Sensors and Materials, Vol. 26, No. 10 (2014) 711–736
MYU Tokyo

S & M 1036

Recent Advances in Potential Nanoparticles and Nanotechnology for Sensing Food-Borne Pathogens and Their Toxins in Foods and Crops: Current Technologies and Limitations

Preeyaporn Koedrith, Thalisa Thasiphu, Kooranee Tuitemwong[1], Rattana Boonprasert[*] and Pravate Tuitemwong[1,**]

Faculty of Environment and Resource Studies, Mahidol University,
999 Phuttamonthon 4 Road, Phuttamonthon District, Nakhon Pathom 73170, Thailand
[1]Food Safety Center, Institute for Scientific and Technological Research and Services (ISTRS),
King Mongkut's University of Technology Thonburi (KMUTT), Bangkok 10140, Thailand

(Received January 20, 2014; accepted September 5, 2014)

Key words: biosensors, fluorescent nanoparticles, food-borne pathogens, nanotechnology, rapid detection

Food-borne pathogens cause infectious diseases in people worldwide. Hence, the development of rapid detection methods for food-borne pathogens is necessary. The application of potentially useful nanoparticles (NPs) results in enhanced sensitivity, improved response time, and increased portability owing to their distinct chemical and optical features. The widely used NPs include quantum dot (QD) NPs, metal NPs, silica NPs, and magnetic NPs as well as potent intrinsic antimicrobial NPs. The NPs can also act as multivalent scaffolds for supramolecular assemblies since their high surface-to-volume ratio enables the functionalization of unique spatial domains, allowing their versatile implementation in various sensing schemes. In this review, we focus on the developments and analytical applications of NPs in chemical and biological sensing within foods and crop matrices. We also discuss advanced tools of NP-based sensitive assays, key requirements, and shortcomings.

1. Introduction

Food-borne diseases are a public health threat with increasing socio-ecomonic costs. In the United States, it has been estimated that 31 main food-borne pathogens

[*]Corresponding author: e-mail: rattana.boo@mahidol.ac.th
[**]Corresponding author: e-mail: pravate.tui@kmutt.ac.th

cause over 9 million cases of illnesses and over 1000 deaths.[1] The cost of managing these problems is over $70 billion annually.[2] For protection against such preventable diseases, federal agencies such as the US Department of Agriculture (USDA), Food and Drug Administration (FDA), and Environmental Protection Agency (EPA) are responsible for adopting effective monitoring technologies and systems globally.

Most of the food-borne diseases are caused by microorganisms such as bacteria, viruses, and parasites. Gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting are commonly associated with microbial infections. For instance, infections by *Cryptosporidium* sp., *Entamoeba histolytica*, *Escherichia. coli*, *Salmonella* sp., *Shigella* sp., and *Vibrio cholerae* result in diarrhea as the major clinical symptom. Hence, food-borne pathogens are of utmost concern in food safety. Table 1 summarizes the major types of microorganism responsible for food-borne diseases in the United States.[3]

Table 1
Major types of microorganism causing food-borne diseases in the United States.

Bacteria			**Viruses**	**Parasites**
Bacillus cereus	*Listeria monocytogenes*	*Staphylococcus aureus*	Astrovirus	*Cryptosporidium parvum*
Bacillus anthracis	*Mycobacterium avium*	*Vibrio cholerae*	Enterovirus	*Cyclospora cayetanensis*
Brucella sp.	*Mycobacterium bovis*	*Vibrio parahaemolyticus*	Hepatitis-A	*Giardia intestinalis*
Campylobacter sp.	Nontyphoidal *Salmonella* sp.	*Vibrio vulnificus*	Hepatitis-E	*Giardia lamblia*
Clostridium botulinum	*Salmonella enterica* serotype Typhi	*Yersinia enterocolitica*	Norovirus	*Taenia saginata*
Clostridium perfringens	*Shigella* sp.	*Yersinia pseudotuberculosis*	Rotavirus	*Taenia solium*
E. coli O157:H7	*Streptococcus* sp.	*Yersinia pestis*		*Trichinella spiralis* *Trichuris trichiura* *Toxoplasma gondii*

Top five food-borne pathogens resulting in the following:

Illness	**Hospitalization**	**Death**
Norovirus	Nontyphoidal *Salmonella* sp.	Nontyphoidal *Salmonella* sp.
Nontyphoidal *Salmonella* sp.	Norovirus	*Toxoplasma gondii*
Clostridium perfringens	*Campylobacter* sp.	*Listeria monocytogenes*
Campylobacter sp.	*Toxoplasma gondii*	Norovirus
Staphylococcus aureus	*E. coli* O157	*Campylobacter* sp.

No global estimate is available on the prevalence of these food-borne diseases owing to their variety and extent. Food-borne diseases cannot be diagnosed precisely since several factors are involved in their etiology, and these diseases may only be manifested after long-term exposure. Thus, the actual number of cases is not obtained by public health authorities under surveillance programs. It has been estimated that 10 and 1% of food-borne diseases are not reported in developed and developing countries, respectively.[4] Although the incidence of nonreporting of food-borne diseases is higher in developing countries, it is still considerably high in developed countries.[5]

Owing to food safety concerns as a global priority, standard methods have recently been developed for detecting and quantifying microorganisms in foods. Nevertheless, these methods are laborious and time-consuming with limited specificity and sensitivity. Currently, nanotechnology enables rapid detection and monitoring of pathogen and toxin contaminations at various steps in the food supply chain, thereby potentially reducing recalls and human health costs, especially those due to lethal food-borne diseases. When a contaminated sample is identified by rapid screening, standard microbiological techniques would still be required to confirm the presence of potential pathogens and/or toxins. Rapid, sensitive testing methods would also improve business efficiency in perishable-product-related food industries owing to the faster release of products without waiting for the results of a long, laborious test of its safety. Although only some types of food are withheld until results are available, sensors capable of detecting contaminated batches will enable the prompt removal of the contaminated raw materials or products from the food chain. Apart from investigative sampling, these rapid methods are also useful for verifying the efficacy of food safety systems such as Hazard Analysis and Critical Control Points (HACCP) and assuring the safety of end products.

In addition to the time delay between sampling and assay results, the aforementioned standard methods have disadvantages owing to a common deficiency: they rely on destructive sampling of food products rather than on-the-food nondestructive sampling. Nondestructive sampling-based safety testing prior to the food products leaving the farm or processing facility is a very powerful tool, particularly where homogenization is impractical. When pathogens are, however, distributed within foods (*e.g.*, ground beef), there is no choice but only destructive testing. Even though the only way to verify that a food is 100% pathogen-free is to utilize the entire food for testing, this has been considered impractical.

Recently, molecular beacon (MB) technology, which utilizes organic fluorophores, has been developed for the detection and quantification of food-borne pathogens in vitro,[6–8] in conjunction with quantitative polymerase chain reaction (qPCR). However, newer fluorescent structures, such as quantum dots (QDs), are attracting interest owing to their higher sensitivity and stability than other fluorophores and the possibility of simultaneous detection of multiple pathogens within a single sample. Numerous reviews have described the application of other nanoparticle-based biosensors (i.e., metallic and magnetic materials) for the detection and imaging of pathogens.[9–12] In

this review, we address the benefits of the molecular nanotechnologies using recently developed nanoparticles (i.e., QDs and metallic and magnetic nanoparticles) as emerging tools for protecting agricultural crops or the food supply chain from the farm to the consumer, their shortcomings, and strategies for further development of nano-biosensors.

2. Current Techniques for Detecting Food-Borne Pathogens

Detection of food-borne pathogens by conventional approaches usually involves microorganism identification by morphological evaluation through selective enrichment, biochemical analysis, and serological confirmation.[13] Owing to these techniques being time-consuming, alternative methods based on different principles of detection have been developed (i.e., chromatography, flow cytometry, electrical conductance, impedance, infrared or fluorescence spectroscopy, and bioluminescence), which require highly qualified staff to operate the advanced complex equipment.[14] The PCR technique that can repetitively amplify even a single strand of a specific nucleic acid target with definite length and sequence by using a thermostable DNA polymerase enables accurate determination, but target-DNA-specific primers need to be designed.[15,16] With advancements in technologies, the cost-effective production of polyclonal antibodies gives rise to the commercialization of immunological detection techniques that enable the detection of various types of food-borne bacterium.[17] Since nucleic acid-based detection is advantageous in terms of specificity, sensitivity, accuracy, speed, and capacity, novel methodologies using DNA chips have thus been established.[18–20] Under practical circumstances, they are difficult for the food industry and crop agriculture owing to their complexity. In addition to pathogen detection, the presence of drugs, flavoring agents, and pesticides at levels above the permitted standard levels needs to be investigated.[21] An increasing need for a rapid, real-time, selective, and low-cost technique for the detection of biotic or abiotic contamination has led to advancements in biosensors.[22]

3. Biosensors as Novel Potential Tools of Pathogen Detection Methods

In principle, a biosensor is defined as an analytical device incorporating a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting and/or imaging any type of sample specimen (Fig. 1).[23] The types of biosensor depend on the method used for signal transduction (Fig. 1) such as optical, electrochemical, thermometric, and piezoelectric.[24,25]

4. Nanoparticles in Biosensors for Food-Borne Pathogen Detection

Potential biosensors using nanoparticles (<100 nm in size) have been developed without the need for cost-ineffective or complicated instruments, allowing the rapid detection of food-borne pathogens on a portable device. Their detection capacity can be improved using immuno-nanoparticles over conventional sensors. Example of potential nanoparticle biosensors for food-borne pathogens are shown in Table 2. For example, the sensitivity of a *Salmonella. enteritidis*-based impedimetric biosensor was improved by incorporating anti-*Salmonella*-antibody-conjugated nanoparticles.[26] By coupling

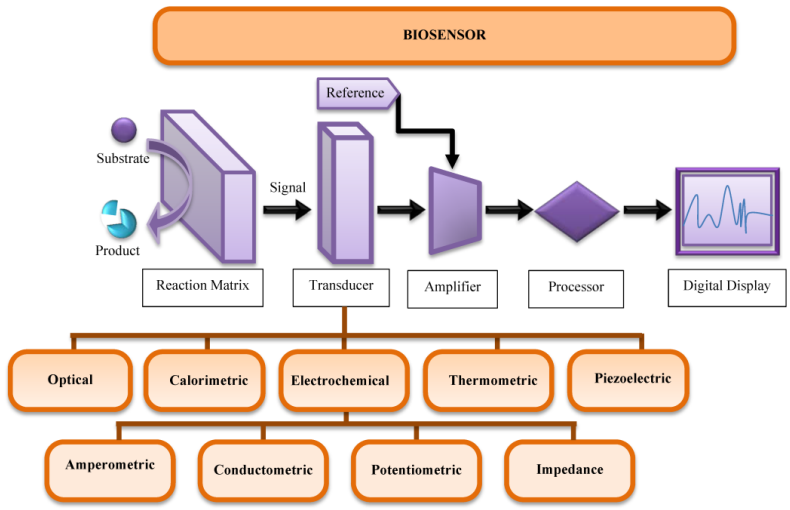

Fig. 1. (Color online) Scheme representing biosensor components consisting of different transducers depending on signal response type (i.e., optical, calorimetric, and electrochemical signals).

immuno-nanoparticles with enzymatic catalysis, an electrochemical immunosensor showed rapid and specific detection of pathogens. A screen-printed electrode coated with an agarose/nano-Au membrane and a horseradish peroxidase (HRP)-conjugated anti-*Vibrio parahaemolyticus* (HRP-anti-VP) antibody exhibited a detection limit of 7.4×10^4 CFU/mL against *Vibrio parahaemolyticus*.[27] Combining immunomagnetic separation with enzymatic *p*-nitrophenyl phosphate hydrolysis by alkaline phosphatase (EC 3.1.3.1) could efficiently detect *E. coli* O157:H7 at levels as low as 10^2 CFU/mL in a colorimetric assay based on the catalysis of "sandwich" structure complexes (antibody-coated micromagnetic beads-*E. coli* O157:H7-antibody-conjugated enzymes).[28]

Magnetic nanoparticles conjugated with an anti-*E. coli* antibody specific to O and K antigens were used to isolate *E. coli* O157:H7 from ground beef.[29] This research revealed that, owing to efficient diffusion and binding kinetics of nanoparticles, no mechanical mixing was required for immunomagnetic separation, suggesting their possible application in microfluidic devices.

