min	El-Boubbou et al. (2007)
E. coli	PBS		Magnetic nanorice	Antibody–antigen	IMS and UVRR		Naja et al. (2007)
E. coli O157:H7	–	2.5×10^3 cells	Liposome	Antibody–antigen	Immunoassay		Ho et al. (2003)
E. coli O157:H7	TBS	1.0×10^2 CFU/ml	Liposome	Antibody–antigen	Immunoassay		Chen et al. (2005)
E. coli O157:H7	0.85% NaCl		Carbon nanotubes	Antibody–antigen	CLSM, SEM		Elkin et al. (2005)
E. coli O157:H7	0.85% NaCl		Carbon nanotubes	Adhesin–receptor	SEM		Gu et al. (2005)
E. coli O157:H7	PBS	8 CFU/ml	Magnetic nanoparticles	Antibody–antigen	IMS + Plating		Varshney et al. (2005)
E. coli O157:H7	0.85% NaCl	40 CFU/0.1 ml	Carbon magnetic nanotubes	Antibody–antigen	IMS + plating		Lin et al. (2006)
Cryptosporidium parvum	PBS	4.5×10^3 oocyst	Quantum dot	Antibody–antigen	Epifluorescence microscopy		Lee et al. (2004)
Pseudomonas aeruginosa	–		Nano-sized membranes	DNA	DNA sensor	<3 h	He and Liu (2004)
Staphylococcus aureus, *Staphylococcus epidermidis*	–	8 CFU/ml, 10 CFU/ml	Magnetic nanoparticles	Antibiotic	IMS + SEM		Gu et al. (2003)
Simultaneous detection							
E. coli O157:H7, *Salmonella* spp., *L. monocytogenes*	TBS	1.5×10^4 CFU/ml, 5×10^4 CFU/ml, 1.2×10^5 CFU/ml	Liposomal nanovesicles	Antibody–antigen	Immunoassay		Chen and Durst (2006)
E. coli O157:H7, *Salmonella* Typhimurium	Saline	1.95×10^3 CFU/ml, 3.35×10^4 CFU/ml	Quantum dot	Antibody–antigen	Fluorescence spectra		Yang and Li (2006)
E. coli, *S.* Typhimurium, *S. aureus*	–		FRET, silica nanoparticles	Antibody–antigen	Confocal microscopy	<30 min	Wang et al. (2007)

Table 1 continued

Micro-organisms	Sample matrix	Detection limit	Nanomaterial	Recognition	Detection methods	Analysis time	References
In food or other matrices							
L. monocytogenes	Milk	10 CFU/ml	Magnetic nanoparticles	DNA	IMS + PCR		Amagliani et al. (2006)
L. monocytogenes	Milk	226 CFU/0.5 ml	Magnetic nanoparticles	Antibody–antigen	IMS + real time PCR		Yang et al. (2007b)
Staphylococcus saprophyticus	Urine	7×10^4 CFU/ml	Magnetic nanoparticles	Antibiotic	IMS + MALDI-MS		Lin et al. (2005)
S. aureus	Urine	7×10^4 CFU/ml	Magnetic nanoparticles	Antibiotic	IMS + MALDI-MS		Lin et al. (2005)
L. monocytogenes	Biofilm		Polymeric nanoparticles	Antibody-Antigen	CLSM		Yang et al. (2007a)
S. Enteritidis	Milk	10^5 CFU/ml	Quantum dot	Antibody-Antigen	Impedimetric biosensor	<3 min	Kim et al. (2007a)
E. coli O157:H7	Ground beef	1 CFU/g	RuBpy-doped silica nanoparticles	Antibody–antigen	Flow cytometer	< 20 min	Zhao et al. (2004)

"–" not specified in the cited reference; *PBS* phosphate-buffered saline; *TBS* tris-buffered saline; *FRET* fluorescence resonance energy transfer; *CLSM* confocal laser scanning microscopy; *TEM* transmission electronic microscopy; *SEM* scanning electronic microscopy; *IMS* immunomagnetic separation; *PCR* polymerase chain reaction; *MALDI-MS* matrix-assisted laser desorption/ionization mass spectrometry; *DEP* dielectrophoresis; *UVRR* ultra-violet resonance Raman

per target result in a unique fluorescent signal for each individual target.