Binding of a pathogenic bacterial cell with very fine nanoparticles can potentially alter their optical, physical and chemical properties, thereby facilitating the real-time detection of pathogens in accordance with the mode of signal transduction or amplification. Anti-*E. coli*-bound gold nanowire arrays (GNWAs) on an anodized porous alumina template were employed for capturing *E. coli* O157:H7.[30] The formation of a bacterial-cell-antibody complex alters the surface properties of the sensor (i.e., capacitance of biomembrane) by which the number of bound *E. coli* cells was estimated by electrochemical impedance spectroscopy (EIS). In this study, the GNWA biosensor was found to detect as few as ten *E. coli* cells in a sensor area of 0.173 cm^2.

Table 2
Important characteristics of potential biosensor-based detection assays using nanoparticles and strategies for biosensor developments for detection of food-borne pathogens including bacteria, viruses, as well as their toxins.

Assay system/principle	Target of assay	Detection limit	Assay duration	Ref.
1. Various types of nanoparticle-based biosensor				
Nanoparticle-enhanced impedimetric biosensors using antibodies immobilized on interdigitated gold electrodes	*Salmonella enteritidis*	10^4 CFU/mL in PBS and 10^5 CFU/mL in milk	3 min	26
Disposable electrochemical immunosensors based on screen-printed electrode (SPE) coated with agarose/ Nano-Au membrane and horseradish-peroxidase-labeled antibody	*Vibrio parahaemolyticus*	7.374×10^4 CFU/mL in buffer solutions	30 min	27
Mannose-encapsulated gold nanoparticles (m-AuNP) based on selective binding of m-AuNP to mannose adhesin FimH of bacterial type 1 pili	*E. coli* O157:H7	Not indicated	Not indicated	28
Immunomagnetic separation with magnetic nanoparticle-antibody conjugates	*E. coli* O157:H7	1.6×10^1 CFU/mL without any enrichment; 8 CFU/ mL with 6 h enrichment from ground beef samples	15 min; 6 h (with enrichment)	29
Glycoconjugate-specific antibody-bound gold nanowire arrays based on enzyme-linked immunosorbent assay (ELISA)	*E. coli* O157:H7	Not indicated	Not indicated	30
Immunological bead-free cell detection using QDs as reporter markers	*E. coli* O157:H7 and *Salmonella sp.*	With 24 h enrichment, 10 CFU/g from artificially contaminated ground beef	24 h	32
Rapid colorimetric gene sensing using biomodification-free gold nanoparticles based on polymerase chain reaction	*Listeria monocytogenes* and *Salmonella enterica*	0.015 ng/mL and 0.013 ng/mL genomic DNA of *L. monocytogenes* and *S. enterica*, respectively	5 min	33
Antibody-conjugated Rubpy dye-doped silica nanoparticles for immunofluorescence microscopy detection on glass slides	*Vibrio cholerae* O1	1 CFU/mL in buffer	10 min	34
Antibody-conjugated fluorescent dye-doped silica nanoparticles with glass slide method under epifluorescence microscope	*E. coli* O157:H7	1.6 CFU/mL in buffer	60 min	36
2. Biosensor developments through nanoparticle-based signal enhancement using metallic nanoparticles, QDs, and nanomaterial complexes				
Colorimetric assay with an anti-salmonella antibody conjugated to oval-shaped gold nanoparticles for label-free detection	*Salmonella typhimurium*	1×10^4 CFU/mL in buffer	5–10 min	40

Colorimetric detection of nucleic acid signature of Shiga-toxin-producing *E. coli* using gold nanoparticles	Enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 (*stx2* an EHEC signature)	1×10^6 copies of target DNA in buffer	Not indicated	42
Circulating-flow piezoelectric biosensors based on gold nanoparticle amplification and verification method (DNA-based QCM sensor coupled with PCR)	*E. coli* O157:H7 (*eaeA*)	1×10^2 CFU/mL from food samples (apple juice, milk, and ground beef)	Not indicated	43
Ultrasensitive immunoassays based on sandwich-type immunoreaction with silver enhancement of nanogold labels coupled with chemiluminescence detection	*S. typhimurium*	5 CFU/mL equivalent target oligonucleotide from actual food samples (poultry & products, pork & products, and ready-to-eat food)	30 min	44
Scanometric assays for on-slide detection with dual enlargement of gold nanoparticles	*Campylobacter jejuni*	1 CFU/mL in buffer	About 2 h	45
SERS system with wavelet-based signal processing and classification module	*Listeria* spp. (including *L. monocytogenes*)	Not indicated (in culture)	Within 24 h	46
SERS based silica-coated magnetic nanoprobes	*Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus*	10^3 CFU/mL in spinach solution and in peanut butter	About 2 h	48
Fluorescence-based immunoassays using QD-labeled antibodies	*L. monocytogenes* [two surface bound proteins, Internalin A (InlA) and Internalin B (InlB)]	1.5×10^4 and 1.2×10^5 CFU/mL in pure culture for direct and competitive formats, respectively	4–8 h	50
Plastic-adherent DNA aptamer-magnetic bead and QD sandwich assay	*C. jejuni*	2.5 and 10 CFU/mL in buffer and various food matrices, respectively	15–20 min	51
Fluorometric assay with indirect QD labeling method based on antibody-antigen and streptavidin-biotin interactions	*E. coli* O157:H7 serotype	2.07×10^7 CFU/mL in PBS buffer	5–6 h	52
Immunoassays for simultaneous detection of two pathogenic bacterial species using semiconductor QDs as fluorescent labels	*E. coli* O157:H7 and *S. typhimurium*	10^4 CFU/mL in PBS buffer	Within 2 h	53
Optical detection using water-soluble CdS QDs as a fluorescent marker of immunoglobulins	*Staphylococcus aureus*	2.5×10^5 CFU/mL in PBS buffer	About 1 h	54
Fluoroimmunoassays of multifood-borne pathogenic bacteria based on functionalized QDs coupled with immunomagnetic separation	*S. typhimurium*, *Shigella flexneri*, and *E. coli* O157:H7	10^3 CFU/mL in food matrix (apple juice and milk)	Within 2 h	55
Quantitative characterization of QD-labeled *E. coli*-specific lambda phage by flow cytometry or fluorescence microscopy	*E. coli*	10^4 CFU/mL in PBS buffer	About 1 h	56

Simultaneous detection of two pathogenic bacterial species by coupling immunomagnetic separation with QDs labeling	*E. coli* and *S. enteritidis*	5×10^2 and 4×10^2 CFU/mL for *E. coli* and *S. enteritidis*, respectively, in PBS	About 1 h	57
Immunoassay based on carbon nanotube-enhanced ELISA	*S. enterica* serovar Typhimurium	10^3 and 10^4 CFU/mL for direct and sandwich ELISA, respectively, in PBS and food samples (commercial UHT milk)	About 4 h	62
Ultrasensitive electrochemical immunoassays of staphylococcal enterotoxin B in food using enzyme-nanosilica-doped carbon nanotubes for signal amplification	Staphylococcal enterotoxin B (SEB)	10 pg/mL in SEB-spiked food samples (watermelon juice, soymilk, apple juice, and pork food)	About 2 h	63
Graphene-based nanoelectronic biosensors using a semiconductor device analyzer	*E. coli* and glucose-induced metabolic activities	10 CFU/mL in PBS buffer	About 2 h	64
Real-time potentiometric biosensors using SWCNTs as transducers and aptamers as biorecognition elements	Pathogenic *E. coli* O157:H7 toward nonpathogenic *E. coli* CECT 657	6–26 CFU/mL in in either PBS or actual samples (milk and apple juice) (with extremely easy pretreatment step of samples)	About 1 h	65
Carbon nanotubes-based chemiresistive biosensors using transduction element of antibody-functionalized SWCNTs aligned in parallel bridging two gold electrodes	*E. coli* O157:H7 and bacteriophage T7	10^3 CFU/mL (10^1 CFU/chip) for *E. coli* O157:H7; 10^3 PFU/mL (10^1 PFU/chip) for bacteriophage	About 5 min (for bacteriophage); about 60 min (for bacteria)	66
Homogenous colorimetric method based on self-assembled DNAzyme-labeled DNA probes with SWCNT conjugates	*Staphylococcus aureus* and its genomic DNA	1×10^5 CFU/mL bacteria; 30 nM target DNA in buffer	About 15–20 h	69
Multiplexed immunoassay platforms using metallic striped nanowires	Three nonpathogenic simulants: 1) *Bacillus globigii* (Bg) spores to simulate *Bacillus anthracis* and other bacterial species, 2) RNA MS2 bacteriophage to simulate Variola (virus for smallpox) and other pathogenic viruses, and 3) ovalbumin (Ova) to simulate protein toxins (*e.g.*, ricin or botulinum toxin)	10^5 CFU/mL, 10^5 PFU/mL, and 5 ng/mL for Bg spores, MS2 bacteriophage, and Ova protein, respectively	Within 3–4 h	73
DNA detection using gold nanoparticle functionalized polyaniline nanofibers	*Staphylococcus aureus* causing maltitis	150×10^{-12} mol/L target DNA in buffer	About 8–10 h	74
Nanowire-labeled direct-charge transfer biosensors	*Bacillus cereus* and *B. anthracis*	10^1 to 10^2 CFU/mL	Within 6 min	75

3. Biosensor developments through magnetic nanoparticles-based pre-concentration				
Assay of using biofunctional magnetic nanoparticles in combination with adenosine triphosphate (ATP) bioluminescence	*E. coli*	20 CFU/mL in bacterial-cell inoculated pasteurized milk	About 1 h	77
Antibody-coated paramagnetic beads with fluorescence detection based on conversion of 4-methylumbelliferyl-beta-D-galactoside to 4-methylumbelliferone by beta-galactosidase	Viable *E. coli*	8×10^4 CFU/mL in PBS	Less than 3 h	78
Magnetic-nanoparticle-based magnetophoresis using biotin-streptavidin system	*E. coli* O157:H7	4.4×10^3 CFU/mL in buffer	About 3 h	79
SERS-based sandwich immunoassay using antibody-coated magnetic nanoparticles	*E. coli*	8 CFU/mL in PBS	Less than 70 min	80
Preanalytical sample processing methods using DNA-aptamer-conjugated paramagnetic beads and detection using quantitative real-time RT-PCR	*S. enterica* serovar Typhimurium	10 CFU/mL in pure culture solution; 10^1 and 10^2 CFU/mL in bacterial-cell-seeded whole carcass chicken rinsate samples using pull-down assay and recirculation format, respectively	About 3–5 h	82
Direct detection by magnetic-nanoparticle-based DNA isolation and PCR relying on paramagnetic-nanoparticle-based isolation of bacterial DNA and listeriolysin O (hlyA) gene-specific PCR	*Listeria monocytogenes*	10 CFU/mL in pasteurized whole milk	Within 7 h	83
Gold-nanorod-based selective identification by two-photon Rayleigh scattering spectroscopy	*E. coli* O157:H7	50 CFU/mL in aqueous solution without any amplification or enrichment	Less than 15 min	84

The high polarizability and dielectrophoretic mobility of single-walled carbon nanotubes (SWCNTs) are used to trap and detect low numbers of bacterial cells in milliliter-sized samples. Attachment of the SWCNTs with both enhanced and reversed bacterial dielectrophoresis (DEP) mobility, leading to rapid assembly of SWCNT-bacterium aggregates (<5 min) in conducting bridges between two electrodes by positive-alternating current DEP.[31] This technique showed a detection threshold of 10^4 CFU/mL of *E. coli*, suggesting that the functionalized SWCNTs may be used as absorbers and transporters of pathogens in biosensors.

A novel immunological bead-free cell detection technique using QDs as reporter markers for food-borne pathogen detection has recently been introduced.[32] The chemical compound (1-ethyl-3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and protein A were utilized as crosslinkers for preparing QD–antibody conjugates. To avoid interference by QD fluorescence, the beads were removed after primary pathogen separation and before fluorescence measurement. The combined approach of EDC–protein A QD labeling and bead-free fluorescence measurement could detect *E. coli* O157:H7 and *Salmonella* sp. as low as 10 CFU/g of artificially contaminated ground beef.

Furthermore, the PCR-based gold nanoparticle colorimetric method using thiol-labeled primers has been established to amplify *hly* and *hut* specific to *Listeria monocytogenes* and *Salmonella enterica*, respectively.[33] A PCR-product-linked gold nanoparticle displays a principally higher salt tolerance than a primer-linked gold nanoparticle; the facilitated gold-nanoparticle-based bacterial targets can be colorimetrically observed by the naked eyes or executively analyzed by spectrum measurement. The detection limits of the genomic DNA of *L. monocytogenes* and *S. enterica* are 0.015 and 0.013 ng/mL, respectively, in artificially contaminated food samples.

Indeed, the potential utilization of antibody-conjugated fluorescent-dye-Rubpy-doped silica nanoparticles for immunofluorescence microscopy detection of various food-borne pathogens (i.e., *Campylobacter jejuni*, *E. coli* O157:H7, and *Vibrio cholerae* O1) has been achieved.[34–36] This methodology has strong promise in improved pathogen detection by signal enhancement.[34] This study indicated that Rubpy-dye-doped silica nanoparticles functionalized with a carboxyl group and conjugated with a *V. cholerae* O1 antibody could be effectively applied in signal amplification to detect a single target bacterial cell.

5. Strategies for Biosensor Development for Food-Borne Pathogen Detection

5.1 *Nanoparticle-based signal enhancement*

Because a bacterial cell is larger than a nanoparticle, a significant number of nanoparticles attach to each a bacterial cell; consequently, signal enhancement occurs (Fig. 2).[37] The effect of signal amplification on the sensitivity of the assay will depend on the optical or electrical properties of each nanoparticle (summarized in Table 2).

5.1.1 *Metallic nanoparticles*

Metallic nanoparticles (i.e., gold and silver) have been used for signal amplification in several biosensing devices. Gold nanoparticles have been widely applied in various optical and electrical assays.[38] Colorimetric assays based on a blue aggregated form and red dispersed solutions of gold nanoparticles were developed for the rapid detection of *S. typhimurium* and multidrug-resistant *S. typhimurium* DT104,[39,40] with sensitivities of 10^4 and 10^3 CFU/mL, respectively. Using SERS, the detection limit of *S. typhimurium* DT104 was decreased to 10 CFU/mL.[41] The electrical properties of the gold nanoparticles were oriented for the development of the piezoelectric biosensor-based real-time detection of a food-borne pathogen, *E. coli* O157:H7. Initially, target-specific single-stranded DNA-functionalized gold nanoparticles were bound to the target

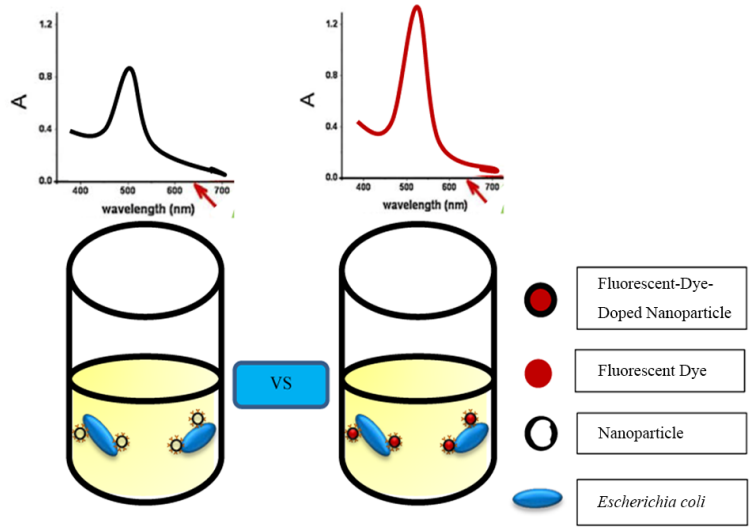

Fig. 2. (Color online) Scheme showing use of fluorescent-dye-doped nanoparticles for signal amplification in food-borne pathogen detection. Thousands of fluorescent dyes such as Rubpy with a high fluorescence signal intensity and a stable orange color can be doped inside each individual nanoparticle, resulting in enhanced signal intensity and eventually improved detection sensitivity. The intensity of the fluorescence signal is measured using an epifluorescence microscope or a confocal laser scanning electron microscope.

DNA and then to a complementary probe immobilized onto the piezoelectric biosensor surface, inducing a frequency shift of the piezoelectric biosensor. Concentrations as low as 1.2×10^2 CFU/mL *E. coli* O157:H7 were detectable.[42]

In addition, signal amplification of gold nanoparticles can be accomplished using silver. For example, a sandwich complex was developed using *Salmonella* sp.-specific antibodies and antibody-coated gold nanoparticles.[43] Subsequently, silver was coated around the gold nanoparticles, which facilitated the signal amplification in the chemiluminescence-based assay with a detection limit of 5 CFU/mL.[44] Similarly, an agressive approach using both gold and silver atoms on-slide was developed for the detection of the food-borne pathogen *C. jejuni*. This cost-effective detection format generated a high-intensity color that can easily be visualized by the unaided eye or determined using an inexpensive scanner.

A previous study using SERS with silver nanoparticles as a signal enhancer showed that six species of the *Listeria* genus including *L. monocytogenes* were distinguishable with the sensitivity of a single bacterial cell.[45,46] Although this label-free assay possesses advantages in terms of simplicity and speed, it is often difficult to ascertain detection of multiple species with high specificity owing to similarity of SERS spectra.[47] The use of SERS for detection of multiple pathogens with silica-coated magnetic probes functionalized with pathogen-specific antibodies was then developed for capture followed by detection using pathogen-specific SERS probes (gold nanoparticles integrated with a Raman reporter).[48] The low sensitivity of detection (10^3 CFU/mL) of

S. typhimurium and *Staphylococcus aureus* in complex food matrices (spinach solution and peanut butter) indicates the potential of the food-based assay format. Nevertheless, batch-to-batch variations in nanoparticle size that can affect the capability of SERS for a quantitative and reproducible assay may be a critical determinant.

5.1.2 *QDs*

QDs are colloidal semiconducting fluorescent nanoparticles that comprise a semiconductor material core (generally, cadmium mixed selenium or tellurium) coated with a semiconductor shell (normally, zinc sulphide). Owing to their unique size-related fluorescence quantums and photostability, QDs are extensively employed to replace traditional fluorescent dyes (*e.g.*, fluorescein isothiocyanate, FITC). Functionalized QDs have been used as DNA labels for probing genomic DNA in fluorescence *in situ* hybridization (FISH) assays.[48] Additionally, QDs have been used as fluorescent labels in various assays (particularly immunoassays) for the detection of food-borne pathogens such as *L. monocytogenes*,[49] *C. jejuni*,[50] *E. coli* O157:H7,[51] *S. typhimurium*,[52] *S. aureus*,[53] and *Shigella flexneri*.[54] A QD-based immunoassay for the sensitive detection of *S. typhimurium* in chicken carcass wash water (LOD, 10^3 CFU/mL) revealed the applicability of QDs in food matrices.[55] As compared with traditional dyes, photostable, bright fluorescence intensities of QDs promoted signal amplification, resulting in lower detection thresholds. For instance, LOD of an immunoassay for *E. coli* O157:H7 detection demonstrated a 16-fold decrease (from 3.33×10^8 to 2.08×10^7 CFU/mL) when QDs served as the fluorescent label instead of FITC.[53]

For the development of functionalization strategies, QDs as labels were coupled to a variety of biorecognition elements, leading to versatility. In addition to antibody immobilized QDs, the detection of *S. aureus* using protein A-functionalized QDs was possible over the range of 2.5×10^5 to 1.0×10^8 CFU/mL.[52] Moreover, genetically-modified bacteriophages coupled to QDs have been utilized for the detection of *E. coli*.[54] Aptamer-linked QDs were employed in a sandwich assay in conjugation with magnetic beads for the detection of *C. jejuni* with reasonable sensitivity.[56] This assay exhibited detection limits as low as 2.5 CFU in buffer and 10–250 CFU in different food matrices. The assay in conjugation with a sensitive handheld fluorometer that could offer rapid (within 15–20 min) portable detection of food-borne contaminants might be recognized as a potential diagnostic tool.