Instead of using immunomagnetic beads, microtiter plates in an array format were developed for simultaneous detection of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* using the G-liposomes conjugated with appropriate antibodies (Chen and Durst 2006). Besides these nanovesicles, inorganic silica nanobeads doped with thousands of fluorescent dye molecules (RuBpy) were developed, and conjugated with anti-*E. coli* O157 antibodies to enable the binding of thousands of nanoparticles onto each bacterial cell via the specific, multiple antibody–antigen recognition (Zhao et al. 2004). This high sensitive protocol allows the simultaneous detection of one bacterial cell per given sample in less than 20 min with a spectrofluorometer in the presence of other pathogens like *S.* Typhimurium and *B. cereus* spores, consistent with the measurements using a simple flow cytometry device. These immunosilica nanoparticles accurately detected ca. 1–400 *E. coli* O157 cells even in spiked ground beef samples. The silica nanobeads were also doped with three amine reactive energy-transfer tandem dyes (FAM-SE, R6G-SE, ROX-SE) in three desired ratios (1:0.5:1, 0.5:1:4, 0.5:0.5:3) to form different FRET nanoparticles (Wang et al. 2007). After conjugated with different antibodies specific to the pathogens, the multiplex detection of pathogens was demonstrated under confocal microscopy displaying three fluorescence emissions, i.e., blue, orange, and purple excited at 488 nm.

Based on the electric responses of nanowires to multiplex pathogen binding via antibody–antigen interactions, Beckman et al. (2005) have recently fabricated an ultra-high density nanowire circuit to decode the combined electric signals for individual pathogenic identification. Using optical signaling such as reflectance and fluorescence of fluorescently labeled antibodies, Tok et al. (2006) developed multi-striped nanowires for efficient and accurate multiplex detection of biowarfare simulants. Antibody-coated nanowires with unique yet easily identifiable encoding patterns were used in a multiplex detection of three nonpathogenic stimulants of *B. anthracis*, Variola, and protein toxins (such as ricin or botulinum toxin) with detection limits of 1×10^5 CFU/ml, 1×10^5 plaque forming units (PFU)/ml, and 5 ng/ml, respectively, reflecting target sizes from 2 μm to 2 nm.

4 Conclusion

Table 1 summarizes the applications of nanomaterials to foodborne pathogen detection. Most of these studies incorporated novel nanomaterials into traditional methods for the improvement of detection speed and sensitivity.

For example, magnetic nanomaterials were most often used to remove the interference from complex food matrices, and concentrate the target cells, which may eliminate the need for time-consuming enrichment via a culture process. Fluorescent nanomaterials were studied for signal enhancement, whereas metal and semiconductor nanomaterials were chosen in the development of biosensors due to their electronic or optical transduction upon biological recognition. As reviewed herein, bioconjugated nanomaterials have exhibited the advantages over conventional (non-nanomaterial-based) methods for specific pathogen detection in nutrient broth, food products and biofilms.

Due to their unique electronic, optical, and catalytic properties, the most promising aspect of exploring nanomaterials is the eventual development of new detection methods. Major efforts have been directed to, but not limited to, the nanomaterial-based biosensors for high-speed, simultaneous and high-throughput detections. Currently, the biosensors generally lack the combination of high sensitivity and high specificity required for detection of ultra-low concentration of foodborne pathogen. As a consequence, research to improve the performance of biosensors should be pursued.

It is noteworthy that nanotechnology in detection of foodborne pathogen is still in its infant phase. Most of the studies have only been conducted on bacterial cultures in nutrient broth with *E. coli* strains being as a model microorganism. Although some nanomaterial-based detection methods were developed for some human pathogens such as *L. monocytogenes*, *Staphylococcus*, *Salmonella* spp. and *P. aeruginosa,* further studies should be given to other foodborne pathogens, such as *Campylobacter, Clostridium botulinum* and *Vibrio* spp. in food products. Some nanomaterial-based procedures for pathogen detection involved the use of impractical and expensive instruments in a laboratory setting, which may not be applicable for industrial applications. In addition, the toxicity of nanoscale materials has been debated extensively, and should be thoroughly evaluated.

Currently, the detection sensitivity by using nanomaterials has not achieved one or a few cells yet. However, all those detection assays were processed without sample enrichment. In terms of detection speed, nanomaterials-based detection methods have the potential for real-time pathogen detection. Those methods may be very useful for pathogen screening of food samples prior to more complicated laboratory analysis. Though there may still have a long way for nanomaterials-based detection assays to be approved as an official method and accepted by customers, the prospect of nanomaterials for rapid and sensitive pathogen detection is promising.

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