The immediate detection of numerous biomarkers due to a broad Strokes shift is considered as one of the major advantages of QD utility, allowing the simultaneous excitation of several different QDs at a single excitation wavelength. The simultaneous detection of *E. coli* and *S. enteritidis* was improved by coupling immunomagnetic separation with QD labeling.[51] Target-specific antibodies coupled to QDs with different emission wavelengths were used to label the captured bacteria, and the detection ranges were found to be 5×10^2 to 5×10^5 CFU/mL for *E. coli* and 4×10^2 to 4×10^5 CFU/mL for *S. enteritidis*. Similarly, three species of food-borne pathogenic bacteria (*S. typhimurium*, *Shigella flexneri*, and *E. coli* O157:H7) were detectable using these multiplex assays in a single test, and the detection limits indicate high possibilities for cost-effective, rapid multiplex devices.[57]

Concerning the extensive application of QDs, challenges still remain. The sensitivity of QDs as labels was decreased when high concentrations of proteins were found in the food matrix.[55] Quenching of the fluorescence signal also occurred when QDs were employed in combination with magnetic beads for bacterial capture.[55] The complete replacement of traditional dyes by QDs is possibly due to their cost and size. Although QDs are in the nanoscale, they are still larger than the common dyes, probably resulting in biorecognition disturbance in multiplex assays.

5.1.3 *Nanotubes, nanofibers, and nanowires*

Carbon nanotubes (CNTs) are fabricated in different forms: SWCNTs are composed of single-atom-thick planar sheets of carbon atoms organized in a honeycomb lattice (graphene) and rolled into a cylinder-like tube; multi-walled carbon nanotubes (MWCNTs) consist of multiple tubes. In recent years, the functionalization of CNTs with proteins, nucleic acids, and antibodies has been extensively carried out.[57] Concerning excellent electrical, physical, and optical properties, the CNT as a transducer has potential for signal amplification.[58–60] By using a SWCNT/horseradish peroxidase (HRP)/antibody bioconjugate in an ELISA-based bioassay against *S. typhimurium*, a 1000-fold increase in detection sensitivity (LOD, 10^4 CFU/mL) was observed as compared with an HRP-labeled antibody (LOD, 10^7 CFU/mL).[61] Ultrasensitive detection (LOD, 10 pg/mL) of staphylococcal enterotoxin B was achieved using an HRP-doped MWCNT-based signal amplification system in complex food matrices (i.e., watermelon juice, soymilk, apple juice, and pork).[62]

Rapid and real-time detection of food-borne pathogens is conceptual, and thus CNT-based electrodes are increasingly becoming nanosensing devices. The sensitive and label-free electrical detection of *E. coli* as low as 10 CFU/mL was accomplished using a graphene-based biosensor.[63] A potentiometric aptamer-based biosensor showed striking changes in electrical potential after the interaction between the aptamer-functionalized SWCNT and the bacterial cell. Within a few minutes, pathogenic bacteria were detectable at as low as 6 and 26 CFU/mL in milk and apple juice, respectively.[64] In addition, the multiplex CNT-based detection against both pathogens and viruses in food demonstrates advancements in food pathogen nanosensors.[65] A CNT-based assay using antibody-functionalized SWCNT aligned with two gold electrodes displayed a detection limit for *E. coli* O157:H7 (10^5 CFU/mL within 60 min) and for bacteriophage T7 (10^3 plaque-forming units or PFU/mL within 5 min).

Interestingly, the CNTs enable protection of molecular probes. The interaction between nucleotide bases of single-stranded DNA (ssDNA) and side walls of SWCNT results in a stable complex that can protect ssDNA from degradation.[66] By hybridization assay using a DNA-labeled probe, the protection of the SWCNT/ssDNA complex could definitely improve the detection sensitivity of *S. aureus* DNA (LOD, 30 nM) compared with the hybridization without SWCNTs (LOD, 160 nM).[67–69] This study has potential implications for molecular techniques in food matrices where the DNA probes can be easily degraded by cellular nucleases.

Recently, CNT-based sensing devices for food-borne pathogens have led to advancements in the sensitive, rapid, and label-free detection of the pathogens in food

matrices. However, human health safety issues of CNTs, similar to other nanoparticles, and the fabrication processes become limiting factors.[69,70] Reduction of CNT toxicity and the improvement of fabrication are somehow required if these devices remain appealing, rendering nano-biotechnologies promising for food-borne pathogen detection.

To complement CNTs, nanowires or nanofibers are considered promising nanomaterials for the electrical detection of analytes; however, electrical conductivity is poor owing to edge effects (atoms on the surface that are not fully bonded to their neighboring atoms) that can restrict the utility of nanowires. Ten *E. coli* O157:H7 cells were detectable using anti-*E. coli*-bound gold nanowire arrays in urine samples. Once a bacteria-antibody complex formed, an alteration in electrical potential was determined by electrochemical impedance spectroscopy.[71] For multiplex immunoassays, a novel biosensing platform using engineered nanowires was established through a sub-micrometer layer of electrodeposited metals within a porous alumina template.[72] After coating the nanowires with target-specific antibodies, sensitive multiplex detection of *B. globiggi* spores (1×10^5 CFU/mL), MS2 bacteriophage (1×10^5 PFU/mL), and ova protein (5 ng/mL) was achieved. *S. aureus* DNA at picomolar concentrations was detectable using gold nanoparticle-modified polyaniline nanofibers.[73] The most potential nanowire-based assay for food-borne pathogen detection is a direct-charge transfer (DCT) biosensor toward *B. cereus*. This biosensor, fabricated using antibodies as sensing element and polyaniline nanowire as molecular electrical transducer, showed sensitivity in pure cultures of 10^1 to 10^2 CFU/mL within 6 min.[74] The rapid, sensitive, and portable biosensor makes it a promising device for in-line or on-site analysis in food supply chains. However, recent findings on the electrical detection of food-borne pathogens have attention on carbon nanotubes rather than nanowires or nanofibers.[75]

5.2 *Magnetic nanoparticle-based preconcentration*

Removal of a pre-enrichment step via magnetic separation technology combined with detection methods in food (i.e., color and opacity) is crucial and can expedite technology in the area of food safety. Magnetic nanoparticles coupled to a biorecognition element (i.e., antibodies, aptamers, or nucleic acids) are used for separating the target analyte from the matrix after a magnetic field is applied (Fig. 3 and Table 2). Owing to the high surface area-to-volume ratios of magnetic nanoparticles with high capture efficiencies, the immunocapture efficiency of *E. coli* using magnetic nanoparticles was higher than those of paramagnetic beads.[76,77] The paramagnetic beads, however, remain to be more extensively used than the magnetic nanoparticles. A magnetic-nanoparticle-based immunocapture for pathogenic bacteria detection is the most common approach applied to *E. coli* O157:H7, with capture efficiencies of 85–97% in buffer and 94% in beef sample.[78–80] A decrease in immunocapture efficiency occurred when more than 10^5 cells were present,[29] reflecting a drawback of quantitative measurement of the pathogen. However, this level far exceeds permissible levels in food.[29] The immunocapture-based nanomagnetic separation becomes the methodology of choice. Multiplex magnetic capture using amine-functionalized magnetic nanoparticles represented the simultaneous detection of *B. cereus*, *E. coli*, *S. aureus*, and *Salmonella* sp. with capture efficiencies of 97.4, 97.0, 66.5, and 55.0%, respectively, whereas the application of aptamer-based magnetic particles was firstly achieved for capturing *S. enterica* from fecal samples and food matrix.[80,81]

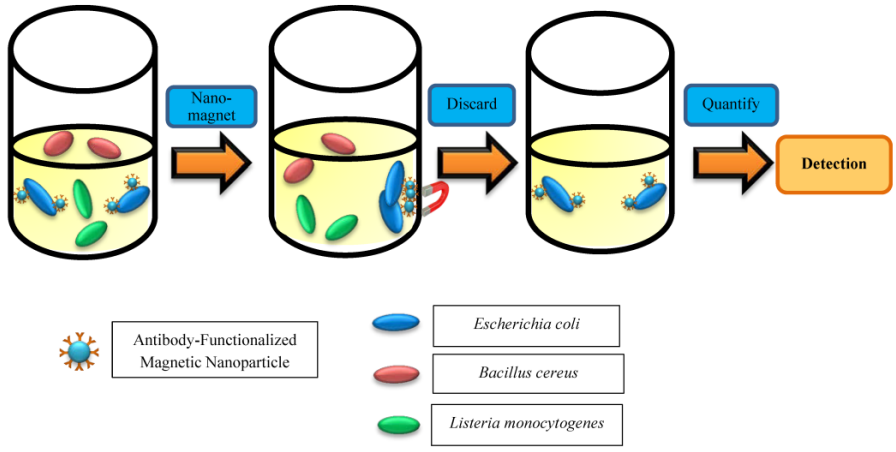

Fig. 3. (Color online) Scheme illustrating magnetic-nanoparticle-based detection method for capturing and concentrating target cells. For instance, selective antibodies specific to bacterial species (*e.g.*, *E. coli*) are bound to the surface of magnetic nanoparticles. Only targeted microorganisms will be bound to the functionalized magnetic nanoparticles. Although a complex matrix (*e.g.*, food, blood, milk, or cereal grains) contains a target analyte as well as several potential interferences such as other bacterial cells, the functionalized magnetic nanoparticles can selectively bind to the target analyte with high capture efficiency. Once a magnetic field is applied, analyte-bound magnetic particles are separated and the supernatant is then carefully discarded. The remaining materials are subjected to quantification assays.

Preconcentration of pathogenic bacteria via nanomagnetic separation coupled with sensitive detection approaches (i.e., chemiluminescence, "real-time" PCR, and QDs) resulted in lower detection limits and faster assays. Targeted paramagnetic nanoparticle-based separation of *L. monocytogenes* DNA extracted directly from milk and subsequently subjected to PCR amplification revealed excellent sensitivity as low as 10 CFU/mL.[82] A chemiluminescent nanomagnetic enzyme-linked immunoassay for the detection of *E. coli* O157:H7 showed a detection limit of 1000 cells/mL.[83] Although magnetic nanoparticles offer the advantage of rapidity, the robust surface chemical immobilization of biorecognition molecules remains a challenge in the case of the more extensive use of magnetic nanoparticles.

5.3 *Improved antimicrobial efficacy of nanoparticles*

The utilization of nanoparticles (i.e., silver, titanium, zinc, and sulfur) as antimicrobial agents has increasingly gained attention.[84,85] The antimicrobial activity of silver nanoparticles against food-borne pathogens (i.e., *Bacillus cereus*, *E. coli*, *L. monocytogenes*, *S. aureus*, and *Salmonella* sp.) was observed.[85-87] Their antiviral and bactericidal activities against methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) were also found. This antimicrobial feature of silver nanoparticles is considered to be due to the antimicrobial property of silver itself rather

than the nanosize; other nanoparticles such as gold do not exhibit an inhibitory effect on growth.[87–91] The toxicity of silver particles at the nanoscale was found to be increased relative to that of silver; this is due to the mechanism by which the nanoparticles, in terms of size and shape, can easily enter into a cell and release silver ions.[92] Regarding environmentally friendly conscientiousness, this has led to the utility of microorganisms for the production of nanoparticles.[93] These biogenic silver nanoparticles produced from *Lactobacillus fermentum* and *Aspergillus clavatus* displayed both antiviral and antibacterial activity comparable to those of chemically produced silver nanoparticles against an *Enterobacter aerogenes*-specific bacteriophage, murine norovirus 1 and Gram-positive and Gram-negative bacteria.[94,95] Silver nanoparticles as antimicrobial agents in various food packages could prolong the shelf life of the product.[89,96]

Numerous potential uses of nanoparticles have been reported in food or food packaging.[97–102] Besides silver nanoparticles, titanium dioxide nanoparticles also have prominent antimicrobial properties owing to UV-mediated photocatalysis.[103–107] Hence, UV irradiation is required for activating their antimicrobial activity. In the presence of UV, titanium dioxide nanoparticles were effective against food-borne pathogens, particularly *L. monocytogenes*, *Salmonella choleraesuis*, and *Vibrio parahaemolyticus*.[103] In food-packages, ethylene vinyl alcohol (EVOH) films thoroughly dispersed with titanium dioxide nanoparticles exhibited photo-activated antimicrobial activities against nine microorganisms, and consequently prevent food poisoning and spoilage.[108] In addition, polypropylene films coated with titanium dioxide nanoparticles conferred antimicrobial properties against *E. coli* on fresh cut lettuce.[109] A number of research studies have reported that combining titanium dioxide nanoparticles with silver nanoparticles could enhance antimicrobial efficacy against pathogens.[110,111]

Other nanoparticles harboring antimicrobial activity include those with magnesium oxide,[112–115] copper and copper oxide,[97,116–124] zinc oxide,[98–102,125–131] cadmium selenide/telluride,[132–134] and chitosan,[135–137] as well as carbon nanotubes.[138,139] Zinc oxide nanoparticles exhibited potent antimicrobial activity against *E. coli* O157:H7, *L. monocytogenes*, and *S. enteritidis*.[140,141] These findings suggest that zinc nanoparticles may distort and damage the bacterial cell membrane, leading to leakage of intracellular components and ultimately cell death.[142] However, zinc nanoparticles suspended in polystyrene film displayed no antimicrobial activities, indicating limitation of its use in packaging. Surface-modified sulfur nanoparticles exhibited fungicidal efficacy against *Aspergillus niger* and *Fusarium oxysporum*.[143] The antimicrobial potential of SCNTs against *B. subtilis* and *E. coli* was also observed. Nanotube networks produced on the surface of a bacterial cell were found to damage the bacterial envelopes, leading to intracellular leakage and cell death.[144]

Several studies have demonstrated the use of various classes of nanoparticles as antimicrobial agents in drinking water sterilization.[145] Instead of sodium ions, silver ions dispersed in either poly(e-caprolactone) or poly(lactic acid) and applied in montmorillonite nanoclays, namely, "silver nanoclays", displayed potential antimicrobial activity.[146,147]

The efficacy of other antimicrobial agents in the presence of nanoparticles has been improved; this offers great promise for the reduction in the amount of pathogens in food. A highly complex structure of antimicrobial nanomaterials has also been developed. For instance, gold nanoparticles covalently attached with vancomycin

exhibited higher bactericidal activity than vancomycin itself against even vancomycin-resistant bacteria.[148] Moreover, carbohydrate (phytoglycogen) nanoparticles loaded with nisin (This *Lactococcus lactis*-produced broad-spectrum antimicrobial peptide is widely used in the manufacture of processed cheeses, meats, and beverages) showed substantial antimicrobial potency against *L. monocytogenes* culture in comparison with uncoated nisin.[149] A carbohydrate (phytoglycogen octenyl succinate) nanoparticle-stabilized emulsion was found to extend the efficacy of the bacteriocin nisin against *L. monocytogenes*.[150] Similarly, lysozyme-carrying nanoparticles functionalized with anti-*L. monocytogenes* showed higher effectiveness for inhibiting *L. monocytogenes* than the free lysozyme.[151] In combination with the high binding affinity of CNTs to bacteria, the antibacterial effectiveness of inherent near-infrared (NIR) laser has been markedly improved whereas the cellular viability was affected neither by CNTs alone nor by NIR irradiations alone.[152] Generally, bacterial cell viability was reduced when bacteria conjugated to gold nanoparticles were exposed to NIR owing to photo-thermal lysis.[153] These early applications of laser-activated nanoparticles as antimicrobial agents represented potential to eliminate disease-causing pathogens.

The utilization of nanoparticles as antimicrobial agents provides great promise. However, the biosafety of nanoparticles is still a health concern.[40] The delineated processes by which nanoparticles kill bacteria and their health effects should be further warranted for their safe and extensive use in food or food processing.

6. Advantages of Nanoparticle Application for Pathogen Detection

6.1 *Fast and real-time detection*

Conventional culture-based methods involve steps of cell proliferation that take 24 h of incubation under laboratory conditions. Although DNA- and protein-based detection methods are quicker, these methods still take a relatively long period to carry out. In the case of a few bacterial cells in a food sample, a step of culture enrichment that may take from 2–3 h to overnight is principally required. The long period for obtaining results reflects cost-ineffectiveness and inconvenience particularly in the food industry.

By using an alternative nanoparticle-based method, the target cells are captured, discarded, or concentrated from sample specimens using biofunctionalized nanoparticles. The formation of a bio-nanoparticle-bacteria complex could then be determined within 2–3 min to hours without bacterial culture and enrichment.[26,154–159] In some cases, the use of impractical and expensive instruments (i.e., scanning electron microscopy (SEM),[160] fluorescence microscopy,[161] confocal scanning laser microscopy) was necessary to reduce the detection time in laboratory procedures.[162]

Furthermore, practical biosensors using nanoparticles have been developed to avoid the need for complicated instruments, allowing for the rapid and convenient detection of food-borne pathogens on a portable device. For instance, a single *E. coli* O157:H7 cell was detectable using a nanoparticle-based sensor in ground beef sample within 20 min as compared with up to 48 h in traditional tests.[156] In principle, the fluorescent silica nanoparticles conjugated with anti-*E.coli* O157 were dispersed to ground beef inoculated with *E. coli* O157:H7. Antibody-conjugated nanoparticles bound to target cells were then determined using a flow cytometer.

The small size of nanoparticles enables them to bind to target bacterial cells, significantly affecting their optical, physical, and chemical properties. This event allows the real-time detection of pathogenic bacteria depending on the mode of signal transduction or amplification. Indeed, several functionalized nanoparticle-based biosensors as pathogenic absorbers and transporters have been established. These nanoparticle-based biosensors require a detection period of within 10 min. For example, the nanoparticle-based impedimetric biosensor consisting of the anti-*Salmonella* nanoparticles immobilized on interdigitated gold electrodes was employed to detect S. *enteritidis* by measuring the impedance alterations after the target cells were bound.[26] By using the nanoparticle-based biosensor, 10^4 CFU/mL of *S. enteritidis* was detectable in phosphate-buffered saline (PBS) within 3 min.

6.2 *Enhanced detection sensitivity*

Food-borne pathogens at even low levels are still harmful to the health owing to their infectious ability and severity. For instance, in the United States, the presence of *L. monocytogenes* in ready-to-eat (RTE) food must be regulated under a "zero-tolerance" policy.[163] Of concern, the "zero-tolerance" policy is implemented owing to the lack of rapid and reliable procedures for the low levels of detection of *Listeria* in foods. The utility of nanoparticles will thus be useful for the rapid and accurate detection of low levels of food-borne pathogens, as discussed and exemplified in a later section.

Fluorescent-dye-doped nanoparticles were developed as sensitive markers for Pathogen Detection owing to their favorable properties (i.e., high fluorescence quantum yields, photostability, and tunable fluorescence bands).[34–36,156,164] A single nanoparticle (<100 nm in size) can contain several hundreds of fluorescent dye molecules, becoming much brighter than a single fluorescent dye molecule.[164] A bioassay based on fluorescent nanoparticles conjugated with anti-*E.coli* O157 was developed for the detection of *E. coli* O157:H7 in ground beef.[156] Owing to the tiny size of the fluorescent nanoparticle compared with the *E. coli* O157:H7 cell, a single bacterial cell was bound with thousands of anti-*E.coli* O157-conjugated fluorescent nanoparticles, resulting in enhanced fluorescent signals from the bacterial surfaces as the single *E. coli* O157:H7 cell was detectable.

Other fluorescent nanoparticles have also been successfully established for the sensitive detection of food-borne pathogens. Protein G-tagged liposomal nanovesicles were applied in an immunomagnetic bead sandwich assay for detecting *E. coli* O157:H7 with a detection limit of 100 CFU/mL.[165] Each liposomal nanovesicle (i.e., liposome) can be filled with several millions of fluorescent dye molecules. With great signal enhancement, liposomal nanovesicles have been used as a potential reporter in immunoassays. Luminescence colloidal semiconductor nanocrystals, namely, QDs, were employed for detecting *Cryptosporidium parvum* oocyst.[166] As compared with the organic fluorescent dye, QDs offer the beneficial feature of a high photobleaching threshold. By combining biotin-tagged *E.coli*-specific bacteriophage with streptavidin-coated QDs, 10 CFU/mL of *E. coli* was detectable within an hour.[157] By using multiple host-specific phages and QDs with different emission colors, this QD-phage-based method extensively provides the possibility of detecting multiple bacterial strains.

The high surface area-to-volume ratio of nanoparticles potentiates them as substances for biomolecule immobilization. A DNA biosensor incorporating nanomembranes was successfully applied for detecting *Pseudomonas aeruginosa*.[155] The surface area of nanomembranes is about 1- or 2-fold larger than that of continuous thin films, allowing increases consequently in the amount of bound DNA as well as response sensitivity. Application of the nanobead as a microfluid platform and its advantage for multiple target pathogen detection have been reported.[167] Slow response time and low sensitivity attributable to low capture efficiency and long diffusion time reflect the limitation of microfluidics. Nanoparticle utilization is one of the research perspectives to overcome these limitations as the large local density of biomolecule-conjugated nanoparticles offers a greater capacity for capturing target bacterial cells in sample specimens, and consequently improves sensitivity and diffusion time.

Owing to the high surface area-to-volume ratio and rapid reactivity, the functionalized magnetic nanoparticles display higher capture efficiencies in immunomagnetic separation compared with microbeads. 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 measured using magnetic nanoparticle-anti-*E. coli* O157 conjugates.[29] In the presence of the background flora *S. typhimurium* DT104 cells, relatively low amounts of *E. coli* O157:H7 (400 CFU/mL) were captured using carbon magnetic nanotubes conjugated with anti-*E.coli* O157 without noticeable species cross reactivity.[168] Nanoparticle-based immunomagnetic separation (IMS) and real-time PCR were combined for a speedy and quantitative detection of *L. monocytogenes*.[169] The capture efficiencies of anti-*L. monocytogenes* magnetic-based IMS were higher than those of Dynabeads®-based IMS when *L. monocytogenes* cells were inoculated into milk samples. By combining with real-time PCR, *L. monocytogenes* DNA was quantitatively detectable in milk samples with *L. monocytogenes* $\geq 2 \times 10^2$ CFU/mL.

Food-borne pathogen detection in biofilms is also a challenge. A microbial biofilm is defined as adherent microorganisms within a polymeric substance in a two- or three-dimensional structure. Several food-borne pathogens can form biofilms with other microorganisms in food and processing environments. Inside biofilms, microbial cells become more resistant to sanitization than planktonic cells and are thus more difficult to eliminate.[170,171] Consequently, food-borne pathogens (i.e., *L. monocytogenes*) formed as biofilms are of serious concern in the food industry. Immuno-nanoparticle-based immunoassays showed higher sensitivity than conventional immunoassays for detecting *L. monocytogenes* in one- or two-species-containing biofilms, including *P. aeruginosa* ATCC 27853.[163] Using the same confocal laser scanning microscopy, nanoparticles coated with anti-*L. monocytogenes* (0–4000 arbitrary units) exhibited a higher intensity of fluorescent signals than anti-*L. monocytogenes* alone (0–250 arbitrary units). Individual *L. monocytogenes* cells at various depths (0–5 μm) of two-species-containing biofilms were effectively detectable using the immuno-nanoparticle-based signal enhancement system.

6.3 *Multiplex detection of food-borne pathogens*

The optical features of nanoparticles (i.e., emissive, absorptive, and light-scattering properties) are associated with their sizes, composition, and shapes. For instance, the smaller the size of the QDs, the higher both the absorption and emission energies of the

QD shift.[172] These characteristics render nanoparticles available for multiplex detection. Semiconductor QDs as fluorescence labels were used in simultaneous immunoassays for detecting the food-borne pathogens *E. coli* O157:H7 (1.95×10^3 CFU/mL) and *S. typhimurium* (3.35×10^4 CFU/mL).[173] Varying the numbers and ratios of different QDs per target generates distinct fluorescent signals for each individual target.

Instead of immunomagnetic beads, microtiter plates in an array format were successfully established for the simultaneous detection of *E. coli* O157:H7, *Salmonella* sp., and *L. monocytogenes* using the G-liposomes conjugated with selective antibodies.[174] Apart from these nanovesicles, inorganic silica nanobeads doped with thousands of fluorescent dye molecules (Rubpy) that were conjugated with anti-*E. coli* O157 antibodies enable the binding of large numbers of nanoparticles onto individual bacterial cells via specific antibody–antigen recognition.[156] Consistent with flow-cytometry-based measurements, these methodologies permit highly sensitive and simultaneous detection of one bacterial cell per given sample within 20 min using a spectrofluorometer in the presence of nontarget pathogens (i.e., *S. typhimurium* and *B. cereus* spores). About 1–400 *E. coli* O157:H7 cells were successfully detected using immunosilica nanoparticles even in spiked ground beef samples. The silica nanobeads were also doped with three amine reactive energy-transfer tandem dyes (FAM-SE, R6G-SE, and ROX-SE) in three ratios (1:0.5:1, 0.5:1:4, and 0.5:0.5:3) to generate different FRET nanoparticles.[159] By conjugation with different pathogen-specific antibodies, multiplex detection of pathogens was conducted under confocal microscopy emitting three fluorescence signals (i.e., blue, orange, and purple) excited at 488 nm.

On the basis of electric responses of nanowires due to multiplex pathogen binding via antibody–antigen interactions, an ultrahigh-density nanowire circuit has recently been developed and the combined electric signals have been decoded for individual pathogenic identification.[175] Using optical signaling (i.e., reflectance and fluorescence) of fluorescently labeled antibodies, multistriped nanowires have been developed for the effective multiplex detection of biowarfare simulants.[73] Antibody-conjugated nanowires with distinct identifiable encoding patterns were employed in the multiplex detection of three nonpathogenic stimulants, namely *B. anthracis*, Variola, and protein toxins (i.e., ricin or botulinum toxin) with detection limits of 1×10^5 CFU/mL, 1×10^5 PFU/mL, and 5 ng/mL, respectively.

7. Conclusions

In this review, we summarize current applications of nanoparticles to food-borne pathogen detection. In numerous studies, novel nanoparticle-based methods are applied to complement or replace traditional methods for the improvement of detection speediness and sensitivity. The utility of nanobiosensors has attracted increasing attention for "real-time" on-site analysis in food industrial or agricultural sectors.

Fluorescent nanoparticles were used for signal amplification whereas metal and semiconductor nanoparticles were selected for the development of biosensors, on the basis of their electronic or optical response as transducing signals. Herewith, bioconjugated nanoparticles have advantages over traditional (non-nanoparticle-

based) methods for specific pathogen detection in both culture media and real sample specimens, including food products and biofilms. Furthermore, magnetic nanoparticles were extensively employed for eliminating disturbance from complex food matrices and for concentrating target microorganisms to avoid a step of time-extensive enrichment via a culture process.

The unique electronic, optical, and catalytic features are focused on for the development of novel nanoparticle-based detection methods. Major attempts have been oriented to the nanoparticle-based biosensors for time-intensive, simultaneous, and high-throughput detections of multiple target species. Interestingly, current nano-biosensor-based methodologies might be implemented for new emerging food-borne pathogen detection. Usually, biosensors lack the combined characteristics of high sensitivity and high specificity that are required for food-borne pathogen detection at an ultralow level. Moreover, research focusing on biosensor capacity improvement is needed.

More than 250 pathogens and their toxins are considered to be transmitted by food. The most important pathogens include *Campylobacter* sp., *L. monocytogenes*, *Salmonella* sp., Shiga-toxin-producing *E. coli*, Norovirus, and *Toxoplasma gondii*. As nanotechnology in detecting food-borne pathogens is still in the early stage, most of those studies have only been conducted on bacterial culture broths with *E. coli* strains being the microorganism. Although nanoparticle-based detection methods have been recently developed for certain pathogens (i.e., *L. monocytogenes*, *Staphylococcus* spp., *Salmonella* sp., and *P. aeruginosa*), further research studies focused on other potential food-borne pathogens (i.e., *Campylobacter* sp., *Clostridium botulinum*, and *Vibrio* sp. products) should be undertaken. Furthermore, more and new emerging pathogens are continuously identified and should be in focus.

Many nanoparticle-based procedures for pathogen detection still require highly complex and expensive instruments in a laboratory setting, which may be impractical in industrial sectors. Importantly, the toxicity of nanoparticles remains to be elucidated.

All these detection assays were currently processed without sample enrichment. In terms of speed, nanoparticle-based detection methods have the advantage of real-time pathogen detection. These methodologies may be very plausible for pathogen screening in food samples prior to highly skillful laboratory analysis. Although nanoparticle-based detection assays still so far need to be officially approved and accepted by customers, the prospect of nanoparticles for rapid and sensitive pathogen detection is probably applicable.

Acknowledgements

This research project was supported by the National Research Council of Thailand. This work was also supported by a grant from Mahidol University, Thailand, and by a grant from the Faculty of Environment and Resource Studies, Mahidol University, Thailand.

References

1 E. Scallan, R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones and P. M. Griffin: Emerg. Infect. Dis. **17** (2011) 7.

2 R. L. Scharff: J. Food Prot. **75** (2012) 123.
3 S. Kumar, N. Dilbaghi, M. Barnela, G. Bhanjana and R. Kumar: BioNanoSci. **2** (2012) 196.
4 World Health Organization: WHO Technical Report No. 7051984 (1984).
5 F. Kaferstein: Bull. World Health Organ. **80** (2002) 759.
6 K. P. Burris and C. N. Stewart: Trends Food Sci. Technol. **28** (2012) 143.
7 A. V. Hadjinicolaou, V. L. Demetriou, J. Hezka, W. Beyer, T. L. Hadfield and L. G. Kostriki: J. Microbiol. Meth. **78** (2009) 45.
8 S. H. Liming and A. A. Bhagwat: Int. J. Food Microbiol. **95** (2004) 177.
9 T. V. Duncan: J. Colloid Interface Sci. **363** (2011) 1.
10 J. Heo and S. Z. Hua: Sensors **9** (2009) 4483.
11 N. Sanvicens, C. Pastells, N. Pascual and M. P. Marco: Trends Anal. Chem. **28** (2009) 1243.
12 P. Tallury, A. Malhotra, L. M. Byrne and S. Santra: Adv. Drug Deliver. Rev. **62** (2010) 424.
13 M. Tietjen and D. Y. C. Fung: Crit. Rev. Microbiol. **21** (1995) 53.
14 T. Humphrey and P. Stephens: Salmonella detection, eds. B. Caballero, L. Trugo and P. Finglas (Academic Press, New York, 2003) pp. 5079–5084.
15 V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa and C. Adley: Biotechnol. Adv. **28** (2010) 232.
16 H. G. Shen, S. Gould, J. Kinyon, T. Opriessnig and A. M. O'Connor: J. Appl. Microbiol. **111** (2011) 1037.
17 J. Chen, J. Tang, J. Liu, Z. Cai and X. Bai: J. Appl. Microbiol. **112** (2012) 823.
18 D. D. L. Bowtell: Nature General Supplement **21** (1999) 25.
19 G. H. W. Sanders and A. Manz: Trends Anal. Chem. **19** (2000) 364.
20 J. Wan, K. King, H. Craven, C. Mcauley, S. E. Tan and M. J. Coventry: Lett. Appl. Microbiol. **30** (2000) 267.
21 S. Nawaz: Pesticides and Herbicides. Residue determination, eds B. Caballero, L. Trugo and P. Finglas (Academic Press, New York, 2003) pp. 4487–4493.
22 V. Polan, J. Soukup and K. Vytras: SAGE-hindawi access to research enzyme research 2010 (2010) doi:10.4061/2010/324184.
23 J. Reyes and R. P. Cavalieri: Biosensors. Encyclopedia of Agricultural and Food Engineering, ed. D. R. Heldman (Marcel Dekker, New York, 2003) pp. 119–123.
24 L. A. Terry, S. F. White and L. J. Tigwell: J. Agric. Food Chem. **53** (2005) 1309.
25 M. S. Thakurm and K. V. Ragavan: J. Food Sci. Technol. **50** (2013) 625.
26 G. Kim, A. S. Om and J. H. Mun: J. Phys. Conf. Ser. **61** (2007) 555.
27 G. Y. Zhao, F. F. Xing and S. P. Deng: Electrochem. Commun. **9** (2007) 1263.
28 C. C. Lin, Y. C. Yeh, C. Y. Yang, C. L. Chen, G. F. Chen, C. C. Chen and Y. C. Wu: J. Am. Chem. Soc. **124** (2002) 3508.
29 M. Varshney, L. Yang, X. L. Su and Y. Li: J. Food Prot. **68** (2005) 1804.
30 M. Basu, S. Seggerson, J. Henshaw, J. Jiang, R. Cordona, C. Lefave, P. J. Boyle, A. Miller, M. Pugia and S. Basu: Glycoconjugate J. **21** (2004) 487.
31 R. Zhou, P. Wang and H. C. Chang: Electrophoresis **27** (2006) 1376.
32 L. Wang, C. S. Wu, X. Fan and A. Mustapha: Int. J. Food Microbiol. **156** (2012) 83.
33 Z. Fu, X. Zhou and D. Xing: Sens. Actuators, B **183** (2013) 633.
34 N. Thepwiwatjit, A. Thattiyaphong, P. Limsuwan, K. Tuitemwong and P. Tuitemwong: J. Nanomaterials **2013** (2013) 1.
35 W. Tansub, K. Tuitemwong, P. Limsuwan, S. Theparoonrat and P. Tuitemwong: J. Nanomaterials **2012** (2012) 1.
36 P. Tuitemwong, N. Songvorawit and K. Tuitemwong: J. Nanomaterials **2013** (2013) 1.
37 T. Soukka, K. Antonen, H. Harma, A. M. Pelkkikangas, P. Huhtinen and T. Lovgren: Clin. Chim. Acta **328** (2003) 45.
38 P. Baptista, E. Pereira, P. Eaton, G. Doria, A. Miranda, I. Gomes, P. Quaresma and R. Franco: Anal. Bioanal. Chem. **391** (2008) 943.

39 M. Vidotti, R. F. Carvalhal, R. K. Mendes, D. C. M. Ferreira and L. T. Kubota: J. Brazilian Chem. Soc. **22** (2011) 3.
40 S. Wang, A. K. Singh, D. Senapati, A. Neely, H. Yu and P. C. Ray: Chem. Eur. J. **16** (2010) 5600.
41 S. A. Khan, A. K. Singh, D. Senapati, Z. Fan and P. C. Ray: Chem. Commun. **47** (2011) 9444.
42 A. Jyoti, P. Pandey, S. P. Singh, S. K. Jain and R. Shanker: J. Nanosci. Nanotechnol. **10** (2010) 4154.
43 S. Chen, V. C. H. Wu, Y. Chuang and C. Lin: J. Microbiol. Methods **73** (2008) 7.
44 Z. Wang, N. Duan, J. Li, J. Ye, S. Ma and G. Le: J. Lumin. **26** (2011) 136.
45 C. Cao, L. C. Gontard, L. L. Tram, A. Wolff and D. D. Bang: Small **7** (2011) 1701.
46 G. C. Green, A. D. C. Chan, B. S. Luo, H. Dan and M. Lin: IEEE Trans. Instrum. Meas. **58** (2009) 3713.
47 M. Kahraman, M. M. Yazici, F. Sahin, O. F. Bayrak, E. Topcu and M. Culha: Int. J. Environ. Anal. Chem. **87** (2007) 763.
48 Y. Wang, S. Ravindranath and J. Irudayaraj: Anal. Bioanal. Chem. **399** (2011) 1271.
49 A. C. Vinayaka and M. S. Thakur: Anal. Bioanal. Chem. **397** (2010) 1445.
50 E. Tully, S. Hearty, P. Leonard and R. O'Kennedy: Int. J. Biol. Macromol. **39** (2006) 127.
51 J. G. Bruno, T. Phillips and M. P. Carrillo: J. Fluoresc. **19** (2009) 427.
52 M. Hahn, J. Tabb and T. Krauss: Anal. Chem. **77** (2005) 4861.
53 L. Yang and Y. Li: Analyst **131** (2006) 394.
54 Z. De Liu, S. F. Chen, C. Z. Huang, S. J. Zhen and Q. G. Liao: Anal. Chim. Acta **599** (2007) 279.
55 Y. Zhao, M. Ye, Q. Chao, N. Jia, Y. Ge and H. Shen: J. Agric. Food Chem. **57** (2009) 517.
56 P. B. Yim, M. L. Clarke, M. McKinstry, S. H. Lacerda, L. F. Pease, M. A. Dobrovolskaia, H. Kang, T. D. Read, S. Sozhamannan and J. Hwang: Biotechnol. Bioeng. **104** (2009) 1059.
57 F. C. Dudak and I. H. Boyaci: J. Rapid Methods Autom. Microbiol. **17** (2009) 315.
58 C. Crean (nee Lynam), E. Lahiff, N. Gilmartin, D. Diamond and R. O'Kennedy: Synth. Met. **161** (2011) 285.
59 C. Lynam, N. Gilmartin, A. I. Minett, R. O'Kennedy and G. Wallace: Carbon **47** (2009) 2337.
60 J. W. Lee, K. M. Shin, C. Lynam, G. M. Spinks, G. G. Wallace and S. J. Kim: Smart Mater. Struct. **17** (2008) 045029.
61 E. Lahiff, C. Lynam, N. Gilmartin, R. O'Kennedy and D. Diamond: Anal. Bioanal. Chem. **398** (2010) 1575.
62 W. Chunglok, D. K. Wuragil, S. Oaew, M. Somasundrum and W. Surareungchai: Biosens. Bioelectron. **26** (2011) 3584.
63 D. Tang, J. Tang, B. Su and G. Chen: J. Agric. Food Chem. **58** (2010) 10824.
64 Y. Huang, X. Dong, Y. Liu, L. Li and P. Chen: J. Mater. Chem. **21** (2011) 12358.
65 G. A. Zelada-Guillen, S. V. Bhosale, J. Riu and F. Rius: Anal. Chem. **82** (2010) 9254.
66 C. Garcia-Aljaro, L. N. Cella, D. J. Shirale, M. Park, F. J. Munoz, M. V. Yates and A. Mulchandani: Biosens. Bioelectron. **26** (2010) 1437.
67 X. Tang, S. Bansaruntip, N. Nakayama, E. Yenilmez, Y. Chang and Q. Wang: Nano Lett. **6** (2006) 1632.
68 R. Yang, Z. Tang, J. Yan, H. Kang, Y. Kim, Z. Zhu and W. Tan: Anal. Chem. **80** (2008) 7408.
69 X. Ding, H. Li, L. Deng, Z. Peng, H. Chen and D. Wang: Biosens. Bioelectron. **26** (2011) 4596.
70 J. Kaiser, M. Roesslein, T. Buerki-Thurnherr and P. Wick: Curr. Med. Chem. **18** (2011) 2115.
71 G. D. Nessim: Nanoscale **2** (2010) 1306.
72 M. Basu, S. Seggerson, J. Henshaw, J. Jiang, R. D. Cordona, C. Lefave, P. J. Boyle, A. Miller, M. Pugia and S. Basu: Glycoconjugate J. **21** (2004) 487.
73 J. B. Tok, F. Y. S. Chuang, M. C. Kao, K. A. Rose, S. S. Pannu, M. Y. Sha, G. Chakarova, S. G. Penn and G. M. Dougherty: Angew. Chem. Int. Ed. **45** (2006) 6900.

74 E. Spain, R. Kojima, R. B. Kaner, G. G. Wallace, J. O'Grady, K. Lacey, T. Barry, T. E. Keyes and R. J. Forster: Biosens. Bioelectron. **26** (2011) 2613.
75 S. Pal, E. C. Alocilja and F. P. Downes: Biosens. Bioelectron. **22** (2007) 2329.
76 F. Patolsky, G. Zheng and C. M. Lieber: Nat. Protoc. **1** (2006) 1711.
77 Y. Cheng, Y. Liu, J. Huang, K. Li, W. Zhang, Y. Xian and L. Jin: Talanta **77** (2009) 1332.
78 F. C. Dudak, I. H. Boyaci, A. Jurkevica, M. Hossain, Z. Aquilar, H. B. Halsall, C. J. Seliskar and W. R. Heineman: Anal. Bioanal. Chem. **393** (2009) 949.
79 H. Huang, C. Ruan, J. Lin, M. Li, L. M. Cooney, W. F. Oliver, Y. Li and A. Wang: Trans. ASABE **54** (2011) 1015.
80 B. Guven, N. Basaran-Akgul, E. Temur, U. Tamer and I. H. Boyaci: Analyst **136** (2011) 740.
81 Y. Huang, Y. Wang and X. Yan: Environ. Sci. Technol. **44** (2010) 7908.
82 R. Joshi, H. Janagama, H. P. Dwivedi, T. M. A. S. Kumar, L. Jaykus, J. Schefers and S. Sreevatsan: Mol. Cell. Probes **23** (2009) 20.
83 G. Amagliani, G. Brandi, E. Omiccioli, A. Casiere, I. Bruce and M. Magnani: Food Microbiol. **21** (2004) 597.
84 A. K. Singh, D. Senapati, S. Wang, J. Griffin, A. Neely, P. Candice, K. M. Naylor, B. Varisli, J. R. Kalluri and P. C. Ray: ACS Nano. **3** (2009) 1906.
85 T. V. Duncan: J. Colloid. Interface. Sci. **363** (2011) 1.
86 P. Dallas, V. K. Sharma and R. Zboril: Adv. Colloid Interface Sci. **166** (2011) 119.
87 H. H. Lara, E. N. Garza-Trevino, L. Ixtepan-Turrent and D. K. Singh: J. Nanobiotechnol. **9** (2011) 30.
88 Q. Feng, J. Wu, G. Chen, F. Cui, T. Kim and J. Kim: J. Biomed. Mater. Res. **52** (2000) 662.
89 B. De Gusseme, L. Sintubin, L. Baert, E. Thibo, T. Hennebel, G. Vermeulen, M. Uyttendaele, W. Verstraete and N. Boon: Appl. Environ. Microbiol. **76** (2010) 1082.
90 S. S. Khan, A. Mukherjee and N. Chandrasekaran: Colloid Surf. B **87** (2011) 129.
91 S. Egger, R. P. Lehmann, M. J. Height, M. J. Loessner and M. Schuppler: Appl. Environ. Microbiol. **75** (2009) 2973.
92 J. S. Kim, E. Kuk, K. N. Yu, J. Kim, S. J. Park, H. J. Lee, S. H. Kim, Y. K. Park, Y. H. Park, C. Y. Hwang, Y. S. Lee, D. H. Jeong and M. H. Cho: Nanomed.-Nanotechnol. Biol. Med. **3** (2007) 95.
93 L. K. Limbach, P. Wick, P. Manser, R. N. Grass, A. Bruinink and W. J. Stark: Environ. Sci. Technol. **41** (2007) 4158.
94 T. N. V. K. V. Prasad, V. S. R. Kambala and R. Naidu: Curr. Nanosci. **7** (2011) 531.
95 M. Saravanana and A. Nanda: Colloid Surf. B **77** (2010) 214.
96 L. Sintubin, B. Gusseme, P. Meeren, B. F. G. Pycke, W. Verstraete and N. Boon: Appl. Microbiol. Biotechnol. **91** (2011) 153.
97 G. Cárdenas, J. Díaz, M. F. Meléndrez, C. Cruzat and A. García: Polym. Bull. **62** (2009) 511.
98 T. Jin, D. Sun, J. Y. Su, H. Zhang and J. Sue: J. Food Sci. **74** (2009) M46.
99 X. Li, Y. Xing, Y. Jiang, Y. Ding and W. Li: Int. J. Food Sci. Technol. **44** (2009) 2161.
100 Y. Liu, L. He, A. Mustapha, H. Li, Z. Q. Hu and M. Lin: J. Appl. Microbiol. **107** (2009) 1193.
101 S. K. Bajpai, N. Chand and V. Chaurasia: J. Appl. Polym. Sci. **115** (2010) 674.
102 Y. Xie, Y. He, P. L. Irwin, T. Jin and X. Shi: Appl. Environ. Microbiol. **77** (2011) 2325.
103 B. Kim, D. Kim, D. Cho and S. Cho: Chemosphere **52** (2003) 277.
104 J. M. C. Robertson, P. K. J. Robertson and L. A. Lawton: J. Photochem. Photobiol. **175** (2005) 51.
105 G. Fu, P. S. Vary and C. Lin: J. Phys. Chem. B **109** (2005) 8889.
106 W. Kangwansupamonkon, V. Lauruengtana, S. Surassmo and U. Ruktanonchai: Nanomed.-Nanotechnol. Biol. Med. **5** (2009) 240.
107 A. Kubacka, M. L. Cerrada, C. Serrano, M. Fernández-García, M. Ferrer and M. Fernández-García: J. Phys. Chem. C **113** (2009) 9182.

108 M. L. Cerrada, C. Serrano, M. Sánchez-Chaves, M. Fernández-García, F. Fernández-Martín, A. Andrés, R. J. J. Riobóo, A. Kubacka, M. Ferrer and M. Fernández-García: Adv. Funct. Mater. **18** (2008) 1949.
109 C. Chawengkijwanich and Y. Hayata: Int. J. Food Microbiol. **123** (2008) 288.
110 Q. Cheng, C. Li, V. Pavlinek, P. Saha and H. Wang: Appl. Surf. Sci. **252** (2006) 4154.
111 T. S. Wu, K. X. Wang, G. D. Li, S. Y. Sun, J. Sun and J. S. Chen: ACS Appl. Mater. Int. **2** (2010) 544.
112 P. K. Stoimenov, R. L. Klinger, G. L. Marchin and K. J. Klabunde: Langmuir **18** (2002) 6679.
113 L. Huang, D. Q. Li, D. G. Evans and X. Duan: Eur. Phys. J. D **34** (2005) 321.
114 L. Huang, D. Q. Li, Y. J. Lin, M. Wei, D. G. Evans and X. Duan: J. Inorg. Biochem. **99** (2005) 986.
115 Y. J. Lin, D. Q. Li, G. Wang, L. Huang and X. Duan: J. Mater. Sci. Mater. Med. **16** (2005) 53.
116 K. Y. Yoon, J. Hoon Byeon, J. H. Park and J. Hwang: Sci. Total Environ. **373** (2007) 572.
117 N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M. D'Alessio, P. G. Zambonin and E. Traversa: Chem. Mater. **17** (2005) 5255.
118 G. Mary, S. K. Bajpai and N. Chand: J. Appl. Polym. Sci. **113** (2009) 757.
119 K. C. Anyaogu, A. V. Fedorov and D. C. Neckers: Langmuir **24** (2008) 4340.
120 L. Esteban-Tejeda, F. Malpartida, A. Esteban-Cubillo, C. Pecharromán and J. S. Moya: Nanotechnology **20** (2009) 505701.
121 G. Ren, D. Hu, E. W. C. Cheng, M. A. Vargas-Reus, P. Reip and R. P. Allaker: Int. J. Antimicrob. Agents **33** (2009) 587.
122 I. Perelshtein, G. Applerot, N. Perkas, E. Wehrschuetz-Sigl, A. Hasmann, G. Guebitz and A. Gedanken: Surf. Coat. Technol. **204** (2009) 54.
123 H. Palza, S. Gutiérrez, K. Delgado, O. Salazar, V. Fuenzalida, J. I. Avila, G. Figueroa and R. Quijada: Macromol. Rapid Commun. **31** (2010) 563.
124 F. Rispoli, A. Angelov, D. Badia, A. Kumar, S. Seal and V. Shah: J. Hazard. Mater. **180** (2010) 212.
125 A. Emamifar, M. Kadivar, M. Shahedi and S. Soleimanian-Zad: Food Control **22** (2011) 408.
126 K. H. Tam, A. B. Djurišić, C. M. N. Chan, Y. Y. Xi, C. W. Tse, Y. H. Leung, W. K. Chan, F. C. C. Leung and D. W. T. Au: Thin Solid Films **516** (2008) 6167.
127 G. Droval, I. Aranberri, A. Bilbao, L. German, M. Verelst and J. Dexpert-Ghys: e-Polymers **8** (2013) 1467.
128 B. A. Sevinç and L. Hanley: J. Biomed. Mater. Res. B **94** (2010) 22.
129 S. Lee: J. Appl. Polym. Sci. **114** (2009) 3652.
130 V. Chaurasia, N. Chand and S. K. Bajpai: J. Macromol. Sci. Pure Appl. Chem. **47** (2010) 309.
131 D. Yuvaraj, R. Kaushik and K. N. Rao: ACS Appl. Mater. Int. **2** (2010) 1019.
132 Z. Lu, C. M. Li, H. Bao, Y. Qiao and Q. Bao: J. Nanosci. Nanotechnol. **9** (2009) 3252.
133 Z. Lu, C. M. Li, H. Bao, Y. Qiao, Y. Toh and X. Yang: Langmuir **24** (2008) 5445.
134 J. H. Priester, P. K. Stoimenov, R. E. Mielke, S. M. Webb, C. Ehrhardt, J. P. Zhang, G. D. Stucky and P. A. Holden: Environ. Sci. Technol. **43** (2009) 2589.
135 L. Qi, Z. Xu, X. Jiang, C. Hu and X. Zou: Carbohydr. Res. **339** (2004) 2693.
136 Y. H. Lu, Y. Y. Chen, H. Lin, C. Wang and Z. D. Yang: J. Appl. Polym. Sci. **117** (2010) 3362.
137 K. Xing, X. G. Chen, M. Kong, C. S. Liu, D. S. Cha and H. J. Park: Carbohydr. Polym. **76** (2009) 17.
138 S. Kang, M. Pinault, L. D. Pfefferle and M. Elimelech: Langmuir **23** (2007) 8670.
139 S. Kang, M. S. Mauter and M. Elimelech: Environ. Sci. Technol. **43** (2009) 2648.
140 C. Costa, A. Conte, G. G. Buonocore and M. A. Nobile: Int. J. Food Microbiol. **148** (2011) 164.
141 A. L. Incoronato, A. Conte, G. G. Buonocore and M. A. Nobile: J. Dairy Sci. **94** (2011) 1697.
142 T. Jin, D. Sun, J. Y. Su, H. Zhang and H. Sue: J. Food Sci. **74** (2009) M46.

143 Y. Liu, L. He, A. Mustapha, H. Li, Z. Q. Hu and M. Lin: J. Appl. Microbiol. **107** (2009) 1193.
144 S. R. Choudhury, M. Ghosh, A. Mandal, D. Chakravorty, M. Pal, S. Pradhan and A. Goswami: Appl. Microbiol. Biotechnol. **90** (2011) 733.
145 Q. Li, S. Mahendra, D. Y. Lyon, L. Brunet, M. V. Liga, D. Li and P. J. J. Alvarez: Water Res. **42** (2008) 4591.
146 A. L. Incoronato, G. G. Buonocore, A. Conte, M. Lavorgna and M. A. Del Nobile: J. Food Prot. **73** (2010) 2256.
147 M. A. Busolo, P. Fernandez, M. J. Ocio and J. M. Lagaron: Food Addit. Contam. Part A **27** (2010) 1617.
148 H. Gu, P. L. Ho, E. Tong, L. Wang and B. Xu: Nano Lett. **3** (2003) 1261.
149 L. Bi, L. Yang, G. Narsimhan, A. K. Bhunia and Y. Yao: J. Controlled Release **150** (2011) 150.
150 S. Liu, A. K. Ng, R. Xu, J. Wei, C. M. Tan, Y. Yang and Y. Chen: Nanoscale **2** (2010) 2744.
151 L. Bi, L. Yang, A. K. Bhunia and Y. Yao: Biotechnol. Bioeng. **108** (2011) 1529.
152 H. Yang, L. Qu, A. Wimbrow, X. Jiang and Y. Sun: J. Food Prot. **70** (2007) 1844.
153 J. Kim, E. V. Shashkov, E. I. Galanzha, N. Kotagiri and V. P. Zharov: Lasers Surg. Med. **39** (2007) 622.
154 L. Qu, P. G. Luo, S. Taylor, Y. Lin, W. Huang, N. Anyadike, T. R. Tzeng, F. Stutzenberger, R. A. Latour and Y. P. Sun: J. Nanosci. Nanotechnol. **5** (2005) 319.
155 F. He and S. Liu: Talanta **62** (2004) 271.
156 X. Zhao, L. R. Hilliard, S. J. Mechery, Y. Wang, R. P. Bagwe, S. Jin and W. Tan: PNAS **101** (2004) 15027.
157 R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, C. Merril, K. Nagashima and S. Adhya: Proc. Natl. Acad. Sci. U.S.A **103** (2006) 4841.
158 K. El-Boubbou, C. Gruden and X. Huang: J. Am. Chem. Soc. **129** (2007) 13392.
159 L. Wang, W. Zhao, M. B. O'Donoghue and W. Tan: Bioconjug. Chem. **18** (2007) 297.
160 H. W. Gu, K. Xu, C. Xu and B. Xu: Chem. Commun. **9** (2006) 941.
161 J. Gao, L. Li, P. L. Ho, G. C. Mak, H. Gu and B. Xu: Adv. Mater. **18** (2006) 3145.
162 H. Yang, L. W. Qu, Y. Lin, X. P. Jiang and Y. P. Sun: J. Biomed. Nanotechnol. **3** (2007) 131.
163 C. W. Donnelly: Nutr. Rev. **59** (2001) 183.
164 W. Lian, S. A. Litherland, H. Badrane, W. Tan, D. Wu, H. V. Baker, P. A. Gulig, D. V. Lim and S. Jin: Anal. Biochem. **334** (2004) 135.
165 C. S. Chen, A. J. Baeumner and R. A. Durst: Talanta **67** (2005) 205.
166 L. Y. Lee, S. L. Ong, J. Y. Hu, W. J. Ng, Y. Feng, X. Tan and S. W. Wong: Appl. Environ. Microbiol. **70** (2004) 5732.
167 H. C. Chang: AIChe. J. **53** (2007) 2486.
168 Y. Lin, X. P. Jiang, T. Elkin, K. A. S. Fernando, L. R. Gu, S. Taylor, H. Yang, E. Jones, W. Wang and Y. P. Sun: J. Nanosci. Nanotechnol. **6** (2006) 868.
169 H. Yang, L. W. Qu, A. N. Wimbrow, X. P. Jiang and Y. P. Sun: Int. J. Food Microbiol. **118** (2007) 132.
170 R. A. N. Chmielewski and J. F. Frank: Compr. Rev. Food Sci. Food Saf. **2** (2003) 22.
171 J. F. Frank and R. A. Koffi: J. Food Prot. **53** (1990) 550.
172 M. Bruchez, M. Moronne, P. Gin, S. Weiss and A. P. Alivisatos: Science **281** (1998) 2013.
173 L. Yang and Y. Li: Analyst **131** (2006) 394.
174 C. S. Chen and R. A. Durst: Talanta **69** (2006) 232.
175 R. Beckman, E. Johnston-Halperin, Y. Luo, J. E. Green and J. R. Heath: Science **310** (2005) 465.